PHYSIOLOGICAL AND ANATOMICAL STUDIES ON *COLEUS AMBOINICUS* Lour. SUBJECTED TO HEAVY METALS STRESS

Thesis submitted to
the University of Calicut in partial fulfillment of
the requirements for the degree of

DOCTOR OF PHILOSOPHY IN BOTANY

By

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CERTIFICATE

This is to certify that the thesis entitled **"PHYSIOLOGICAL AND ANATOMICAL STUDIES ON *COLEUS AMBOINICUS* Lour. SUBJECTED TO HEAVY METALS STRESS"** submitted by **Sudheeshna P. K.** 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 2018-2024 and no part thereof has been submitted for the award of any other degree.

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This is to certify that all the corrections mentioned by the adjudicators in the thesis entitled **"PHYSIOLOGICAL AND ANATOMICAL STUDIES ON *COLEUS AMBOINICUS* Lour. SUBJECTED TO HEAVY METALS STRESS"** submitted by **SUDHEESHNA P. K.** has been incorporated as per the adjudication report. It is also certified that the contents in the thesis and the CD submitted are one and the same.

Pattambi
Date:

Dr. HUSSAIN K.

DECLARATION

I, **Sudheeshna P.k.** hereby declare that the work presented in the thesis entitled **"PHYSIOLOGICAL AND ANATOMICAL STUDIES ON *COLEUS AMBOINICUS* Lour. SUBJECTED TO HEAVY METALS STRESS"** 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.

S.N.G.S. College Pattambi **Sudheeshna P.K.**

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"To my Father, My Hero –

Your love is the foundation upon which
my achievements stand."

ABSTRACT

The present study investigated the heavy metal-induced stress and tolerance potential of *Coleus amboinicus* Lour., a widely used medicinal herb belongs to Lamiaceae family. It is a large fleshy succulent perennial herb with aromatic pubescence and inherent medicinal properties due to the presence of many phytochemicals and is known by a bunch of vernacular names ranging from Indian borage to “Panikoorka” in Malayalam.The plant's structural and functional alterations were assessed through analyses of roots, stems, and leaves exposed to heavy metal stress. Rooted propagules were treated with Al (500 µM), Cr (150 µM), Cu (80 µM), and Hg (10 µM) in Hoagland nutrient medium. The immediate response to metal exposure was observed in morphology and anatomy. The effects of heavy metals on development, growth, and metabolism were evaluated using a range of parameters, including stem, root, and leaf morphometry. Tolerance and stomatal indices were calculated to assess heavy metal impacts. Additionally, biochemical analyses were performed to quantify and qualify changes in protein, phenolic, proline, chlorophyll, and carotenoid pigment content. To investigate the heavy metal-induced oxidative stress, the activities of antioxidant enzymes, including catalase and superoxide dismutase, and malondialdehyde (MDA) production were analyzed in plant parts. Additionally, scanning electron microscopy (SEM) was employed to confirm anatomical changes in plant organs. Furthermore, SEM-energy dispersive X-ray spectroscopy (SEM-EDX) was used to determine the distribution and localization of heavy metal ions in the plant tissues. The bioaccumulation pattern of *C. amboinicus* for heavy metals was assessed using Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES). Given the plant's medicinal importance, Gas Chromatography-Mass Spectrometry (GC-MS) was employed to analyze the occurrence and distribution of bioactive secondary metabolites.

Morphological analysis revealed slight growth retardation in root length across all metal treatments compared to the control, with no significant differences

between metals. The tolerance index percentage showed significant reduction in all metal treatments compared to the control, with negligible differences between metals. The reduction in photosynthetic pigments like chlorophyll content along with increase in carotenoids provides the plant with protection from photo oxidative damage.

In *C. amboinicus* treated with Hg, there is an increased number of stomata and a wider stomatal opening; and also, the presence of lenticels. These traits are directly linked to the removal of volatile forms of Hg, which is an indicator of toxicity sequestration. An interesting observation in the Cu treatment is the removal of oil globules from stem and leaf. SEM images give the clear evidences for the removal of oil globules. The possible reason for the removal of oil globules may be due to the reduction of a secondary metabolite named alpha-bergamotene after Cu treatment. It is a major component of essential oils in various plants and it contributes to the aromatic profile and therapeutic properties of essential oils. The enhanced cell wall thickness plays vital role in efficient sequestration of toxic levels of Cu^{2+} in the cell wall, thereby preventing the entry of toxic ions to the cytoplasm and enhancing Cu stress tolerance in *C. amboinicus*. Anatomical examination revealed that plants treated with chromium, copper, and mercury exhibited significant structural changes, including a broken epidermal layer and alterations in vessel size and shape. In contrast, aluminium-treated plants showed minimal anatomical changes, likely due to aluminium's essential role as a micronutrient with a negligible stimulatory effect on plant growth. According to GCMS results, effect of heavy metals resulting in the absence of many secondary metabolites which is vital for the antibacterial property of *C. amboinicus* whereas occurrence of some new bioactive components especially Tetradecanoic acid,12-methyl-methyl ester shows same properties.

Key word: *Coleus amboinicus*, Heavy Metals, Bio accumulation, Lenticel, Oil globules

സംഗ്രഹം

കോലിയസ് അംബോയിനിക്കസ് ലൂർ എന്ന ഔഷധ സസ്യത്തിന്റെ ഹെവി മെറ്റൽ പ്രേരിത സമ്മർദ്ദവും സഹിഷ്ണുതയും അന്വേഷിക്കുന്നതിനാണ് ഇപ്പോഴത്തെ പഠനം നടത്തിയത്. ലാമിയേസി കുടുംബത്തിൽ പെടുന്ന വ്യാപകമായി ഉപയോഗിക്കപ്പെടുന്ന ഒരു ഔഷധസസ്യമാണിത്, മിക്കവാറും എല്ലാ ഭ്രപ്രദേശങ്ങളിലും ധാരാളമായി വളരുന്നു. ധാരാളം ഫൈറ്റോകെമിക്കലുകളുടെ സാന്നിധ്യം കാരണം കുട്ടികളിലെ പനി, ജലദോഷം തുടങ്ങിയ അസുഖങ്ങൾക്ക് ഈ സസ്യത്തിന്റെ നീര് മരുന്നായി ഉപയോഗിക്കുന്നു. ധാരാളം ജലാംശം അടങ്ങിയ ഈ സസ്യം ഇന്ത്യൻ ബോറേജ് മുതൽ മലയാളത്തിൽ "പനിക്കൂർക്ക" വരെയുള്ള ഒരു കൂട്ടം പ്രാദേശിക നാമങ്ങളിൽ അറിയപ്പെടുന്നു. ഹെവി മെറ്റൽ സമ്മർദ്ദത്തിന് വിധേയമായ ചെടികളിലെ ഘടനാപരവും പ്രവർത്തനപരവുമായ മാറ്റങ്ങൾ വിലയിരുത്തുന്നതിനായി ഈ ചെടി പഠന വിധേയമാക്കി. ഈ പഠനത്തിൽ, ഹോഗ്‌ലാൻഡ് പോഷക മാധ്യമത്തിൽ വേരുന്നിയ പ്രോപ്പഗ്രൂലുകളെ Al, Cr, Cu, Hg എന്നിവയ്ക്ക് വിധേയമാക്കി. ഈ ലോഹത്തോടുള്ള ചെടിയുടെ ഉടനടി പ്രതികരണം രൂപശാസ്ത്രത്തിലും ശരീരഘടനയിലും കാണപ്പെടുന്നു. വികസനം, വളർച്ച, എന്നിവയിൽ കനത്ത ലോഹങ്ങളുടെ സ്വാധീനം തണ്ട്, വേര്, ഇല എന്നിവയുടെ അളവുകൾ ഉൾപ്പെടെ വിവിധ അളവുകൾ ഉപയോഗിച്ച് അന്വേഷിച്ചു. കനത്ത ലോഹങ്ങളുടെ ഫലങ്ങളെക്കുറിച്ച് പഠിക്കാൻ ടോളറൻസ് ഇൻഡക്സും സ്റ്റോമറ്റൽ ഇൻഡക്സും ഉപയോഗിച്ചു. കൂടാതെ, പ്രോട്ടീൻ, ഫിനോളിക്സ്, പ്രോലിൻ, ക്ലോറോഫിൽ, കരോട്ടിനോയിഡ് പിഗ്മെന്റുകൾ എന്നിവ ഗുണപരമായും അളവിലും വിശകലനം ചെയ്തു. ROS-ന്റെ ജനറേഷൻ ഹെവി മെറ്റൽ വിഷബാധയാൽ പ്രേരിപ്പിച്ചതിനാൽ, കാറ്റലേസ്, സൂപ്പർഓക്സൈഡ് ഡിസ്മ്യൂട്ടേസ് പ്രവർത്തനങ്ങൾ, എംഡിഎ ഉൽപ്പാദനം തുടങ്ങിയ സ്കാവെഞ്ചിംഗ് എൻസൈമുകളുടെ പ്രവർത്തനവും റൂട്ട്, തണ്ട്, ഇലകൾ തുടങ്ങിയ വിവിധ അവയവങ്ങളിൽ പരിശോധിച്ചു. പ്ലാന്റിന്റെ വിവിധ ഭാഗങ്ങളിൽ ശരീരഘടനാപരമായ മാറ്റങ്ങൾ വിശദീകരിക്കുന്നതിനുള്ള ഒരു സ്ഥിരീകരണ നടപടിയായി, SEM ചെയ്തു. ന്യൂട്രിയന്റ് മീഡിയത്തിലേക്ക് ചേർത്ത ഹെവി മെറ്റൽ അയോണുകളുടെ പങ്കാളിത്തവും വിതരണ രീതിയും കൃത്യമായി കണ്ടെത്തുന്നതിന്, SEM-EDX സാങ്കേതികതയും ചെയ്തു. ICP-OES ഉപയോഗിച്ച് ഘന ലോഹങ്ങളിലേക്കുള്ള C. ആംബോനിക്കസിന്റെ ബയോകുമുലേഷൻ സാധ്യതകളും വിശകലനം ചെയ്തു. ഇതൊരു ഔഷധ സസ്യമായതിനാൽ, ജിസി-എംഎസ് ഉപയോഗിച്ച് ബയോ ആക്റ്റീവ് സെക്കൻഡറി മെറ്റബോളിറ്റുകളുടെ സംഭവവും വിതരണവും വിശകലനം

ചെയ്യ. ആൻ്റിഓക്സിഡേഷൻ ഉപകരണം, ഓസ്മോട്ടിക് അഡ്ജസ്റ്റ്മെന്റ്, ഫോട്ടോസിന്തറ്റിക് എഫിഷ്യൻസി, ബയോഅക്യുമുലേഷൻ എന്നിവ മോഡുലേറ്റ് ചെയ്യകൊണ്ട് Al, Cr, Cu, Hg എന്നിവയുടെ ഉയർന്ന സാന്ദ്രതയെ C. അംബോനിക്കസിന് സഹിക്കാൻ കഴിയുമെന്ന് ഫലങ്ങൾ സൂചിപ്പിക്കുന്നു.

ക്ലോറോഫിൽ പോലുള്ള ഫോട്ടോസിന്തറ്റിക് പിഗ്മെൻ്റുകളുടെ ഉള്ളടക്കം കുറയുകയും കരോട്ടിനോയിഡുകളുടെ വർദ്ധനവ് ഫോട്ടോ ഓക്സിഡേറ്റീവ് നാശത്തിൽ നിന്ന് ചെടിയെ സംരക്ഷിക്കുകയും ചെയ്യുന്നു. Hg ഉപയോഗിച്ച് ചികിത്സിക്കുന്ന സസ്യങ്ങളിൽ, സ്റ്റോമറ്റയുടെ എണ്ണം വർദ്ധിക്കുകയും വിശാലമായ സ്റ്റോമറ്റൽ തുറക്കുകയും ചെയ്യുന്നു; കൂടാതെ, ലെന്റിസെല്ലുകളുടെ സാന്നിധ്യം സസ്യത്തെ സംരക്ഷിക്കുന്നു. ഈ സ്വഭാവസവിശേഷതകൾ Hg യുടെ അസ്ഥിര രൂപങ്ങളുടെ രക്ഷപ്പെടലുമായി നേരിട്ട് ബന്ധപ്പെട്ടിരിക്കുന്നു, ഇത് വിഷാംശം വേർതിരിക്കുന്നതിന്റെ ഒരു സൂചകമാണ്. Cu ചികിത്സയിലെ രസകരമായ ഒരു നിരീക്ഷണം തണ്ടിൽ നിന്നും ഇലകളിൽ നിന്നും ഓയിൽ ഗ്ലോബ്യൂളുകൾ നീക്കം ചെയ്യുന്നതാണ്. ഓയിൽ ഗ്ലോബ്യൂളുകൾ നീക്കം ചെയ്യുന്നതിനുള്ള വ്യക്തമായ തെളിവുകൾ SEM ചിത്രങ്ങൾ നൽകുന്നു. Cu ചികിത്സയ്ക്ക് ശേഷം ആൽഫ-ബെർഗാമോട്ടീൻ എന്ന ദ്വിതീയ മെറ്റബോളിറ്റിന്റെ കുറവായിരിക്കാം ഇത് നീക്കം ചെയ്യാനുള്ള കാരണം. വിവിധ സസ്യങ്ങളിലെ അവശ്യ എണ്ണകളുടെ ഒരു പ്രധാന ഘടകമാണ് ഇത്, അവശ്യ എണ്ണകളുടെ സുഗന്ധവ്യഞ്ജന പ്രൊഫൈലിനും ചികിത്സാ ഗുണങ്ങൾക്കും ഇത് സംഭാവന ചെയ്യുന്നു. സെൽ ഭിത്തിയിലെ Cu2+ ന്റെ വിഷാംശ അളവ് കാര്യക്ഷമമായി വേർതിരിക്കുന്നതിൽ വർദ്ധിപ്പിച്ച കോശഭിത്തി കനം സുപ്രധാന പങ്ക് വഹിക്കുന്നു, അതുവഴി സൈറ്റോപ്ലാസത്തിലെ വിഷ അയോണുകളുടെ സാന്നിധ്യം തടയുകയും C. അംബോനിക്കസിൽ Cu സ്ട്രെസ് ടോളറൻസ് വർദ്ധിപ്പിക്കുകയും ചെയ്യുന്നു. അംബോനിക്കസിന്റെ ആൻറി ബാക്ടീരിയൽ സ്വഭാവത്തിന് അത്യന്താപേക്ഷിതമായ നിരവധി ദ്വിതീയ മെറ്റബോളിറ്റുകളുടെ അഭാവത്തിൽ ഘനലോഹങ്ങളുടെ പ്രഭാവം സംഭവിക്കുന്നു, അതേസമയം ചില പുതിയ ബയോ ആക്റ്റീവ് ഘടകങ്ങൾ പ്രത്യേകിച്ച് ടെട്രാഡെകാനോയിക് ആസിഡ്, 12-മീഥൈൽ-മീഥൈൽ എസ്റ്ററിന് സമാനമായ ഗുണങ്ങൾ കാണിക്കുന്നു.

സൂചക പദങ്ങൾ: കോളിയസ് അംബോയിനിക്കസ്, ഹെവി മെറ്റലുകൾ, ബയോ അക്യുമുലേഷൻ, ലെൻ്റിസെൽ, ഓയിൽ ഗ്ലോബ്യൂൾസ്

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ABBREVIATIONS

Al	-	Aluminium
As	–	Arsenic
BCF	-	Bioconcentration factor
CAT	-	Catalase
Cd	-	Cadmium
Chl	-	Chlorophyll
Co	–	Cobalt
Cr	-	Chromium
Cu	-	Copper
DW	-	Dry weight
EDTA	-	Ethylenediaminetetraacetic acid
EDX	-	Energy dispersive X-ray analysis
FW	-	Fresh weight
GCMS	-	Gas chromatography and mass spectrometry
H_2O_2	-	Hydrogen peroxide
Hg	-	Mercury
KBr	-	Potassium bromide
MC%	-	Moisture content %
MDA	-	Malondialdehyde
MDHA	-	Monodehydroascorbate
Mn	–	Manganese
MT	-	Metallothionein
Ni	–	Nickel
$^{\bullet}O2^{-}$	–	Superoxide
Pb	-	Lead
PC	-	Phytochelatin

POD	-	Guaiacol peroxidase
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	-	Superoxide dismutase
TBA	-	Thiobarbituric acid
TCA	-	Trichloroacetic acid
TF	-	Translocation factor
Zn	–	Zinc

INTRODUCTION

In nature, plants are exposed to a wide variety of environmental stresses and heavy metal stress is one of the common stresses that limit plant growth and development. The group of heavy metals in general consist of essential and non-essential elements for plants. The essential metals include Fe, Mn, Zn, Cu, Mo, Ni etc. All the rest are toxic like Cd, Cr, Pb, Hg etc. Aluminium is not a heavy metal but in some respect its effect shows similarities with heavy metal toxicity. Toxicity is always the question of concentration. The essential nutrient elements may be available at an optimal concentration range. For non-essential, certainly, there is no optimal concentration range. Since most of the non-essential metals are toxic, even at very low concentration to plants, whereas the optimal concentrations of essential metal can be termed 'physiological' and above the optimal concentration the essential metals impose toxicity to plants.

Heavy metals stand out among other pollutants as one of the major environmental hazards. Once released into the environment, they can never be degraded/ removed either chemically or biologically and hence they are ultimately indestructible. Heavy metal contamination of soil and water is becoming a more serious problem as a result of careless disposal of toxic metal compounds that are produced from a variety of sources, including industrial and agricultural activities, in to land regions, surface water, and ground water.

High concentrations of heavy metals in soil and water results in the increased absorption and translocation of metal ions by plants which negatively affect growth, as these metals interfere with metabolic functions such as photosynthesis, respiration and degeneration of main cell organelles, ultimately leading to plant senescence and death. A wide range of metabolic and physiological alterations are driven when plants are exposed to heavy metals. The effects of various heavy metals on plant metabolism vary, and overall growth retardation is the visual response. Plants growing in metal enriched soil absorb metal ions to varying levels, in response to external and internal factors. Considerable interest has been focused on the pattern

of absorption, translocation and accumulation of heavy metals, because experimental studies show subtle differences in absorption and translocation by tolerant and intolerant genotypes.

A review of the botanical literature reveals various aspects of absorption, translocation, metabolism and resultant impact of heavy metals, demonstrating the extent of variations between the genotypes and nutrients as well as non-essential metals in the field and laboratory studies ranging from trace nutrient elements to toxic heavy metals (Salt *et al*., 1998; Cseh, 2002; Memon and Schroder, 2009; Solanki and Dhanker, 2011; Pradhan, 2017b; Kumar *et al*., 2021; Babangida *et al*., 2021; Yan *et al*., 2021).

Aluminium is one of the most abundant and potent toxic metals in acidic soils, which constitute nearly 40% of the world's arable lands (Foy *et al*., 1978; Kochian, 1995; Kochian *et al*., 2005). At neutral or weakly acidic pH, Al exists in the form of insoluble aluminosilicate or oxide which is non-toxic to plants. When the soil becomes more acidic, Al is solubilized into a phytotoxic form and soluble Al is classified into several groups such as free or mononuclear forms of Al^{3+}, polynuclear Al and as a low molecular-weight complex (Kochian, 1995). Aluminium is one of the mineral soil components and can be rendered free from mineral soil via lowered pH of the environment (Vitorello *et al*.,2005). The release of metal occurs in the form of Al (III). Al (III) is toxic to plants and is counted as the most toxic form of Al (Rout *et al*.,2001; Sathyaseelan and Karthika, 2019).

While the levels of Al are expressed in its compound forms in some studies (Wang *et al*., 2016; Li *et al*., 2018), others expressed it in a trivalent form which gives a better representation of the amount of Al plant roots are exposed to (Awasthi *et al*., 2017; Jaskowiak *et al*., 2018; Sun *et al*., 2020). For example, low Al concentration of 0.25 and 0.5 mM did not affect Trifolium and tomato (*Solanum lycopersicum*) seedling root growth whereas high concentrations of 1.25 mM remarkably restricted root growth (Bortolin *et al*., 2020; Ofoe *et al*., 2023). In barley (*Hordeum vulgare*), low concentrations between 5-20 µM had no significant effect on root grow while concentrations of 40 and 60 µM reduced root growth. Similarly,

exposure of plants to low Al doses for a short period inhibited root growth whereas no inhibition effect was noticed with higher Al concentrations for long-period exposure (Zhou *et al*., 2011). These suggest that different plant species have different response mechanisms to Al toxicity.

Cytological, morphological and physiological aspects of Al toxicity in plants has been extensively documented (Kochian, 1995; Kochian *et al*., 2005; Delhaize and Ryan, 1995; Horst *et al.*, 1999; Kollmeier *et al*., 2000; Marienfeld *et al*., 2000; Poschenrieder *et al*., 2008). According to Delhaize and Ryan (1995) and Kochian (1995), there are two strategies behind the Al tolerance mechanism, exclusion of Al from the root apex and internal tolerance once Al enters the plant symplasm. The exclusion mechanism involves secretion of Al chelating ligands binding of Al with the cell wall and mucilage, a plant induced pH barrier in the rhizosphere or root apoplasm, selective permeability of the plasma membrane and Al^{3+} efflux (Watanabe *et al.*, 2005).

In general, Al interferes with cell division in root tips and lateral root formation and increases cell wall rigidity (Kochian, 1995; Posehneider *et al*., 2008). Root tips and elongation region of plants are considered to be the primary sites of Al ion toxicity (Taylor, 1995; Rengel, 1996). Aluminium also alters biochemical processes, impairing DNA synthesis, root respiration, enzyme inactivation, and the intake and translocation of critical elements, in addition to structural modifications (Foy, 1992).

The metal actively interferes with plant cell cellular and molecular events (Kochian *et al*., 2005), such as cell growth (Matsumoto, 2000; Silva *et al*., 2012). For example, in the case of *N. tabacum* cells, a 50 micromolar dose of the metal reduces the post-treatment growth by 70%, while a 100 micromolar dose nearly eliminates growth (Abdel-Basset *et al*., 2010). In addition to growth inhibition, another cellular response against Al is callose (1,3-glucan) expression (Chang *et al*., 1999; Larsen *et al*., 1996). According to Horst *et al*. (1997), callose expression is the most concrete sign of the genotypic toxicity of Al. Together with other toxic effects, the metal is also discovered to prevent tubulin polymerization, which promotes a

delay in microtubular disassembly in the mitotic division of plant cells (Vardar and Ünal, 2007).

The release of Al chelators (for instance, malate and oxalate) from plant roots to the environment is counted among known strategies plants utilize against Al. While this is an extracellular mechanism to cope with the metal, there are also intracellular routes to combat the metal. One of these intracellular routes is the chelation of Al with organic acids in the cytoplasm and the subsequent imprisonment into the vacuole.

Chromium has been found to have a variety of toxic effects on plants(Clijster and Van Assche, 1985; Bishnoi *et al*., 1993). According to those authors, the impact of Cr on physiology of plants depends on the metal speciation that causes the absorption, subsequent mobilization of the metal, and toxic effects. Toxic effects of Cr have been reported in growth and development (Rout *et al*., 1997; Iqbal *et al*., 2001). The mechanism of enhanced accumulation of Cr in roots provides some sort of natural tolerance to plants towards Cr toxicity (Shanker *et al*., 2005; Singh *et al*., 2013; Ali *et al*., 2015; Pradhan, 2017b).

The wide distribution of Cr in soil, water, and biological materials has made it a serious pollutant in the ecosystem over the past few decades (Gill *et al*., 2015). With a specific density of 7.19 g/cm^3, Cr is the 21st most prevalent heavy metal in the Earth's crust and ranks seventh among all metals (Economou-Eliopoulos *et al*., 2013). Chromium ions are released into the atmosphere from industries like chrome plating, cement plants, steel production works, manufacture of dyes and paints, mining, leather tanning, textile industry, aircraft industry, wood preservation, mud drilling, and upon leaching from improper sanitary landfills (Coetzee *et al*., 2020; Haider *et al*., 2022). Chromium has high redox potential and can exist in a range of valence states from (-II) to (IV), in which Cr (0), Cr (III), and Cr (VI) are the stable forms in nature (Jiang *et al*.2015). Chromium in different oxidation states shows different chemical, toxic and epidemiological characteristics, and Cr (VI) is 100 times more toxic than Cr (III) because of its higher oxidation potential, solubility, and mobility (Liang *et al*., 2021).

Another important effect of Cr on plants is induction of free radical scavenging enzymes such as catalase, peroxidase and superoxide dismutase which are involved in the detoxification of Cr toxicity (Prasad, 1998; Shanker *et al.*, 2004).

Plants absorb Cr in both its valence states, i.e., Cr (III) and Cr (VI) (Shahid *et al.*, 2017). The cation exchange sites in plant cell walls allow the passive entry of Cr (III) (Singh *et al.*, 2013; Park, 2020), while sulphate and phosphate carriers actively transport Cr (VI) into plant cells (Gill *et al.*, 2017; Xu *et al.*, 2021). Chromium (III) may have beneficial effects on plant growth with an enhanced yield at low concentrations, even if it is not necessary for plants (Paiva *et al.*, 2009; Helena, 2012). However, Cr (III) at high concentrations and Cr (VI) can have deleterious effects on plant physiological processes such as development, seed germination, mineral nutrition, photosynthesis, biomass production, metabolism, and crop productivity and eventually cause plant death (Chebeir *et al.*, 2016; Anjum *et al.*, 2017; Shahid *et al.*, 2017; Jobby *et al.*, 2018; Singh *et al.*, 2020). Chromium toxicity reduces plant growth by inducing ultrastructural modifications of the cell membrane and chloroplast, modulation in cell division and cell cycle, degradation of chlorophyll, water and minerals imbalance, affecting transpiration and nitrogen assimilation and alters enzymatic activities (Reale *et al.*, 2016; Anjum *et al.*, 2017; Masciarelli *et al.*, 2017; Zaheer *et al.*, 2020). High Cr concentrations in the soil are taken up and translocated to shoots of plants, where it is stored and eventually enter the food chain and have adverse effects on human health (Giri and Singh, 2017). It is a powerful epithelial irritant and can cause bronchitis, dermatitis, and tuberculosis (Saud *et al.*, 2022). Therefore, it is crucial to develop effective methods for removing Cr from the environment.

Mercury (Hg) is unique in that occurs in the environment in several physical and chemical forms. In well oxygenated soil environments, the soluble $HgCl_2$, Hg(OH)Cl, and $Hg(OH)_2$ are the predominant Hg species (Schuster, 1991). High level of Hg^{2+} becomes strongly phytotoxic to cells and induce visible injuries and physiological disorder (Orcutt and Nilsen, 2000; Ortega-Villasante *et al.*, 2005; Zhou *et al.*, 2007).

Toxicity of Hg has been reported in many plants and even in very low concentrations it causes hazards to plant growth (Sandmann and Boger, 1983; Kagi and Hapke, 1984; De *et al*., 1985). Translocation and accumulation of Hg are very feeble in plants and no plants have been reported as hyperaccumulator of this element (Henry, 2000; Raskin and Ensley, 2000). According to those authors, transgenic plants of *Arabidopsis thaliana* are known to accumulate Hg. Velasco-Alinsug *et al*. (2005) reported *Chromolaena odorata* as an accumulator of Hg and the mode of sequestration is by phytovolatilization and hence this plant has been recommended for phytoremediation technology. Although phytoremediation potential of *Bacopa monnieri* has already been reported by cultivating this plant in nutrient medium artificially contaminated with heavy metals like Cd, Cr, Hg and Pb (Sinha, 1999; Hussain, 2007). Studies on accumulation pattern of different heavy metal by naturally growing *Bacopa monnieri* collected from different polluted habitats confirmed the phytoremediation potential of this medicinal plant (Hussain *et al.,* 2010). In *Vigna mungo* the 'cycling' of Hg between growth media, plant and atmosphere involves absorption, chelation and localization in the roots and / or excretion through trichomes (Hussain *et al*., 2010).

Copper (Cu) is an essential micronutrient for plants and is a component of plastocyanin, constituent of many enzymes like ascorbic acid oxidase, tyrosinase, uricase, cytochrome oxidase, phenolase and laccase (Mengel and Kirkby, 2001). Copper is a redox-active transition metal that plays a key role in a variety of functions in plant growth, development and metabolism including CO_2 absorption, electron transport, cellular transportation, mitochondrial respiration, ATP generation, protein trafficking and hormone signalling (Demirevska-Kepova *et al*., 2004; Paz-Ferreira *et al*., 2014; Marques *et al*., 2018).

Given the potential of Cu as an essential micronutrient for plants, higher concentrations of Cu is also toxic to growth and development of plants and cause suppressed root growth and leaf chlorosis (Baker and Walker, 1989). Copper-induced root abnormalities range from the disruption of root epidermis, decreased root hair proliferation to severe root deformation (Sheldon and Menzies, 2005).

According to Burkhead *et al*. (2009) Cu delivery is accomplished by the concerted action of a set of evolutionarily conserved transporters and metallochaperones. Those authors further stated that as a result of regulation of transporters in the root and the rarity of natural soil with high Cu level, very few plants in nature will experience Cu in toxic excess in their tissues. An appraisal of biochemical behaviour of Cu in soil-plant system with esteem to their quantity and speciation by Kumar *et al*. (2021) highlights nutrient uptake, functions of protein transportation and detrimental effect of Cu and mechanism of detoxification.

Coleus amboinicus is a potential medicinal herb belonging to the family Lamiaceae. It is a large fleshy succulent perennial herb with aromatic pubescence and inherent medicinal properties due to the presence of many phytochemicals (Arumugam *et al*., 2016). A perusal of literature on *C. amboinicus* reveals that, the plant grows in natural habitat and is a widely cultivated medicinal purposes. This plant grows naturally and is distributed in tropical and warm regions of Asia, Africa, and Australia and is known by a bunch of vernacular names ranging from Indian borage to “Panikoorka” in Malayalam (Retief, 2000).

In accordance with the molecular taxonomic revision proposed by A.J. Patron (2019), the plant commonly known as *Plectranthus amboinicus* has been reclassified and renamed as *Coleus amboinicus*. This taxonomic change reflects a reassessment of the plant's botanical characteristics and genetic makeup.

In Ayurveda, particularly in formulations like Gopichandhanadi Gulika for children and Pulileham, the plant *Coleus amboinicus*, holds significant medicinal value (Sivarajan and Balachandran,1994). Its inclusion in these traditional Ayurvedic preparations underscores its therapeutic properties and historical usage in promoting health and well-being, especially among children. *Coleus amboinicus* is renowned for its anti-inflammatory, antimicrobial, and digestive properties, making it a valuable component in Ayurvedic remedies aimed at addressing various health concerns. Its incorporation into formulations like Gopichandhanadi Gulika and

Pulileham highlights its longstanding presence and continued relevance in Ayurvedic medicine for pediatric health and overall wellness.

Coleus amboinicus possess therapeutic and nutritional properties attributed to its natural phytochemical compounds which are highly valued in the pharmacological industry (Lukhoba *et al*., 2006). The plant is found to be effective against respiratory, cardiovascular, oral, skin, digestive and urinary diseases. Studies have already been undertaken and shown numerous pharmacological properties including analgesics, antioxidant, anti-inflammatory, antimicrobial, antifungal, anticancer, antiepileptic and wound healing (Arumugam *et al*., 2016). The plant is used to treat chronic coughs, asthma, bronchitis (Morton, 1992), insect bites (Jain and Lata, 1996), oral disease (Santos *et al*., 2015), skin infections (Harsha *et al*., 2003; Selvakumar *et al*., 2012), wound healing (Warriner and Burrell., 2005; Sunitha *et al*., 2010; Jain *et al*., 2012).

Morallo *et al*. (1990) evaluated the biological activity of *C. amboinicus* and reported a variety of bioactive compounds and most of them are volatile in nature and responsible to cure a variety of ailments and these constituents majorly contribute to the various therapeutic properties. The phytochemical analysis of aqueous extracts of the roots and leaves of *C. amboinicus* revealed the presence of carbohydrates, sterols, and glycosides. According to Arumugam *et al*. (2016), the phytochemicals in *C. amboinicus* includes flavonoids, esters, phenolics, monoterprenoids, diterpernoids, triterprenoids, and sesquiterprenoids and these phytochemicals attribute antibacterial, antihelminthic, allelopathic, antifungal, antiepileptic, larvicidal, antioxidant, anti-inflammatory, and analgesic properties to the plant.

According to Castillo and Gonzalez. (1999) and Singh *et al*. (2002) carvacrol and thymol respectively are the main active constituents of *C. amboinicus*. The major volatile constituents of *C. amboinicus* are carvacrol (Roja *et al*., 2006; Rout *et al*., 2012), thymol and eugenol (Park *et al*., 2016). Non-volatile compounds such as

phenolics and flavonoids have been identified from *C. amboinicus* (Khare *et al*., 2011).

An exhaustive review by Arumugam *et al*. (2016) on *C. amboinicus* reported 76 volatile and 30 non-volatile compounds under different classes of phytochemicals. In addition to carvacrol and thymol, eugenol, chavicol, and ethyl salicylate were reported. Moreover, plant contains a lot of vitamin C and flavonoids including cirsimaritin and sitosterol G glucoside. Due to its inherent phytochemicals, this herb has distinct medicinal and nutritional properties. As described above the plant contain a number of phytochemicals of varying potential and hence it is an important ingredient of many Ayurvedic preparations.

Prominent importance of *C. amboinicus* in modern medicine has been reported by Steinar *et al*., (2003) and Leonor *et al*. (2005). According to those authors, the antioxidant properties of *C. amboinicus*, assessing its ability to scavenge free radicals and protect against oxidative stress. These studies may investigate the plant's potential role in reducing oxidative damage and associated diseases.

An anatomical study of the vegetative organs of *C. amboinicus* was made by Mauro *et al*. (2008) which showed non-glandular trichomes on the foliar leaf and also sessile and pedungulate glandular trichomes were present. This study is justified by the popular utilization of this plant as Phyto-therapeutic agents.

Hullatti and Bhattacharjee (2011) investigated the pharmacognostical evaluation of plant parts by using different parameters and morpho-anatomical studies, physiochemical properties and fluorescence analysis were done to set up the quality control parameters for the raw material. The microscopic features and the quantitative standards were used for laying down pharmacopeial standards. The exomorphology and histomorphology of different plant parts of *C. amboinicus* and phytochemical study by Sreedharren *et al*. (2010) and observations will enable to standardize the botanical identity of the drug in crude form. Muthukumarana and Dharmadasa (2014) describes the comprehensive pharmacognostic aspects of *C.*

amboinicus by means of physical and chemical yield parameters. Morphological, anatomical, TLC profiles, essential oil contents and its composition were carried out. Presence of higher physical growth of the plant, higher content of essential oil scientifically validates the traditional claims of harvesting at fully maturity stage.

Coleus amboinicus grows easily in a well-drained, semi-shaded location. It is found to grow well under tropical and subtropical locations. It was also found to adapt well in cooler climates if grown in a pot and brought indoors, or moved to a warm, sheltered position during winter (Arumugam *et al*., 2016). Since it grows under natural habitat chance exists to get exposed to soil contaminants in general and heavy metals in particular. Studies on the impact of heavy metal pollution and resultant changes in the quality and quantity of bioactive secondary metabolites have not yet been done elaborately in this plant. So also, the pharmacological properties of each and all bioactive secondary metabolites are not interpreted to correlate their role and principle with the traditional medicinal use of *C. amboinicus*. The present study is proposed to unravel the structural and functional aspects of selected heavy metals- Al, Cr, Cu and Hg on *Coleus amboinicus* -an important medicinal plant on one hand and the impact of these heavy metals on the qualitative and quantitative aspects of secondary metabolites on the other hand.

Two distinct but complementary lines of investigation are pursued in this study. In the first line, effect of Al, Cr, Cu and Hg on the morphology and metabolism of *C. amboinicus* is carried out. The results revealed the significant difference in the concentration of each metal to impart toxicity and bioaccumulation of *C. amboinicus*. In the second line of the study, distribution and characterization of the bioactive secondary metabolites included since *C. amboinicus* is an important medicinal plant containing large number of therapeutically used volatile compounds. Another complementary aspect of the investigation is the elucidation of differences in the qualitative and quantitative aspects in *C. amboinicus* subjected to Al, Cr, Cu and Hg in order to assess the stimulation and/or deterioration of the bioactive potentials due to these heavy metals. An effort is made to establish a relationship

between the reported therapeutic properties related to a specific disease or treatment and the role that one or more individual or group of bioactive chemicals play in the treatment and curing of such illnesses. The following are the investigation's objectives, which are based on the findings and concepts mentioned above.

1. Study of the effect of selected heavy metals like Al, Cr, Hg and essential metal Cu on structural and functional aspects during growth under nutrient culture method.

2. Evaluation of growth pattern and modification of different organs due to heavy metal toxicity.

3. Anatomical study using histochemical staining, light microscopy, Scanning Electron Microscopy and EDX analysis to pinpoint the structural changes as well as translocation and accumulation pattern of heavy metals in the plant body.

4. Toxicity of metals on growth rate, distribution of biomass and water relations.

5. Effect of heavy metals on the synthesis and distribution of primary metabolites - carbohydrates, proteins and pigments.

6. Evaluation of tolerance mechanism of *C. amboinicus* towards each heavy metal by analysing morphological modifications and activity of stress induced enzymes SOD and CAT.

7. Bioaccumulation potential of *C. amboinicus* by analyzing the absorption and translocation of Al, Cr, Hg and Cu in various parts-root, stem and leaves to evaluate the tolerance mechanism.

8. Analysis of bioactive secondary metabolites distribution using GC-MS.

9. Evaluation and interpretation of each bioactive secondary metabolites separated by GC-MS, emphasising their phytochemical potential and comparing the role of individual molecules with the principles underlying in

the medicinal use or practice of *C. amboinicus* for different types of diseases and treatments.

10. Elucidation of stimulatory/ inhibitory role of Al, Cr, Hg and Cu on the synthesis and quality of secondary metabolites and detection of beneficial effects of the metals, if any, in improving the medicinal property of *C. amboinicus*.

REVIEW OF LITERATURE

Metals such as Al, As, Cd, Co, Cr, Cu, Pb, Mn, Hg, Ni, Se and Zn have been considered as the major environmental pollutants and the impact and mechanism of phytotoxicity have already been established and excellently reviewed (Foy *et al*., 1978; Lepp, 1981; Fitter and Hay, 1983; Kochian, 1995; Shaw, 1995; Salt *et al*., 1998; Orcutt and Nilsen, 2000; Cseh, 2002; Fodor, 2002; Pilon-smits., 2005; Memon and Schroder, 2009; Solanki and Dhankar, 2011; Babangida *et al*., 2021; Yan *et al*., 2021).

Heavy metals impart negative influence on physiological activities like photosynthesis, respiration and nutrient absorption, resulting in the reductions of plant growth, dry matter accumulation and yield (Devkota and Schmidt, 2000). Levitt (1980) suggested that heavy metals in the plant environment operate as stress factors and they cause physiological changes and, in the process, they reduce vigour, or in the extremes totally inhibit plant growth. Different heavy metals of supra-optimal concentrations have been shown to inhibit various metabolic processes in plants resulting in their reduced growth and development (Davies *et al*., 1991; Bernier *et al*., 1993; Lang *et al*., 1995; Shaw, 1995). At cellular and molecular level, metal toxicity causes denaturing of enzymes and damage to DNA and also forms the increased production of free radicals (Cseh, 2002; Khatun *et al*., 2008; Posmyk *et al*., 2009).

Availability and toxicity levels of heavy metals, responses and adaptive strategies of plants to metal toxicity and phytoremediation technology have been extensively discussed and excellently reviewed by several authors (Basberg-pahlsson, 1989; Rauser, 1990; Steffens, 1990; McNeily, 1994; Prasad, 1997; Salt *et al*., 1998; Pilon-Smits, 2005).

High rate of absorption and accumulation of toxic metal leads to visible injuries and physiological disorders in plants (Zhou *et al*., 2007). Metal ions bind to the water channel protein, which induces stomatal closure and it impairs the uptake

of water by the root tissues (Zhang *et al*., 2001). These metals could damage enzymes, polynucleotides, transporters, and cell membrane (Panda and Patra, 2000). Cell cycle impairment and DNA damage were also observed during heavy metals toxicity (Soares *et al*., 2019). Moreover, metal ions induce the accumulation of H_2O_2 and MDA content in a cell that results in the development of oxidative stress (Chen *et al*., 2015). Excess accumulation of different essential elements like Cu, Ni, Co, Zn and Fe also become toxic to plants, leading to plant growth retardation (Lewis *et al*., 2019; Lwalaba *et al*., 2020; Tadaiesky *et al*., 2020).

Aluminium (Al)

Aluminium is the third most abundant element occurring almost 8% of the earth's crust. It is a major constituent of mineral soils where it is present as oxides and alumino-silicate-mineral and other precipitated forms like gibbsite (aluminium hydroxide). These hydroxyl rich minerals only partially dissolve in acidic environments, releasing Al into the soil solution where it equilibrates into a variety of chemical species that depend on the presence of Al ligands and these species co-exist in harmony with one another (Delhaize and Ryan, 1995). There are different types of soluble Al, including free or mononuclear forms of Al^{3+}, polynuclear Al, and Al as a low molecular weight complex (Kochian, 1995). Oxides and silicates of aluminium are harmless to plants (Poschenrieder *et al*., 2008) whereas Al^{3+} and Al $(H_2O)_6{}^{3+}$ ions are toxic to plants (Horst *et al*., 2010).

The estimated activity of the Al^{3+} ion has been cited by many researchers as the best predictor of toxicity, however there is some evidence supporting the toxicity of the monomeric hydroxyl cations Al $(OH)^{2+}$ and Al $(OH)^{+}$ as well as the polynuclear hydroxy complexes of Al (Foy *et al*., 1978). Aluminium bioavailability and consequent toxicity, is mainly restricted to acid environments and acid soils (with a pH of 5.5 or lower) are among the most important limitations to agricultural production. In nature, Al is present not only in acid soils but in alkaline ones also. According to Kochian (1995) and Kochian *et al.* (2005), Al is one of the most abundant and potent toxic metals in acidic soils.

Aluminium toxicity is the primary environmental stress, limiting crop productivity in acid soils, which comprise up-to 40% of the world's arable lands (Taylor, 1995; Kochian, 1995). In acidified fresh water, this metal may reach concentrations of 0.3-1.6 mM (Panda and Matsumoto, 2007) and seriously disrupt the metabolism of hydrophytes (Ciamporova, 2002). In higher plants, Al phytotoxicity is linked to sites of reactions that involve a variety of physiological functions. Evidences indicate that Al toxicosis is primarily associated with disruption of root structure and general functions of plants (Taylor, 1991; Ryan *et al*., 1992).

It has been widely established that Al causes cytotoxicity in plants (Kochian, 1995; Delhaize and Ryan, 1995; Kollmeier *et al*., 2000; Marienfeld *et al*., 2000). Aluminium exists as either irreversible or reversible macromolecular complexes in the cytoplasm of living cells. Aluminium-sensitive plants absorb more Al than aluminium-tolerant plants, thus the chief mechanism behind Al tolerance is Al exclusion (Kochian,1995; Matsumoto, 2000). Marienfeld *et al*. (2000), reported localization of Al in the root tips of *Zea mays* and *Vicia faba* when plants were grown in Al supplied medium. According to Delhaize and Ryan (1995) and Marienfeld *et al*. (2000), even though Al is not an essential element, in small doses it stimulates growth and other desirable effects.

Common responses of plants to Al include cellular and structural changes, increased rate of diffusion resistance, reduction of stomatal size and decreased photosynthetic rate (Meng *et al*., 2017). Aluminium ions exert their effect primarily on the root system (Huang *et al*., 2014a) and are found to induce abnormalities like dwarfing of roots (Ryan, 2002; Riaz *et al*., 2018; Yang *et al*., 2019).

The initial and dramatic symptom of Al toxicity is inhibition of root elongation which can occur within 1-2 hours after exposure to Al (Delhaize and Ryan, 1995). The initial symptom of the toxicity in plants is inhibition of root and changes in root morphology such as roots appear characteristically stubby and brittle, swelling in root tips, atrophy of root hair and lateral roots become thickened and brown (Huang *et al*., 2014). The root system as a whole became coralloid in

appearance (Foy, 1984). Ryan *et al*. (1993) and Liugany *et al*. (1994) suggested that only the terminal 2-3 mm of maize root needed to be exposed to Al to cause inhibition of root growth. According to Budikova (1999) partial root growth inhibition is the impact in maize root tissues due to Al treatment and root cap length and area reduction are other effect.

Most evident significance of Al toxicity in root growth inhibition which takes place by damage of root apical cells inducing root cap cell elongation zone (Poschenrieder *et al*., 2008). Reduced biomass as a result of root damage, causes toxicity symptoms to appear in the shoot system also (Mossor-Pietraszenska, 2001). An exhaustive review of Silva (2012), on the mechanisms underlying Al toxicity and resistance in plants reveals that potential targets of Al toxicity include cell wall, plasma membrane, root cytoskeleton and DNA, associated with root growth and signal transduction pathways also are involved. The primary symptoms of Al toxicity in plants are rapid inhibition of root growth and disruption of root morphology (Buchanan *et al*., 2015). Such reduction in root growth has been widely used as a marker in evaluating Al toxicity or Al tolerance plants (Awasthi *et al*., 2017). Root tips are the most sensitive part of the root system and respond to micromolar concentrations of Al (Huang *et al*., 2014).

Decreased shoot growth was observed in rice after Al treatment (Fageria, 1982); and in coffee (Pavan and Bingham, 1982). In some plants purpling of stems also is observed (Foy, 1992). Aluminium has been reported to promote plant resistance to biotic (pathogens and herbivores) and abiotic stresses including nutrient deficiency and ion toxicity (Kaur *et al*., 2016; Bojó rquez-Quintal *et al*., 2017). The toxic or beneficial effect of Al on plant growth depends largely on the growing conditions, Al concentration and duration of exposure, plant species and physiological age (Huang *et al*., 1992; Bojó rquez-Quintal *et al*., 2017; Aguilera *et al*., 2019; Ofoe *et al*., 2022).

Chlorophyll synthesis is another target of Al toxicity in plants. Decrease in quantity of chlorophyll pigments was reported in buck wheat (Sung and Kwon, 1980). In rice, chlorophyll content decreased due to Al and the ratio between

chlorophyll a and b was declined, accompanied by a marked decrease in gross photosynthesis and photosynthetic rate (Sarkunan *et al*., 1984). According to those authors, decreased protein and RNA content have also been induced by Al and it caused abnormal distribution of ribosomes on the endoplasmic reticulum, leading to disturbed protein synthesis and reduced RNA synthesis also.

According to Anderson (1995), Al interfered with the synthesis of starch and proteins, decreased translocation of sugars, increased peroxidase and decreased cytochrome oxidase activity in rice. Godbold (1994) suggested that Al specifically disrupts DNA synthesis and displaces calcium in the apoplast of Al exposed cells, impairing cell division and cell elongation. According to Vazquez *et al*. (1999), Al ions can rapidly cross the plasma membrane in plants that are tolerant to the metal.

Root apex and the surrounding mucilage or root exudate accumulate greater amounts of Al than other parts of the root and tissue damage is more severe in this region than in the mature tissue (Ryan *et al*., 1993). The root apex plays a central role in the mechanism of Al toxicity, as it is the target site for Al-induced root growth inhibition (Ahn *et al*., 2002).

Patch-Clamp study on the physiology of Al toxicity and tolerance resulted in the identification and characterization of Al^{3+}- induced anion channels in maize (Pineros and Kochian, 2001). Plasma membrane malate channels were first identified from wheat root cells employing Al^{3+} stimulated secretion of malate as a screening method (Ryan *et al*., 2002). Using the Patch-Clamp technique to root cell protoplast from wheat plants, Sasaki *et al*. (2004), recorded flow of malate currents from Al tolerant plants but not in Al sensitive plants.

Earlier Huett and Menary, (1980) opined that Al can enter the plant by moving into meristematic cells and the symplasm via the cortex, hence bypassing the endodermal barrier. Silva *et al*. (20), reported that Al is able to penetrate the cell symplasm relatively fast and bind to nuclear molecules, presumably leading to decrease in mitotic activity.

The effects of Al on the mineral nutrition of plants may either be due to interactions in the growth medium, or to physiological antagonism inside the plant (Andersson, 1988) according to whom, Al partly blocks the uptake of macro nutrients such as calcium, phosphate, potassium and magnesium and micronutrients such as manganese.

According to Palani *et al*. (2018), the medicinal plants used for the human consumption or disease management should be collected from clean environment and processed carefully. A study was conducted to assess the Al contamination in Mettur, an industrial town of Tamil Nadu, India which has many Al industries and the total Al content in the soil of the region where medicinal plants were cultivated was found to be 16700 mg kg-1 and selected plants were *Centella asiatica*, *Bacopa monneri* and *Euphorbia hirta*. All the three plants were found to accumulate level of Al which is above the maximum permissible limits prescribed by World Health Organization and thus, it may cause Al toxicity among the consumers of herbal medicines.

Aluminium can rapidly suppress cell division, destroy the cell structure, reduce nutrient and water uptake, and inhibit root elongation growth in Solanaceae plants (He *et al*., 2019). Aluminium avoidance, the alteration of nutrient element distribution, and increase of rhizosphere pH value contributes to Al tolerance in Solanaceae plants. Moreover, secretion of organic acids, enhancement of antioxidant capacity, and induction of Al-tolerant genes also play an important role in Al tolerance of Solanaceae plants.

According to Dixit *et al*. (2019), the interactive role of Brassinosteroids and Ca, regulating plant growth at the physiological, biological and molecular level, focusing mainly on the brassinosteroids induced Ca signalling participate in regulating reactive oxygen species suggesting an elevation in ROS generation confer plant Al resistance. Their findings provide further potential for the relevance of brassinosteroids and Ca in phytoremediation and Al detoxification in crops.

Exogenous applications of auxin, cytokinin and abscisic acid have shown significant effect on Al-induced root growth inhibition while ethylene and cytokinin act synergistically with auxin in responding against Al toxicity (Ranjan *et al*., 2021).

The harmful impacts of Al on morphological, anatomical, physio-biochemical, and molecular aspects of the plant were reviewed by Rahman and Upadhyaya (2021) and discussed the strategies to reduce the toxic effects of Al in plant and various Al-responsive genes which can be used in genetic manipulation for better crop development.

According to Wei *et al*. (2021), the transcription factors (TFs) and plant hormones are involved in the adaptation to Al stress. Specifically, it addresses methods to give plants resistance to Al stress, like transgenic breeding, and reduce Al toxicity by using small chemicals and plant growth-promoting rhizobacteria (PGPRs). The theoretical foundation for increasing plant productivity in acidic soils is given in this study. A review of the global extension and probable cause of Al in the environment and mechanisms of Al toxicity in plants are followed by detailed emphasis on tolerance mechanisms, identifying and categorized the important transporters that secrete organic acids and outlined their role in Al stress tolerance mechanisms in crop plants (Chauhan *et al*., 2021).

A study conducted by Li *et al*. (2023), provides an effective method for screening key genes by combining QTLs, transcriptome sequencing, and metabolomic analysis, but also lists key genes for exploring the molecular mechanism of Al tolerance in rapeseed seedling roots. Root tissues from seedlings of Al-resistant lines and Al-sensitive lines from the RIL population were harvested for transcriptome sequencing and metabolome determination. By combining the data on quantitative trait genes (QTGs), differentially expressed genes (DEGs), and differentially accumulated metabolites (DAMs), key candidate genes related to Al tolerance in rapeseed were determined.

Chromium (Cr)

Chromium is a naturally occurring element in the Earth's crust and is found in rocks, soil, water, and air. In the atmosphere, Cr occurs in hexavalent and trivalent forms. The trivalent form is more stable and less toxic than the hexavalent form. Chromium enters the atmosphere through both natural and human activities. (Panda and Patra,2000; Han *et al*., 2004; Shanker *et al*., 2004,2005: Panda and Choudhury, 2005). Natural sources, as well as various anthropogenic activities, are responsible for the release of Cr in the soil, air, and water which ultimately lead to Cr pollution. Chromium is used in manufacturing stainless steel, dyes and pigments, leather tanning, paper and pulp industry.

Chromium is unique among regulated toxic elements in the environment as different Cr species exists specifically (Cr III) and (Cr V1) (Kimbrough *et al*., 1999). Chromium occurs in the environment in its material form as (Cr III) and (Cr V1) is generated by oxidation of (Cr III) during various industrial process (Kotas and Stasicka, 2000; Pradhan *et al*., 2017). According to Pradhan *et al*. (2017), hexavalent Cr develops intracellular toxicity through a variety of mechanism including reduction of Cr (V1) to Cr (III), generation of reactive oxygen species, Cr-DNA complex formation and protein denaturation.

Chromium is a hazardous heavy metal that cause adverse impact on the growth and development of plants and affects several physiological processes such as growth, photosynthesis, status of mineral elements, water balance and nitrogen metabolism (Shanker *et al*., 2004; Vernay *et al*., 2007; Gangwar and Singh, 2011). Chromium toxicity symptoms in plants include inhibited growth of roots and leaves, inhibition of enzyme activities and mutagenesis (Clijsters and Van Assche, 1985; Bishnoi *et al*., 1993; Shanker *et al*., 2005). In plant tissues, the Cr (VI) is converted to Cr (III) that has the tendency to bind to the cell walls, which hinders the further transport of Cr within the plant tissues (Kabata-Pendias *et al*., 2015).

Chromium imparts toxic effects in water relations of plants (Vazques *et al*., 1987), accumulation of Cr in the roots and resultant deleterious effect on root growth (Bishoni *et al*., 1993) mineral metabolism, growth and development (Rout *et*

al., 1997), seed germination (Peralta *et al*., 2001) enzymatic activities and translocation of sugars (Zeid, 2001). Even though Sharma and Aery (2012) noticed stimulatory effect of low concentration of Cr on plants, most of the research on Cr registered inhibitory and toxic effect on plant growth (Amin *et al*., 2013). Case studies on the toxic effect of Cr on several plants show structural damage of different organs of the plant body and impaired the growth and development. Toxicity of Cr is normally affecting root growth due to the close contact and severity of reaction with Cr (Moral *et al*., 1995). According to Samantaray *et al*. (1997), Cr toxicity affect root length and damage the architecture of the entire root system. Chromium is reported to affect root growth more adversely than any other heavy metals and root length and dry weight also were reduced due to Cr treatment (Iqbal *et al*., 2001).

Chromium is found to be accumulated mainly in the roots and poorly transported to the shoots (Moral *et al*., 1994,1995; Samantaray and Das,1997) possibly due to the spatial localization in the specific sub cellular compartment in the root cells as suggested earlier by Barcelo *et al*. (1985). Samantaray and Das (1997) reported the accumulation of Cr by mung bean plants up-to 70 ppm in their roots when the plants were grown in chromate mine waste. According to Pulford *et al*. (2001) Cr is poorly translocated to aerial parts and held predominating in the roots.

Plant species such as, *Phragmitis karka*, *Scirpus lacustris* and *Bacopa monnieri* exhibit high potential to absorb, translocate and concentrate Cr in their tissues (Yadav *et al*., 2005). Those authors further stated that about 99% of the absorbed Cr is retained in the root tissue because most plants show low Cr concentration in the shoot tissue even when grown in Cr rich soil. So, the food chain is well protected against the Cr toxicity. The extent of damage caused by Cr on plants depends on its bioavailability, mobilization and subsequent accumulation in the tissues. Compared to other heavy metals, the mobility of Cr in the plant roots is low. Therefore, the concentration of Cr in the roots is sometimes 100 times higher than in the shoots. (Gupta *et al*., 2016). The translocation of Cr from the roots to the

aerial shoots is very limited and it depends on the chemical form of Cr inside the tissue and the distribution and translocation of Cr within plants depend upon the plant species, the oxidation state of the Cr ions, and also its concentration in the growth medium (Shahid *et al*., 2017).

Morphological and physiological changes manifested by plants due to Cr toxicity have been reported by many authors. Due to Cr application mung bean plants showed severe stunted growth and leaf chlorosis (Rout *et al*., 1997). According to Samantaray *et al*. (1998) Cr reduces chlorophyll and carotenoid synthesis indirectly by the inhibition of iron and zinc transport to the leaves in general. In *Nymphaea alba*, Cr was found to inhibit the production of chlorophyll (Vajpayee *et al*., 2000). Treatment of cauliflower with Cr resulted in decreased water potential and reduction in trachea vessels diameter (Chatterjee and Chatterjee, 2000). According to Shanker *et al*. (2005) biomass production and yield were generally affected by Cr IV in many plants. Biomass production was found to be reduced in *Oryza sativa* plants as a consequences of Cr toxicity (Panda, 2007).

Chromium stress has been reported as one of the important factors that affect photosynthesis (Shanker *et al*., 2005). According to Panda and Choudhury (2005), Cr-induced oxidative stress triggered the degradation of photosynthetic pigments, which resulted in a decline in growth. High Cr concentrations also disrupted the ultrastructure of chlorophyll and had an impact on photosynthesis.

Photosynthetic capacity of plants is compromised under Cr stress due to interaction with biosynthesis of chlorophyll molecules by inhibiting vital enzymes contributing in photosynthesis (Zlobin *et al*., 2015). According to those authors, excessive Cr affects photosynthetic system by targeting the Calvin cycle enzymes, photosynthetic electron transport and thylakoid membrane. Therefore, gradual decrease in the net photosynthetic rate can be observed in the plants treated with higher concentration of Cr.

Different strategies developed by plants against Cr toxicity include chelation of Cr with ligands, reduction of Cr(VI) to Cr(III), compartmentation of Cr in vacuoles and activation of antioxidant enzymes (Shanker *et al*., 2005; Singh *et al*.,

2013; Daud *et al*., 2014; Ali *et al*., 2015; Prado, 2016). Phytochelatins are among the most important plant molecules involved in detoxification of Cr in the plants (Shanker *et al*., 2005; Singh *et al*., 2013). Phytochelatins bind Cr and other heavy metals in the cytosol followed by their sequestration into vacuoles (Saud *et al*., 2022).

Enhanced synthesis of ROS and the regulation of antioxidant enzyme activity for mitigation of Cr toxicity have been reported in plants like *Arabidopsis thaliana* (Eleftheriou *et al*., 2015), *Glycine max* (Balasaraswathi *et al*., 2017), *Oryza sativa* (Yu *et al*., 2019) and *Tetrana qataranse* (Usman *et al*., 2019). The role of Cr-induced ROS in phytotoxicity was exhaustively reviewed by Pradhan *et al*. (2017) and Wakeel *et al*. (2020) taking into account ROS synthesis, the enzymatic antioxidant system, lipid peroxidation, DNA damage and gene toxicity, ultrastructural changes at the cellular and subcellular levels, and changes in plant photosynthetic processes. According to those authors, Cr alter the enzymatic antioxidant system, which in turn trigger cytotoxic, genotoxic, ultrastructural, and photosynthetic alterations in plants.

Chromium stress causes plants to produce ROS like H_2O_2 and O_2, which are indicators of lipid peroxidation and lead to an increase in MDA content (Panda *et al*., 2003). According to Panda (2007), root cells of rice seedling undergo oxidative stress resulting in the formation of ROS when exposed to Cr. This author further stated that, membrane transporters like sulphate carriers are involved in the translocation of Cr. The oxidative stress and generation of ROS in the isolated chloroplasts of *Pisum sativum* as a result of various concentrations of Cr toxicity has been explained by Pandey *et al*. (2009).

When exposed to Cr (III) stress, *Chamaemelum nobile* plants showed increased accumulation of Cr mainly in the roots, which contained high concentrations of ROS, nitric oxide and thiols. At higher concentration of Cr (III), SOD activity specifically was increased in the roots, while level of H_2O_2 showed irregular trend under different concentrations of Cr due to the altered activities of various peroxidases (Kováčik *et al*., 2014). According to Sharma *et al*. (2022), the

reactive oxygen species (ROS) that are formed when Cr reacts with lipids, membranes, DNA, proteins, and carbohydrates and all are responsible for damage caused by Cr. ROS regulate plant growth, programmed cell death (PCD), cell cycle, pathogen defence, systemic communication, abiotic stress responses, and growth. Plants accumulate Cr mostly through the root system, with very little movement to the shoots.

Bioaccumulation of Cr is generally occurred mainly in the roots due to the spatial localization in the specific subcellular compartments in the roots (Moral *et al*., 1994, 1995; Samantaray and Das, 1997). Pulford *et al*. (2001), stated that Cr is poorly translocated to aerial parts of the plant body and held predominating in the root. Shanker *et al*. (2005) reported characteristic of *Albizia amara* as a potential Cr accumulator and recommended the plant for phytoremediation of Cr. Distribution and bioaccumulation of Cd and Cr are reported to be species-specific in *Vigna* varieties (Ratheeshchandra *et al*., 2010).

Amin *et al*. (2019) reported that while conducting experiment with six biofuel plant species, reported that *Cyamopsis tetragonoloba*, *Glycine max, Avena sativa*, *Abelmoschus esculentus*, *Sesamum indicum* and *Guizotia abyssinica*, were subjected to eight Cr concentrations (0.5, 2.5, 5, 10, 25, 50, 75 and 100 mg kg^{-1} soil) to investigate Cr toxicity, tolerance and accumulation resulted in significant reduction of chlorophyll content and seed germination. Accumulation of Cr was higher in roots than shoot in all the studied plants.

Studies on genetic and transcriptional regulation of plants have shown the various detoxification genes get up-regulated and confer tolerance in plants under Cr stress and the ability of the plant to withstand Cr toxicity by accumulating Cr inside the plant has been recognized as one of the promising bioremediation methods for the Cr contaminated regions (Srivastava *et al*., 2021).

Soil microbial community plays a key role in governing Cr speciation and behaviour in soil (Saleem *et al*., 2022). According to those authors, a number of factors have been identified to influence Cr toxicity on activated sludge, such as pH, biomass concentration, presence of organic substances or other heavy metals,

acclimation process, exposure time, etc. Inside plants, Cr provokes numerous deleterious effects to several physiological, morphological, and biochemical processes. Chromium induces phytotoxicity by interfering plant growth, nutrient uptake and photosynthesis, inducing enhanced generation of reactive oxygen species, causing lipid peroxidation and altering the antioxidant activities.

Copper (Cu)

Copper is an essential redox-active transition metal that is required for a number of processes in plant growth and development, including the uptake of CO_2, transport of electrons, cellular transportation, production of ATP in the mitochondria, protein trafficking, and hormone signaling (Demirevska-Kepova *et al*., 2004; Ferreira *et al*., 2015; Taiz *et al*., 2015). Several proteins and enzymes involved in photosynthesis and respiration, such as plastocyanin, cytochrome c oxidase, Cu/Zn superoxide dismutase, polyphenol oxidase, laccase, and ascorbate oxidase, depend on copper ions to perform their activity (Burkhead *et al*., 2009).

Optimal concentration of Cu necessary for healthy plant growth and development comparatively very low, while larger concentrations are toxic (Taiz *et al*., 2015). Some plants develop resistance to heavy metals and thrive in soils with Cu contamination. Thomas *et al.* (1998), reported that the halophyte *Mesembryanthemum crystallinum* could cope up with high exogenous doses of Cu, probably through the synthesis of osmoprotecting compounds. Copper hyperaccumulators such as *Commelina communis* and *Elsholtzia splendns*, have been identified as interesting candidates for phytoremediation of Cu contamination (Wang and Zhong, 2011). According to Chai *et al*. (2014), *Spartina alterniflora* can withstand a certain amount of Cu stress by storing the majority of the metal in the underground portions and having a favorable relationship with Cu bioaccumulation and detoxification.

Primary impact of Cu toxicity is the abnormalities in roots such as disruption of piliferous layer of root, reduced root hair proliferation to severe root deformation (Sheldon and Menzies, 2005). Likewise, the reduced root surface area induced by Cu stress has a detrimental impact on nutrient absorption (Kumar *et al*., 2021).

According to Liu *et al*. (2014), root cell membrane degradation may lead to Cu-induced inhibition of root activities in maize seedlings. Toxicity symptoms includes chlorosis and necrosis, as well as stunting and inhibition of root and shoot growth (Yruela, 2009; Chen *et al*., 2015; Jung *et al*., 2015). Excessive Cu buildup causes nutritional deficits and interferes with cellular respiration, nitrate reductase activity and the catalytic cycle of peroxidase in plants (Martins *et al*., 2016).

Excess Cu in photosynthetic organisms cause replacement of magnesium in chlorophylls, preventing energy transfer from chlorophylls to PSII in low light, or it directly blocks the PSII reaction centre during high light (Küpper *et al*., 2002; Laporte *et al*., 2020). According to Küpper *et al*. (2009), Cu-induced inhibition of photosynthesis in *Crassula helmsii* plants was due to the negative impact of Cu on PSII reaction centers. There has been a significant decrease in photosynthetic gas exchange and chlorophyll fluorescence characteristics observed in the leaves of *Avicennia germinans* and *Bruguiera cylindrica* during Cu stress (Gonzalez-Mendoza *et al*., 2013; Sruthi and Puthur, 2019).

Another important symptom is loss of leaf area during the excess of Cu resulting in lignin accumulation in the xylem, which leading to cell wall thickening and hardening, which cause a negative impact on cell development and leaf expansion by reducing its elasticity (Kumar *et al*., 2021). Moreover, Cu toxicity results in oxidative stress in plants, owing to an increase in the generation of extremely toxic and highly reactive free radicals of oxygen.

Since Cu is both an essential cofactor and a toxic element, involving a complex network of metal trafficking pathways, many mechanisms are adapted and developed in plants to regulate Cu homeostasis in accordance with the level of Cu in the environment. These strategies prevent the metal from accumulating in its freely reactive state (metal detoxification pathways) and ensure their proper targeting to the specific metalloproteins (Yruela, 2005). Redox cycling between Cu^{+} and Cu^{2+} may produce extremely toxic hydroxyl radicals, causing significant lipid peroxidation and membrane denaturation (Burkhead *et al*., 2009)

Copper form complexes with organic and inorganic ligands in the soil (manganese and iron oxides) through cation exchange, biosorption, adsorption, or precipitation to reduce the amount of free metal ions (Cui *et al*., 2017). Therefore, the elemental distribution of Cu in the soil profile is greatly influenced by soil organic matter with a higher negative charge and cation exchange capacity (Zhang *et al*., 2020). At the soil-root interface in the rhizosphere, it has been observed that Cu bioavailability has been associated to physical, chemical, and biological features (Kumar *et al*., 2021).

Feil *et al*. (2020) suggested that the deliberate use of cupric fungicides has led to the accumulation of copper Cu in soil, exceeding safety limits and causing toxicity in plants. This study investigates the impact of different Cu concentrations on plant growth, nutrient content, and phosphorus (P) uptake mechanisms in cucumber plants. At high Cu concentrations (*i.e.* above 25 μM), the shoot and root growth resulted stunted and the P influx rate diminished. Furthermore, two P transporter genes (i.e. CsPT1.4 and CsPT1.9) were upregulated at the highest Cu concentration, albeit with different induction kinetics. Overall, these results confirm that high Cu concentrations can limit the root acquisition of P, most likely via a direct action on the uptake mechanisms (e.g. transporters).

Alves *et al*. (2023) investigates the impact of Cu applications on the oxidative and nitrogen metabolisms of tomato plants grown in organic farming systems. The study examines the effects of Cu spraying and Cu treatment in the root substrate on various parameters such as AOX mechanisms, nitrogen use efficiency (NUE), and enzyme activities. The results indicate that even small amounts of Cu in the rhizosphere and Cu spraying can induce stress responses in tomato plants, leading to changes in total ascorbate levels and a decrease in GS activity. These findings suggest that excessive Cu application could potentially be harmful in organic horticultural production.

Patrícia *et al*. (2023) analysed the effect of excess Cu on chlorophyll concentration and secondary metabolite profile in *Lantana fucata* leaves, finding a decrease in chlorophylls and key compounds in secondary metabolism, but an

increase in phenolics, suggesting a defence strategy against Cu exposure. Thus, the presence of excess Cu in the soil may have triggered an increase in the amount of reactive oxygen species in the plants, which that led to the synthesis of antioxidant compounds, as a defence strategy.

Mercury (Hg)

Among metals, Hg is unique in that it occurs in different physical and chemical forms in the environment. In well oxygenated soil, predominant Hg species are soluble $HgCl_2$, Hg (OH)Cl, and Hg $(OH)_2$ (Schuster, 1991). According to Heaton *et al*. (2005) in the soil, Hg occurs in different forms, the electrochemically uncharged or volatile Hg, the mercuric ion (Hg^{2+}) which predominates in many Hg contaminated soils and the methylated or methyl mercury (MeHg) that is bioconcentrated in plants. The high solubility in water and easiness with which Hg shifts to the gaseous phase are the two of most important properties of Hg. Even trace quantity of Hg, a non- essential metal imposes detrimental effects on plant growth and development. The high level of Hg^{2+} is strongly phytotoxic to cells and induces visible physiological disorders (Lepp, 1981; Orcutt and Nilsen, 2000; Cseh, 2002; Ortega-Villasante *et al*., 2005; Zhou *et al*., 2007). According to Kabata-Pendias, (2001) the maximum allowable concentration of Hg in the soil is 0.5-5 mg/kg.

Toxicity of Hg has been reported in many plants and even in very low concentrations it causes hazards to plant growth (Sandmann and Boger, 1983; Kagi and Hapke, 1984; De *et al*., 1985). Various forms of growth retardation and physiological changes have been reported in plants by Hg toxicity. In *Cyperus rotundus* and *Chloris barbata* root growth inhibition occurred due to Hg treatment and the rate of inhibition was increased with the increase in concentration of Hg (Lenka *et al*., 1993).

Shekar *et al*. (2011) studied the effect of Hg on growth and yield of *Solanum lycopersicum* plants and it was noticed that at a lower concentration of Hg, plants showed an enhanced percentage of germination, plant height, root length, early flowering, more pollen viability and increase in chlorophyll content.

Sahu *et al*. (2012) reported that $HgCl_2$ (2.5 μM) caused an increase of total protein contents in roots and leaves of *Triticum aestivum* and the increase in total protein pool under Hg stress was presumed as one of the mechanisms of tolerating Hg stress. In *Vigna radiata*, exposure to Hg cause ultrastructural deformities such as; the deformation of nodule structure, breakdown of spongy parenchyma cells and decrease in intercellular spaces (Mondal *et al*., 2015). Marrugo-Negrete *et al*. (2016) investigated the effect of Hg on the *Jatropha curcas* plants in hydroponic cultures fortified with different Hg concentrations and reported a reduction in biomass with stunted growth and partial or complete inhibition of photosynthesis.

It has been suggested that Hg is phytotoxic and the toxicity is due to its strong affinity to acidic and thiol groups of proteins and nucleotides, thus interfering with the function of metabolites/organelles. Mercury forms stable complexes with a variety of organic ligands and has exceptional affinity for sulfhydryl groups of proteins (Falchuk *et al.*, 1977; Nath *et al*., 1993). According to Jain and Puranik (1993) in *Zea mays*, one of the mechanisms by which Hg exerts the toxic effects is by interaction with essential –SH group of enzymes and structural proteins and another impact of Hg is, it competes with other metals such as Cu or Zn within the cell (Marschner, 1983).

Mercury is, an inhibitor of enzymes and proteins in biological systems and all Hg compounds are highly toxic to plants in general and aquatic plants in particular (De Fillippis, 1979; Baker and Walker, 1989; Reed and Gadd, 1990). Increased synthesis of malondialdehyde in plants treated with Hg was reported due to the inhibition of enzymes of photosynthetic carbon reduction cycle (Van Assche and Clijsters, 1990; Shaw, 1995)

Maitani *et al*. (1996) observed a reduction in the relative root elongation of *Rubia tinctorium* when treated with 10μM Hg^{2+}. Prasad and Prasad (1987) and Parmer *et al*. (2002) in *Phaseolus* seedlings have reported decline of chlorophyll content due to Hg toxicity and according to them the decline of which is linked to the photosynthetic productivity. According to Prasad *et al.* (1991) Hg has got direct effect on photosynthetic electron transport causing generation of singlet of oxygen

and superoxide radicals. *Phaseolus arvens*, when exposed to mercuric chloride showed enhanced lipid peroxidation and activities of antioxidant enzymes- catalase, guaicol peroxidase and ascorbate peroxidase (Shaw, 1995).

Translocation and accumulation of Hg are very feeble in plants and no plants have been reported as hyperaccumulator of this element (Henry, 2000; Raskin and Ensley, 2000). According to those authors, transgenic plants of *Arabidopsis thaliana* are known to accumulate Hg. An investigation conducted on *Berkheya coddii* and *Atriplex cansescens*, revealed elevated Hg translocation and accumulation (Moreno *et al*., 2004), according to whom there is a potential for induced Hg accumulation enabling phytoremediation in those plants. Moreno *et al*. (2005) carried out experiments on *Brassica juncea* in plant growth chambers to investigate Hg accumulation and volatilization and estimated Hg concentration in shoot and root and volatilization rate and suggested that, volatilization is a dominant pathway for Hg removal from the accumulator plant parts. Based on the experimental studies in *Brassica juncea*, Moreno *et al*. (2005) concluded that this plant exhibits phytofiltration potential of Hg from waste water, contaminated with Hg and loss of Hg from the plant-soil system occurs by accumulation and volatilization. *Azolla corolimiana* was also reported as an accumulator of Hg (Bennicelli *et al*., 2004).

According to Velasco-Alinsug *et al*. (2005), specific Hg binding peptides are present in *Chromaleana odorata* treated with Hg. Detection by using HPLC studies revealed that these proteins contain a series of five to nine cysteine residues repeatedly attached to the long chain of Hg-binding peptides. *Atriplex conodocarpa* and *Australodanthonia caespitose* show potential for Hg phytoextraction in Hg-contaminated biosolids due to their ability to translocate Hg from roots to above-ground tissues (Lomonte *et al*., 2010).

Aquatic plants such as *Eichhornia crassipes*, *Pistia stratiotcs*, *Scirpus taebernaemontani* and *Colocasia esculenta* are capable of removing Hg from water (Skinner *et al*., 2007). According to those authors, the higher the concentration of Hg in water, the greater the amount of Hg removed by the plants. The roots of *Pistia* exhibited the largest uptake and accumulation potential over all plants followed by

Eichhornia, Colocasia and Scirpus. Roots of these plants showed more Hg accumulation because roots are the only plant structure submerged in the experimental water, containing Hg and those authors opined that storage of Hg in the roots may be a strategy of exclusion since roots are usually at the base of the plants (Skinner *et al*., 2007). Velasco-Alinsug *et al*. (2005) further reported *C. odorata* as an accumulator of Hg and hence this plant has been recommended for phytoremediation technology since the accumulated Hg content form an insoluble Hg binding protein, cinnabar. The ability of the plant to accumulate and sequestrate Hg is primarily attributed to the production of Hg binding proteins.

Mercury heavy metal pollution significantly inhibits water hyacinth growth and causes genotoxic alterations in the plant's DNA, making it a useful molecular marker for detecting genotoxic effects of pollution (Malar *et al*., 2014). There was a positive correlation between heavy metal dose and superoxide dismutase, catalase, and peroxidase antioxidative enzyme activities which could be used as biomarkers to monitor pollution in *Eichhornia crassipes*.

Azevedo *et al*. (2018) determined the extent of Hg-induced cytotoxicity and genotoxicity, in *Pisum sativum* L. The results showed the cytostatic effects which delayed S-phase at lower doses and arrests at G1 at higher concentration and further decrease of mitotic index and cell proliferation index. According to those authors, DNA fragmentation, strand breakage, clastogenic parameter and micronuclei also were observed at higher concentration in roots. This study clearly indicates that Hg disrupt the cell cycle of the plants and may cause genetic variations.

Marigold and *Amaranthus* roots can accumulate relatively higher Hg levels in contaminated soils, making them suitable for non-food chain crops in Hg-contaminated soils (Sinduja *et al*., 2018). Four different concentrations of Hg were spiked to a soil along with control. Relatively higher amount of Hg was found accumulating in the roots of Marigold (3.35 µg/g) and Amaranthus (3.35 µg/g) and the plants did not express any visual symptoms of toxicity. Regarding the partition of Hg in different plant parts, it ranked in the order of roots > shoots > leaves.

Rodríguez-Alonso *et al*. (2019) reported that, Hg exposure in holm oak seedlings has no overall detrimental effects, but root morphology is modified, with Hg accumulation varying based on time, organ, and treatment dose. When comparing Hg build-up in the different organs, highest concentrations of the metal were detected in the roots, followed by the leaves and stems. The Hg accumulation pattern was positively correlated with time and Hg dose, whereas negative correlation was observed with growth stage.

Exogenous salicylic acid in lemon balm plants mitigates Hg toxicity through coordinated alternations in plant metabolic processes, providing insights into Hg tolerance mechanisms (Safari *et al*., 2019). Their results collectively indicate the ameliorative effects of exogenous SA in Hg toxicity through coordinated alternations in plant metabolic processes which provide insights to better understand mechanisms of Hg tolerance in lemon balm plant.

According to Kumari *et al*. (2020), the detailed application of whole-cell biosensor, nanotechnology, phytoremediation, plant-assisted microbial remediation and significance of modern biotechnological techniques such as transposon-mediated In-situ molecular breeding for effective removal of Hg indicates the recent developments in environmental Hg bioremediation.

Fischer and Brodziak-Dopierala (2022) reported no statistically significant differences in the concentration of Hg among the different spice plants. However, there were statistically significant differences when comparing samples of spices purchased in stores and those grown independently. The Hg content in self-cultivated herbs may be influenced by growing conditions and species variability. The highest concentration of Hg was found in peppermint, with 9.39 µg/kg.

The translocation of Hg from roots to shoots and grains is generally low, and the different Selenium species used for biofortification influence the accumulation and translocation of Hg. The interaction of Se species with Hg at the root is not simply additive, suggesting the importance of species formation in the translocation process (Manivannan *et al*., 2023). The study investigates the speciation of Hg in wheat plants grown under different Se-biofortification treatments and finds that the

1:1 Se mixture treatment helps reduce Hg levels and the presence of toxic methylmercury in grains.

Coleus amboinicus

Coleus, one of the most common medicinal plants, used to treat various ailments. Disorders of the digestive system are treated using 21 species of *Coleus*. *Coleus amboinicus* is the most frequently used species and is used to treat a wide variety of digestive problems. This species is used to treat stomach pain, nausea, vomiting, diarrhoea, mouth and throat infections and is used as purgatives, carminatives and as antihelmintics. For instance, *Coleus amboinicus* is popular in the treatment of dyspepsia, indigestion, diarrhoea and as a carminative in India and Africa (Morton, 1992; Gurib-Fakim *et al*., 1996; Jain and Lata, 1996).

Coleus amboinicus is the most frequently cited species for the treatment of burns, wounds, sores, insect bites and allergies. It is used in Brazil for the treatment of skin ulcerations caused by *Leishmania braziliensis* (Franca *et al*., 1996). It is also used to treat burns and as a poultice for centipedes and scorpion bites in Malay (Morton, 1992). In India, the juice of the leaves is used to treat skin allergies (Harsha *et al*., 2003). *Coleus amboinicus* is also frequently cited in the treatment of chronic coughs, asthma, bronchitis and sore throat in India and the Carribean (Morton, 1992; Jain and Lata, 1996), and in Cuba it is used to treat catarrhal infections and asthma (Castillo and Gonzalez, 1999; Cano and Volpato, 2004). The leaves of *Coleus amboinicus* have been found to have bronchodilator activity in guinea pig and anti-Mycobacterium tuberculosis activity (Carbajal *et al*., 1991).

Coleus amboinicus is important in Asia and South America for the treatment of fevers (Morton, 1992; Harsha *et al*., 2003) and as a cure of cholera in Rodrigues (Gurib-Fakim *et al*., 1996). It also has antimicrobial activity (Bos *et al*., 1983; Castillo and Gonzalez, 1999) and is reported to have antiviral activity against Herpes simplex virus-1 (Hattori *et al*., 1995) and anti-HIV inhibition activity (Kusumoto *et al*., 1995). The leaves are frequently utilized in the treatment of urinary diseases in the Amazon and India (Jain and Lata, 1996; Yoganarasimhan, 2000). This species is also reported to relieve kidney troubles, treat vaginal

discharges and is drunk after childbirth (Morton, 1992). *Coleus amboinicus* is used for treating stiff neck and backache (Githinji, 1990; Githinji and Kokwaro, 1993). It is used in the Caribbean, to treat congestive heart failure (Morton, 1992). It is prescribed in cases of epilepsy and convulsions (Morton, 1992; Castillo and Gonzalez, 1999) and meningitis (Neuwinger, 2000). Species of *Coleus* are also used to treat sensory disorders associated with ear and eye problems. For example, *Coleus amboinicus* seed oil is used in the treatment of acute edematous otitis acuta in Polynesia, whereas in India its leaves are rubbed onto the eyes to alleviate conjunctivitis (Morton, 1992).

A number of species including *Coleus amboinicus*, have cytotoxic and anti-tumour promoting activity and can be used in the treatment of cancer (Bhakuni *et al*., 1969). *Coleus amboinicus* have been used against snakebites in India, Gabon and Kenya (Yoganarasimhan, 2000). It is used to prevent or alleviate inflammation (Kuebel and Tucker, 1988; Morton, 1992; Prudent *et al*., 1995; Chifundera, 2001).

The leaves of *Coleus amboinicus* is used in food stuffings, for flavouring and marinating beef and chicken (Kuebel and Tucker, 1988; Bodner and Gereau, 1988; Brown, 1997), to mask odor of strong smells associated with goat, fish and shellfish (Morton, 1992) and to spice dishes containing tomato sauces. The leaves are sometimes eaten raw with bread and butter and in India, they may be added to beer and wine (Morton, 1992).

Coleus amboinicus has scented leaves and these are often rubbed into the hair and body after bathing (Morton, 1992). In the Amazon, the leaves are mixed with sugar and used as an intoxicant (Jain and Lata, 1996), while in Tonga and Martinique the leaves are used in the cleaning of textiles to perfume them (Prudent *et al*., 1995). It is also used as insect repellants (Prudent *et al*., 1995).

This herb has the ability to prevent or decrease the risk of infection and its complications in diabetic patients (Warriner and Burrell, 2005). Application of a paste prepared using *C. amboinicus* showed an enhanced wound healing ability by immune-stimulation in diseased giant murrels (Sunitha *et al.*, 2010). Likewise, *C. amboinicus* leaves and root derived paste (10%) has been shown to exhibit thorough

epithelialization on the excision wound in albino rats after 12 days of application (Jain *et al.*, 2012). The use of polyherbal suspension prepared from *C. amboinicus* and *Punica granatum* was shown to exhibit good wound healing properties in laboratory mice (Soni *et al*., 2011). Further, ethanolic extract of *C. amboinicus* reduced the wound area by up to 76.6% in diabetic mice induced by monosodium glutamate. It was observed that the plant extract promoted wound healing by increased wound contraction, enhancing collagen deposition and reducing the wound epithelialization period (Muniandy *et al*., 2014).

In Africa, *C. amboinicus* is used as a remedy for headaches (De Padua, 1988). The aqueous extract of *C. amboinicus* leaves showed an analgesic and anti-inflammatory property, mainly modulated by controlling inhibition of proinflammatory mediators (Chen *et al.*, 2014). It is also used to treat musculo-skeletal conditions such as a stiff neck and backache (Githinji,1993).

Senthilkumar and Venkatesalu (2010) reported the possible use of *C. amboinicus* essential oil as a low-cost eco-friendly resource for inhibiting the malarial vector mosquito population. Likewise, Lima *et al*. (2011) reported larvicidal activity of the essential oil of *C. ambonicus* against the mosquito (*Aedes aegypti*) which is a chief vector of dengue, yellow fever and dengue haemorrhagic fever. In another study, the essential oil of *C. amboinicus* was shown to act as a good larvicidal agent against the mosquito, *Anopheles gambiae* after 48 h (Verma *et al*., 2012).

In an investigation by Baranitharan *et al*. (2014), the highest larvicidal activity against *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* was found in the ethyl acetate leaf extracts of *C. amboinicus*. More recently, Jayaraman *et al*. (2015) have reported the larvicidal potential of different solvent extracts of *C. amboinicus* leaves against *Aedes aegypti*, *Culex quinquefasciatus*, and *Anopheles stephensi*. *C. amboinicus* zinc oxide nanoparticles (Pam-ZnO NPs) showed 100% mortality of fourth instar mosquito larvae of *Anopheles stephensi*, *Culex quinquefasciatus* and *Culex tritaeniorhynchus* at the concentration of 8 and 10 g/ mL.

In India, the juice of the leaves is used to treat skin allergies (Harsha *et al.*, 2003). It is also used to treat burns in Asian regions (Jain and Lata, 1996). When the leaf paste is baked on a flame and applied to cuts or burns, it acts as an antiseptic and promotes healing (Bhat *et al.*, 2012). Essential oil of *C. amboinicus* also inhibits the growth of dandruff-causing fungus *Malassezia furfur*, and was tested using the agar diffusion method and compared against Ketaconazole-based shampoo as the standard (Selvakumar *et al*., 2012).

Recently the effect of salinity stress on this plant was studied by Kotagiri and Kolluru (2017). They could find that all *Coleus* species showed a decrease in their growth and physiology during their exposure to the salinity stress expect two species namely, *Coleus aromaticus* and *Coleus amboinicus* have shown better tolerance to the salinity stress with respect to their morphology, carbohydrate content, decreased water potentials and water uptake capacity. Thus, they were regarded as salt tolerant. Another study on *Coleus aromaticus* done by Kumari and Prasad (2014) for analysing the effect of UV-B Pre-treatment on essential oil composition and secondary metabolites, which revealed that the former being stressful to other plants have enhanced the level of secondary metabolism in this medicinal plant by nourishing its nutrient or medicinal value.

The effect of water stress on the physiology and biochemistry of two different *Coleus* species were studied, *Coleus forskholii* and *Coleus amboinicus*. The study-imposed drought stress by withholding water supply until leaf water potentials reached specific levels. The study found marked variations in the antioxidative defence system and osmolyte accumulation between the two species, with *C. amboinicus* showing greater tolerance to drought stress compared to *C. forskholii* (Chaitanya and Prathyusha, 2019).

Secondary metabolites

Secondary metabolites have been analysed and screened in several medicinal plants such as *Erythrina sandwicensis* (Saidu *et al*., 2000), Euphorbia kansuii (Yu *et al*., 2005), *Helichrysum* (Aliyangoro and Okoh, 2010), *Artimesia princeps* (Ryu *et*

al., 2011), *Acanthes ilicifolius(*Singh and Aeri, 2013), *Breynia disticha*(Abid and Touquer, 2015), *Moringa oliffera* (Igwe *et al*., 2015), *Clerodendrum inerma* (Thilagavathi *et al.,* 2015), *strobilanthes alternata* (Devarajan, 2023) and *Ricinus communis*(Sameena, 2022). The identification and estimation of bioactive secondary metabolites of plants in general, and medicinal plants in particular, are the focus of almost all investigations using GC-MS.

Essential oil of *Coleus amboinicus* leaves is particularly rich in phenolic monoterpenes such as Thymol and Carvacrol, which are speculated to exert various pharmacological properties (Lukhoba *et al*., 2006; Can Baser *et al*., 2008; Roshan *et al*., 2010; Khare *et al*., 2011). The volatile constituents of *C. amboinicus* leaves extracted with head space solid phase microextraction (HS-SPME) analysed using GC-MS and electron impact ionization method revealed the presence of Linanol as the major component (Rout *et al*., 2012; Asiimwe *et al.,* 2014).

The chemical constituents of *C. amboinicus* essential oil differed with the collected samples from diverse geographical places, environmental factors and different seasons. In India, *C. amboinicus* essential oil was reported to possess volatiles such as Carvacrol (43.1%), Thymol (7.2%), Eugenol (6.4%), Chavicol (5.3%) and Et-salicylate (3.2%) (Dutta.,1959). Baslas and Kumar (1981) reported that the constituents observed by them are Thymol (41.30%), Carvacrol (13.25%), 1,8-Cineole (5.45%), Eugenol (4.40%) and β-Caryophyllene (4.20%). According to Singh *et al*. (2002), the compounds eugenol and methyl eugenol were first detected in *C. amboinicus* oil from Andhra Pradesh.

Likewise, analysis of essential oil obtained from wild *C. amboinicus* plants in Bangalore, India showed the presence of 36 compounds (Mallavarapu *et al*., 1999). They reported that the quality of essential oil will be superior when collected during September. The oil content was found to be higher in the plants harvested during September in comparison to the plants harvested during May. GC-MS study carried out by Roja *et al*. (2006) to identify the chemical constituents of *C.*

amboinicus oil from the leaves of the tissue culture plants, in vitro root cultures as well as parent plants revealed that the presence of similar volatile constituents, though the parent plants and root cultures contained 21 compounds in comparison to the only 15 compounds noticed in the tissue culture plants.

MATERIALS AND METHODS

Choice of the plant

Coleus amboinicus Lour. Previously named as *Plectranthus amboinicus* was selected for the present investigation. The twigs approximately 15 cm length with 4-5 pairs of fully opened leaves were collected and cultivated in the Botanical garden of SNGS College Pattambi.

Earthen pots half filled with potting mixture were used for cultivation. Cuttings were planted and irrigated with water and maintained under greenhouse condition. Twigs were collected from most profusely growing plants. Healthy plants were maintained for the availability of planting materials throughout the period of experimentation.

Nutrient culture studies

Healthy cuttings of 15-20 cm length consisting of 3-4 nodes and approximately 4 pairs of unfolded leaves were selected for culture studies under Hoagland nutrient medium. Twigs were grown in water for root initiation. After 12-15 days, rooted propagules with 4-5 roots were transferred to Hoagland medium artificially contaminated with known quantities of Al ($AlCl_3$), Cr ($K_2Cr_2O_7$), Hg ($HgCl_2$) and Cu ($CuSO_45H_2O$)

Chemicals

Either AR or GR grade chemicals were purchased from MERCK, BDH, SRL and GLAXO companies.

Composition and preparation of nutrient solution

Modified Hoagland solution prepared according to Epstein (1972) 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 0.1 N HCl or NaOH.

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

Compounds	Molecular weight g/mol	Concentration of stock solution		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

Heavy metals selected for the study include aluminium (Al), chromium (Cr), copper (Cu) and mercury (Hg). For the standardization of heavy metal concentrations that induce toxicity symptoms so as to impart about 50% growth retardation, rooted cuttings were grown in different concentrations of aluminium chloride ($AlCl_3$), potassium dichromate ($K_2Cr_2O_7$), copper sulphate ($CuSO_45H_2O$) and mercuric chloride ($HgCl_2$). The concentrations of metal salts in which the rooted propagules of *C. amboinicus* survived but exhibited approximately 50% growth retardation were selected are given in Table II

Table 2

Concentration of selected heavy metals used in study

Name of metal	Name and formula of metal salt	Molecular mass of the salt	Concentration (µM) selected
Aluminium (Al)	Aluminium chloride ($AlCl_3$)	133.34	500
Chromium (Cr)	Potassium dichromate ($K_2Cr_2O_7$)	294.19	150
Copper (Cu)	Copper sulphate ($CuSO_4\,5H_2O$)	249.68	80
Mercury (Hg)	Mercuric chloride (Hg Cl_2)	271.5	10

Treatment with heavy metals

One Molar stock solution of heavy metal salts- Al, Cr, Hg and Cu were prepared and required dilutions were made. The optimal concentrations were determined by trial-and-error method. Rooted propagules were grown in different concentrations such as

Aluminium chloride – 500 µM

Potassium dichromate-150 µM

Mercuric chloride- 10 µM

Copper sulphate -80 µM

Experimental setup

Fifty mL of each of the solution was taken in glass culture tube of 25×150 mm size. Rooted propagules (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 as given in Table 2. The hydroponic system was maintained under greenhouse conditions. Plants grown in Hoagland solution without any heavy metal salt served as the control.

Sampling

Cultured plants of treatments and control were sampled at comparable intervals of 4 days up to 20 days of growth. At each interval (0, 4, 8, 12, 16 and 20 days), a minimum of five plants in each treatment were sampled.

Morphological measurements

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

Root, shoot length and leaf area

The sampled propagules were taken for the measurements of root and shoot length and leaf area was measured manually, using a graduated scale. Measurements of not less than five propagules each were recorded. Leaf area measurements were made using fully grown leaves by sketching the outline of the leaf on graph paper and measured the leaf area.

Tolerance index percentage

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

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

Stomatal index

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

$$\text{Stomatal index} = \frac{\text{Number of stomata per unit area}}{\text{Number of stomata} + \text{number of epidermal cells per unit area}} \times 100$$

Anatomical studies

Root, stem and leaves of control as well as treated plant parts were collected on 20th day and fixed in FAA (100 mL FAA contains 10 mL formalin, 5 mL acetic acid, 50 mL alcohol and 35 mL distilled water). Free-hand cross sections of root, stem and leaves were taken and stained with toluidine blue according to Khasim (2002). Photomicrographs were taken using compound microscope (LEICA DM2000 LED microscope attached with LEICA DMC4500 camera).

Scanning Electron Microscopy (SEM) and Energy-Dispersive Xray (EDX) Analysis

The anatomical studies of root, stem, and leaves of *C. amboinicus* cuttings sampled on 20th day of treatment were evaluated using field emission scanning electron microscope (FESEM). Free-hand sections of samples were fixed in 2.5% glutaraldehyde, prepared in 0.1 M phosphate buffer (pH 6.9). The sections were dehydrated by passing through ascending 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.

Quantitative compositional analysis of metals of treatment and other essential elements in the tissues of root, stem, and leaves of *C. amboinicus* were evaluated using FESEM attached with Energy Dispersive X-ray spectroscope (Ametek EDAX Octane Plus, New Jersey, USA).

Leaf micromorphological characters

Micromorphology of stomata and trichomes of *C. amboinicus* leaves was 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 0.1M 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 Parameters

Sampling

Samples for the physiological/biochemical studies was done at an interval of four days (4, 8, 12, 16 and 20^{th} days) of growth. A minimum of ten plants were harvested randomly from each treatment, washed thoroughly in distilled water and blotted to dryness. For biochemical analyses, root, stem and leaves of individual plants were cut and pooled together, randomized and kept separately. From the randomized samples of each component ie, root, stem and leaf. Samples for each biochemical analysis were weighed in triplicate immediately after cutting.

Dry weight

Separate samples of root stem and leaves were weighed in pre- weighed containers using electronic balance. Fresh weight obtained was recorded and the weighed samples were then placed in hot air oven at 100ºC for one h followed by at 60º C. Dry weight of each sample was taken on the next day and drying and weighing were repeated until values become constant. Dry weight percentage was calculated by using the following formula.

$$\text{Dry weight percentage} = \frac{\text{Dry weight}}{\text{Fresh weight}} \times 100$$

Total chlorophyll and carotenoid contents

Leaf pigments such as total chlorophyll and carotenoid contents were estimated according to the methods of Arnon (1949) and Lichtenthaler and Wellburnn (1983) respectively. Fifty milligrams of fresh leaf sample were weighed using electronic balance and ground in 80% acetone using prechilled glass mortar

and pestle. The homogenate was centrifuged at 5000 rpm for 10 min at 4°C using a refrigerated centrifuge (Sorvall legend X1R, ThermoFisher Scientific, India) and the supernatant was collected. The residue was re-extracted with 80% acetone and centrifuged. The process was repeated until the pellet became colourless. The final volume of the pooled supernatant was noted, and the absorbance was recorded at 646, 663, 750 and 470 nm against the solvent blank (80% acetone) using UV-VIS spectrophotometer (Shimadzu Corp A12425780917). Total chlorophylls (*Chl a* + *Chl b*) and carotenoids present in the extract was calculated as micrograms of the pigments per gram fresh weight using the following formula,

$$\text{Chlorophyll } a + b = \frac{20.12\,(A_{646} - A_{750}) + 8.02\,(A_{663} - A_{750})}{\text{Fresh weight of the sample}} \times \text{volume}$$

$$\text{Carotenoids} = \frac{1000\,(A_{470}) + 3.27\,(\text{Chl } a - \text{Chl } b)}{\text{Fresh weight of the sample} \times 229} \times \text{volume}$$

$$\text{Chlorophyll } a = \frac{12.69(A_{663} - A_{750}) - 2.69(A_{646} - A_{750})}{\text{Fresh weight of the sample}} \times \text{volume}$$

$$\text{Chlorophyll } b = \frac{22.9(A_{646} - A_{750}) - 4.68(A_{663} - A\,750)}{\text{Fresh weight of the sample}} \times \text{volume}$$

Where,

A663 - Absorbance at 663 nm

A645 - Absorbance at 645 nm

Primary Metabolites

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.

Analysis of Starch

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 colour 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 milliliter 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 free aminoacids

Total free aminoacids was estimated according to the method of Lee and Takahashi (1966).

Extraction: one gram of fresh tissue was homogenized in 80% alcohol using a clean glass mortar and pestle. The homogenate was transferred to a round bottomed flask fitted with vertical condenser and refluxed on a boiling water bath for 2 hours, centrifuged and the supernatant was collected. The residue was re-extracted with 80% alcohol and after each centrifugation the supernatant was combined with the original supernatant. The combined supernatant was then evaporated to dryness over a boiling water bath (60ºC), eluted using a known quantity of 6% Isopropanol. This extract was used for the estimation of total free amino acids using ninhydrin reagent.

Estimation: To 0.2 mL of the sample, 3.8 mL of Ninhydrin-Citrate-Glycerol mixture was added. After shaking well, the mixture was heated in a boiling water bath for 10 minutes and cooled to room temperature, by keeping under tap water. Within 1 hour, the optical density of the resultant solution was measured at 570 nm using a spectrophotometer (Shimadzu Corp A12425780917).

The reagent blank was prepared by mixing 0.2 mL water and 3.8 mL ninhydrin-citrate-glycerol. Glycine was used as the standard.

Estimation of total protein

Total protein of *C. amboinicus* samples estimated according to the method of Lowry *et al*. (1951). Bovine Serum Albumin (66 KDa) was used as the standard.

Extraction: Two hundred milligram each of root, stem and leaves from the randomized samples of each treatment and control were weighed separately. The weighed tissues were homogenized in 5 mL of phosphate buffer (pH 7) using pre-chilled glass mortar and pestle. A known volume of the homogenate was pipetted in a centrifuge tube and equal volume of 10% trichloroacetic acid (w/v) was added and kept in a refrigerator for 1 h for flocculation. The protein precipitate was collected by centrifugation for 10 min. The supernatant was decanted off and the residue was washed twice with cold 2% trichloroacetic acid (w/v) and centrifuged. The precipitants were washed with diethyl ether, 30% perchloric acid (v/v) and 80% acetone (v/v) in order to remove lipids, starch and pigments respectively. The pellet was digested with 5 mL of 0.1N NaOH and boiled for 5 min in water bath, cooled and centrifuged. The supernatant was transferred to test tubes and used for protein estimation.

Estimation:

Reagents

A- 2% Sodium carbonate in 0.1N sodium hydroxide

B- 0.5% Copper sulphate in 1% potassium sodium tartarate

C- Alkaline copper sulphate solution: Prepared by mixing 50 mL A and 1 mL of B reagents prior to use.

D- 1N Folin-Ciocalteau reagent

Suitable aliquots were taken in duplicates from each extract. 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 min, and 0.5 mL 1N Folin-Ciocalteau reagent was added and mixed immediately. The tubes were kept

for 30 min in dark for colour development. Absorbance was read at 700 nm using a spectrophotometer (Shimadzu Corp A124257 80917)

Proline

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

Extraction: One-gram fresh tissue each of sample such as root, stem and leaves each of the experimental and control plants was homogenized in 10 mL of 5% aqueous sulfosalicylic acid using a clean glass mortar and pestle. The homogenate was transferred to centrifuge tubes and centrifuged for 10 min 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 6M orthophosphoric acid.

Estimation: From the supernatant, 2 mL was taken in test tubes and equal volume of glacial acetic acid and ninhydrin were added. 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. After 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 using a micropipette and brought to room temperature. The colour intensity of the solution was measured at 520 nm using spectrophotometer (Shimadzu Corp A124257 80917). L-proline was used as the standard.

Secondary Metabolites

Total phenolics content

The estimation of total phenolics content was carried out according to the method of Folin and Denis (1915) using Folin-ciocalteu reagent.

Extraction: One hundred milligram of fresh tissue was weighed and homogenized in 5 mL of 80% ethanol using pre-chilled mortar and pestle. The homogenate was

centrifuged for 10 min, and the supernatant was collected. The pellet was re-extracted using 80% ethanol, the pooled supernatant was used to estimate total phenolics content.

Estimation: From the supernatant, a known volume of aliquot was pipetted out and made up to 3 mL using distilled water. To this, 0.5 mL of 1 N Folin Ciocalteu reagent was added and mixed well. After 3 min, 2 mL of 20% sodium carbonate was added. The mixture was thoroughly mixed and kept for 1 min for colour development. The optical density of the solution was read at 650 nm using UV-VIS spectrophotometer (Shimadzu Corp A124257 80917). Catechol was used as the standard.

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 5mL 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 millilitre of the supernatant was mixed with an equal aliquot of 0.5% of Thiobarbituric acid (TBA) in 20% trichloroacetic acid (TCA) (w/v). The solution was heated at 95ºC for 24 minutes, cooled and then centrifuged at 3000 rpm for 10 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 substracting absorbance value at 600 nm and then the MDA content was calculated using an extinction coefficient of 155 mM-1 cm-1.

Analysis for bioactive compounds by Gas chromatography and mass spectrometry (GCMS)

The sample preparation and extraction were carried out according to Canini *et al.* (2007) with some modifications.

Sample preparation: Fresh leaves of treatment and control plants sampled at sixth interval (20th day) were collected, washed with distilled water. The samples were cut into small pieces and shade dried at room temperature for 3-4 weeks. After drying, the samples triturated into fine powder with a mechanical grinder and stored in airtight containers until use.

Preparation of extract: Five grams of powdered root and leaf samples were extracted in soxhlet apparatus with 20 mL of aqueous methanol (v/v) at 75°C for 24 h. After cooling, the extract was filtered to remove the residues and was concentrated in rotary evaporator at 40°C. The crude extract was stored in air-tight vials at -20°C until use.

GCMS analysis: The identification of bioactive compounds in the methanolic extracts was carried out using GCMS (Shimadzu QP2010S, Kyoto, Japan). An ELITE-5MS column (30 m length × 0.25 mm ID × 0.25 µm thickness) was used for GC separation. At start, the column oven temperature was maintained at 80°C for 4 min and then increased to 260°C for 6 min. The specifications of the instrument were, injection mode - split less; injection temperature - 260°C; sample injection volume - 1 µl; total flow - 54.01 ml/min; column flow - 1.00 ml/min; pressure - 65.2 kPa; solvent cut time - 6.50 min; purge flow - 3.0 mL/min; and detector gain mode - relative. The compounds in the samples were identified by comparing with the mass spectra on WILEY 8 and National Institute of Standard and Technology (NIST 11) libraries using GCMS Solutions software.

Enzymatic Antioxidants

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 prechilled 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 µmol of H_2O_2 per min.

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.

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

Quantitative estimation of heavy metals

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 4^{th},12^{th} and 20^{th} 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. 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 equation,

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

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

Statistical analysis

The results of the study were statistically examined using one-way ANOVA. All significant treatment effects were determined by using Dunnett's test at $p < 0.05$. Data are average recordings from three independent experiments, each with five replicates (i.e., $n = 15$). The data represent mean ± standard error.

RESULTS

Growth Parameters

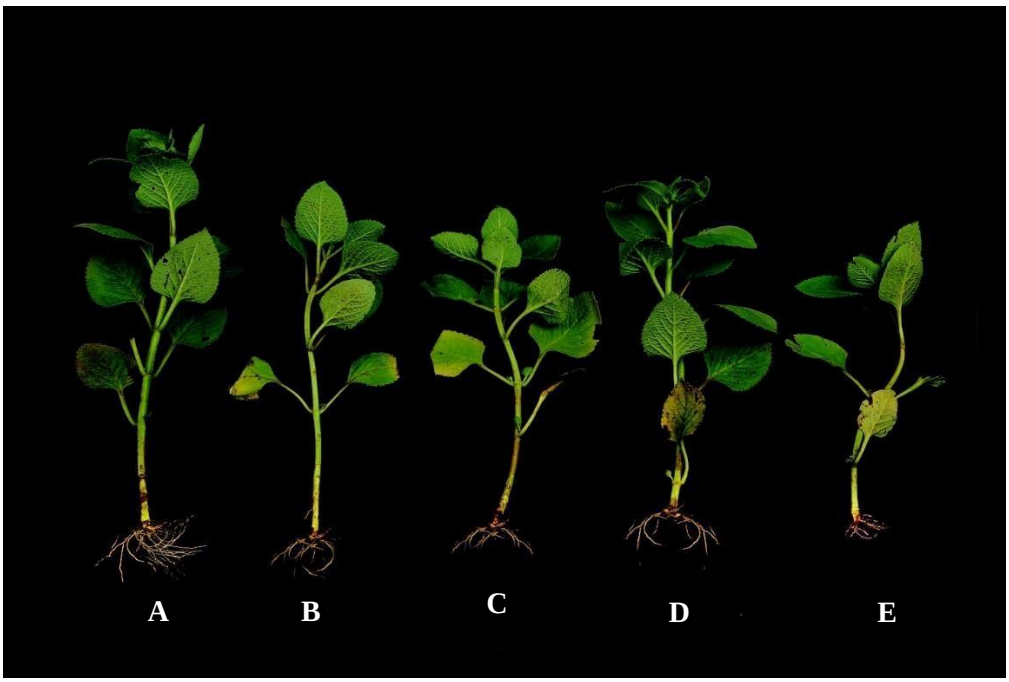
Morphological measurements such as root length, stem length and leaf area provided a simple method to assess the impact of Al, Cr, Cu and Hg on the growth and development of *Coleus amboinicus*. Visible symptom of growth retardations due to the toxicity of Al, Cr, Cu and Hg were observed from 4th day onwards in comparison with the control (Fig. 4). Growth retardation rate and pattern were more or less similar in the case of all these heavy metals except Hg.

Plants treated with Al showed a gradual reduction in root length compared to the control, whereas minimum retardation of root growth was observed in plants treated with Cu. Plants treated with Cr resulted in the decline of root growth and the decrease was significant in between the intervals. Maximum growth retardation was noticed in root length, shoot length and leaf area as a result of the Hg treatment. Retardation of root growth between the treatments was in the order Hg < Al < Cr < Cu. (Table 3, Fig. 1)

During growth, Al stress resulted in retardation of stem elongation. Treatments with Cr and Cu showed only negligible growth retardation and stem length was minimum in plants treated with Hg. The order of growth retardation was Hg < Cr < Al < Cu. (Table 4, Fig. 2).

Due to the toxicity of Al, Cr, Cu and Hg, *Coleus amboinicus* growth was evidently retarded in terms of leaf area also. Plants treated with Al and Cu exhibited only negligible difference in leaf area compared to the control and the leaf growth retardation trend was more or less similar in plants treated with Cr and Hg (Table:5, Fig. 3).

Fig. 1 Growth performance of *Coleus amboinicus* plants treated with metals like Aluminium, Chromium, Copper and Mercury after 20 days of growth.



A-Control, B-aluminium, C-chromium, D- copper, E-mercury

Figure 2. Lenticel production after Hg treatment

A-control, B- -mercury

Table 3: Effect of Aluminium, Chromium, Copper and Mercury on rootlength in *Coleus amboinicus*.

Root length in cm.

Metals	Interval- days					
	0	**4**	**8**	**12**	**16**	**20**
Control	4.5±0.40	8.21±0.32	10.53±0.34	13.67±0.88	15.81±0.5	17.92±0.2
Aluminium (500µM)	4.21±0.61	7.42±0.44	9.6±0.57	10.73±0.34	11.53±0.2	13±0.86
Chromium (150µM)	4.32±0.5	8.32±0.53	9.9±0.42	11.57±0.82	12.72±0.99	13.53±0.8
Copper (80µM)	4.67±0.43	6.73±0.49	8.95±0.55	10.71±0.68	12.3±0.73	14.81±0.5
Mercury (10µM)	4.12±0.32	6.25±0.35	6.9±0.63	7.61±0.55	8.71±0.53	9.89±0.6

Values given are mean of 5 replicates ±S.E

Fig. 3 Effect of Aluminium, Chromium, Copper and Mercury on root length in *Coleus amboinicus*

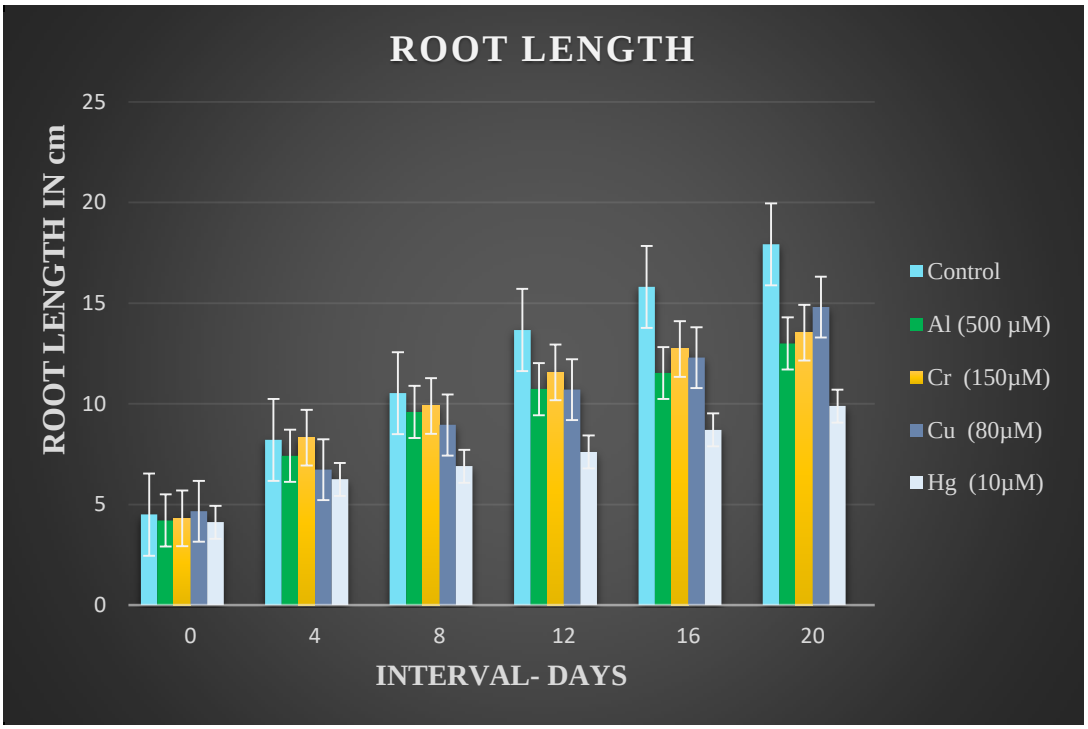


Table 4: Effect of Aluminium, Chromium, Copper and Mercury on stem length in *Coleus amboinicus*.

Stem length in cm

Metals	**Interval -days**					
	0	**4**	**8**	**12**	**16**	**20**
Control	17.82±1.3	20.21±1.2	24.53±2.4	27.67±1.8	30.81±2.5	35.92±3.4
Aluminium (500µM)	18.21±1.9	21.42±2.4	24.6±1.7	26.73±2.4	29.53±1.2	32±1.6
Chromium (150µM)	18.32±1.2	19.32±1.3	21.9±2.2	25.57±2.2	27.72±1.9	30.53±1.8
Copper (80µM)	18.67±1.3	21.73±2.9	23.95±1.5	26.71±2.8	29.3±2.3	33.81±2.1
Mercury (10µM)	17.12±1.2	19.25±2.5	21.2±1.3	23.61±1.5	25.71±1.3	26.89±2.6

Values given are mean of 5 replicates ±S.E

Fig. 4 Effect of Aluminium, Chromium, Copper and Mercury on stem length in *Coleus amboinicus*

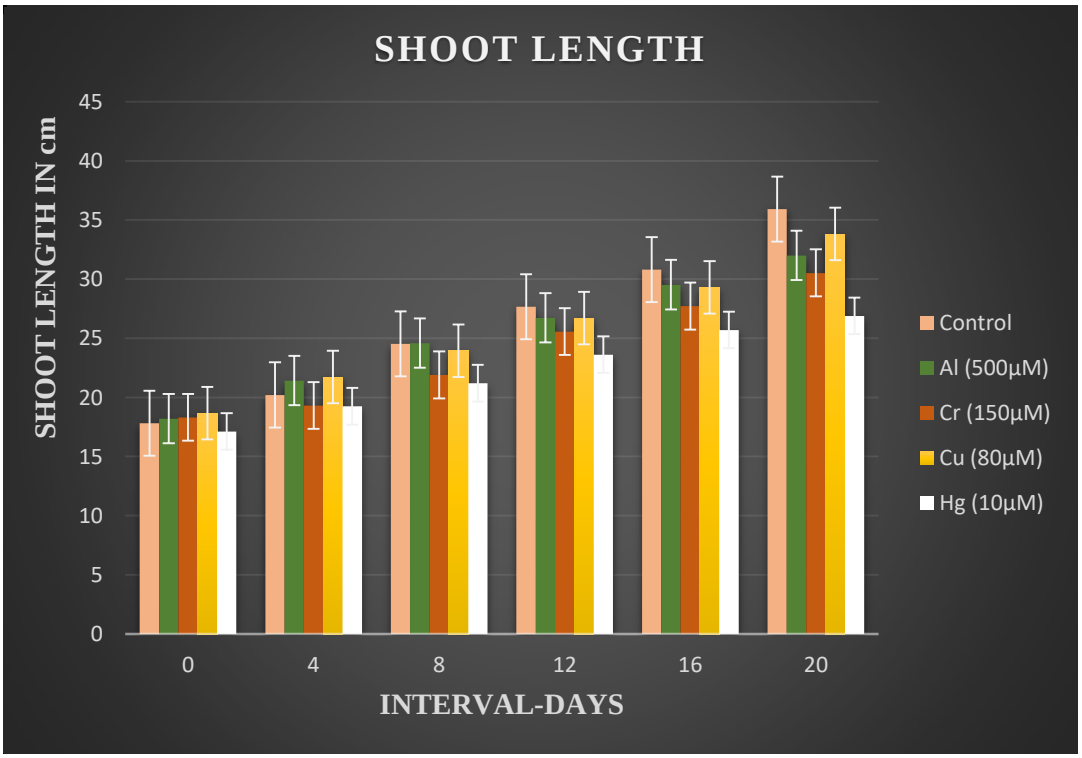


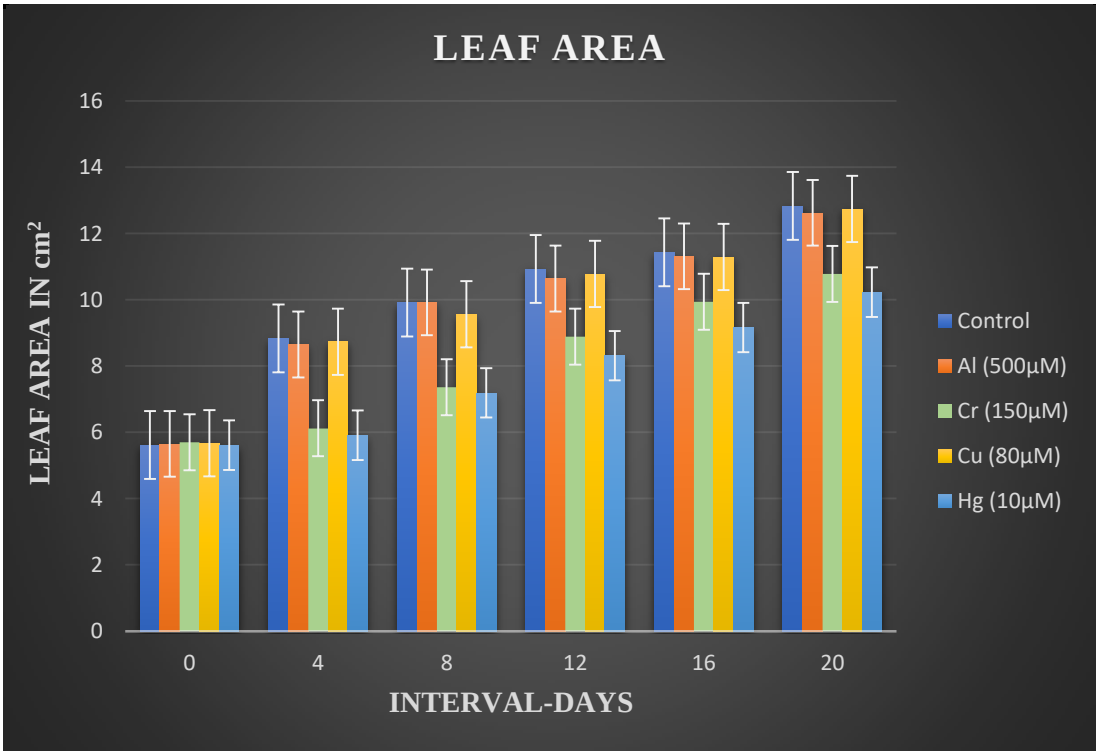
Table 5: Effect of Aluminium, Chromium, Copper and Mercury on leaf area in *Coleus amboinicus*.

Leaf area in cm^2

Metals	Interval -days					
	0	4	8	12	16	20
Control	5.62±0.21	8.83±0.12	9.91±0.14	10.93±0.08	11.43±0.05	12.83±0.04
Aluminium (500µM)	5.65±0.29	8.65±0.04	9.92±0.17	10.64±0.04	11.31±0.12	12.62±0.16
Chromium (150µM)	5.7±0.3	6.12±0.13	7.36±0.02	8.89±0.02	9.94±0.09	10.78±0.18
Copper (80µM)	5.67±0.43	8.73±0.09	9.56±0.15	10.78±0.18	11.29±0.13	12.74±0.21
Mercury (10µM)	5.61±0.32	5.91±0.05	7.19±0.13	8.31±0.05	9.16±0.03	10.23±0.06

Values given are mean of 5 replicates ±S.E

Fig. 5 Effect of Aluminium, Chromium, Copper and Mercury on leaf areain *Coleus amboinicus*



Tolerance Index

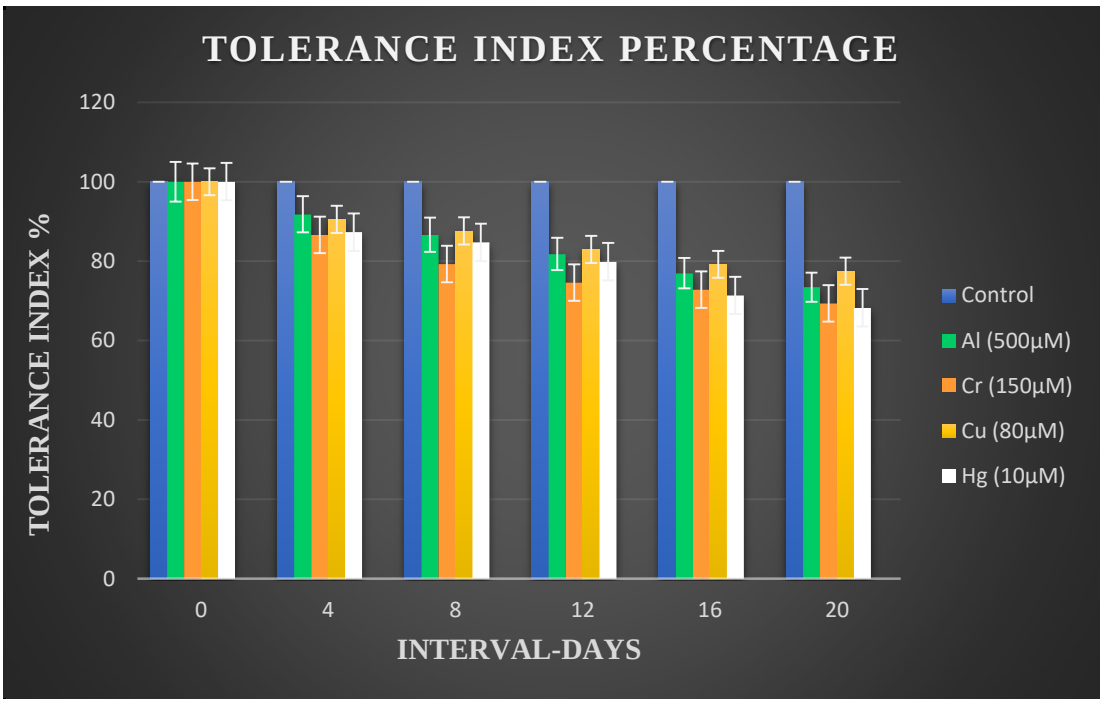
Tolerance index calculated by comparative root length of the control and treatments showed a steady decline in the values during growth in plants treated with all the metals treatments (Table 6, Fig.6). In plants treated with heavy metals, the tolerance index percentage difference in between intervals was negligible in comparison with the control. Tolerance index values of Al and Cu during initial days that is on 4^{th} day was maximum compared to Cr and Hg and both these metals exhibited significant reduction (more than 10%) of tolerance index compared to the control whereas Al and Cu showed more tolerance (Fig. 6). Similarly, both Al and Cu treatment resulted in significant reduction of tolerance index values during growth and at each interval the difference was significant. In the case of Cr, the trend in the reduction of tolerance index values was almost equal to Al and Cu whereas between each interval the reduction of tolerance values was not as much significant in Cr and Hg treatments. In the case of Hg treatment, on 20^{th} day there was a significant decrease in tolerance index ($P < 0.01$) when compared with other treatments.

Table 6: Effect of Aluminium, Chromium, Copper and Mercury on tolerance index of *Coleus amboinicus* during growth

Metals	**Interval- days**					
	0	**4**	**8**	**12**	**16**	**20**
Control	100	100	100	100	100	100
Aluminium (500µM)	100	91.8±0.13	86.63±0.17	81.78±0.04	76.94±0.12	73.42±0.16
Chromium (150µM)	100	86.59±0.13	79.27±0.02	74.61±0.02	72.83±0.09	69.32±0.18
Copper (80µM)	100	90.53±0.09	87.59±0.15	82.93±0.18	79.16±0.13	77.43±0.21
Mercury (10µM)	100	87.31±0.05	84.73±0.13	79.85±0.05	71.36±0.03	68.24±0.06

Values given are mean of 5 replicates ±S.E

Figure: 6 Effect of Aluminium, Chromium, Copper and Mercury on tolerance index percentage pertaining to root length in *Coleus amboinicus* during growth



Anatomical Studies

Control

Root

Cross section of the root of *Coleus amboinicus* plants grown in Hoagland nutrient solution showed a distinct piliferous (rhizodermal) layer, cortex, and vasculature (Fig. 7A). The piliferous layer was characterized by an undulated margin with large number of root hairs and the cellular details were not clear due to dense staining (Fig. 7B). Phloem was made up of tiny, thin-walled and lightly stained cells whereas xylem cells were thick walled and densely stained. The pith was narrow and made up of parenchymatous cells.

Scanning electron microscopic images of *C. amboinicus* exhibited unevenly distributed piliferous layer of roots and individual cells were not clearly seen but root hairs were mostly seen (Fig. 12A). Cells of the cortex were compactly arranged

and well-defined configuration. The magnified view showed well organized xylem vessels (Fig. 12B).

Stem

Anatomy of stem exhibited a typical pattern of dicot stem consistingof distinct epidermal layer with thick walled cells and epidermal hairs (Fig. 7C). Cortex was conspicuous and consisted of thin-walled cells. Vasculature showed small phloem cells and xylem cells were thick walled and deeply stained (Fig. 8D). Medullary rays consisted of thick-walled angular cells. Xylem vessels were large and thick walled (Fig. 7D). Conspicuous pith consisted of thin-walled parenchyma cells.

The SEM images of *C. amboinicus* stem shows typical structure of dicot stem. Epidermis and epidermal hairs can be clearly captured in detail in the full view (Fig. 12C), but in magnified view cellular details were completely seen (Fig. 12D). Cortex cells were arranged in well-organized manner. Vascular tissues consisted of protoxylem and metaxylem. Pith region was wide and cells were compactly arranged.

Leaf

Upper and lower epidermis of leaf midrib consisted of thick walled cells, thin walled mesophyll cells and many layered palisade cells (Fig. 7E). Epidermal hairs (trichomes) were present on both upper and lower epidermis. Vasculature consisted of thin-walled small phloem cells and angular thick-walled xylem vessels. In the lower epidermis, vast mesophyll cells which were thin walled and plenty intercellular spaces also were present. (Fig. 7F).

SEM images of leaf showed upper and lower epidermis, vasculature and mesophyll tissues (Fig. 12E). Epidermal cells were thin and cuticle found overlapped and epidermal hairs were clearly seen in SEM images. In the magnified view mesophyll cells were clearly seen (Fig. 12F).

Treatment with Aluminium

Root

Compared to control, root hairs disappeared in *C. amboinicus* roots due to Al treatment (Fig. 8A). Structure of piliferous cells were not distinctly seen because cells were damaged and densely stained. Unlike the control, endodermis was very distinct and showed specific cell wall thickening. Cortex region was conspicuous (Fig. 8B). Structure of vascular tissue was almost similar to the control.

SEM studies showed treatment with Al, resulted in damaged piliferous layer and root hairs were absent (Fig. 13A). Other structural details remained unchanged compared to control. Noticeable observation of Al-treated root was the elaborated cortex with compactly arranged cells. Xylem vessels were similar to that of control. Well organized medullary rays consisting of thick-walled angular cells was observed (Fig. 143).

Stem

Stem of *C. amboinicus* plants treated with Al showed almost similar structure compared to the control (Fig. 8C). Stelar region, particularly xylem become more distinct, consisting of more number of xylem vessels than the control (Fig. 8D).

SEM images of the stem treated with Al showed more or less similar configuration compared to control (Fig. 13C). Epidermal hairs were comparatively lower than the control. All cell types including cortex, vasculature and pith were distinctly seen. Vasculature was conspicuous and cells were well arranged in the stem (Fig. 13D).

Leaf

Leaves of *C. amboinicus* treated with Al showed typical structure of dicot leaf and the appearance was almost similar to control (Fig. 8E). The midrib region of the lower epidermis was found to be reduced in size so also the vasculature and hence tissue differentiation was meagre compared to the control.

SEM images of leaf in *C. amboinicus* treated with Al show epidermis with epidermal hairs similar to control leaf hairs and it appear as damaged. Vasculature consisted of conspicuous xylem vessels (Fig. 13E). In the magnified view, it was clearly seen that the mesophyll cells were slightly and irregularly broken (Fig. 13F).

TREATMENT WITH CHROMIUM

Root

Absence of root hair was an important impact of Cr treatment and piliferous cells layer appeared damaged and hence cellular details were obscure. Noticeable anatomical characteristic of the roots in the plant treated with chromium was the conspicuous stelar region consisting of large number of large xylem vessels and pith was reduced (Fig. 9A).

SEM image of root section of *C. amboinicus* treated with Cr revealed, completely damaged piliferous layer and root hairs were totally absent (Fig. 14A). Noticeable character of the plants treated with Cr was the conspicuous stelar region consisting of medullary rays showing angular and thick-walled cells (Fig. 14B). In the medullary rays large number of big vessels were seen embedded. Pith was narrow compared to control.

Stem

In stem tissues of *C. amboinicus* plants treated with Cr, structure of epidermis, cortex and vasculature were almost similar to control plants (Fig.9C) and structural details were distinct and well arranged (Fig. 9D).

Stem of *C. amboinicus* treated with Cr showed compactly arranged epidermal cells and epidermal hairs were comparatively reduced in number than the control (Fig. 14C). Phloem cells were small and xylem vessels are surrounded by medullary rays the cells of which were angular and thick walled. Pith was similar to that of the control. Deposits were also seen in medullary region (Fig. 14D).

Leaf

Leaf anatomy of plants treated with Cr showed a typical dicot leaf structure. But abundant occurrence of epidermal hairs/trichomes on both epidermis (Fig. 9E) was an important impact of chromium treatment. Structure of vascular tissues were not distinct and unevenly distributed. Xylem vessels were thick walled and angular in shape (Fig. 9F).

SEM image of *C. amboinicus* leaf treated with Cr showed only negligible changes in the anatomy compared to the control. Epidermis was clear and epidermal hairs were present (Fig. 14E). Vascular region was conspicuous in contrast to control. Mesophyll cells were thick walled but appeared as damaged by breaking the cell walls (Fig. 14F) and some of the cells contained some deposits and were unevenly distributed.

Treatment with Copper

Root

Plants treated with Cu resulted in the complete disappearance of root hairs. Piliferous layer cells were damaged (Fig.10A). Densely stained particles were distributed which appear as embedded all over the sections except cortex (Fig. 10B) and hence vascular tissues were not distinct. Pith was reduced compared to the control.

SEM images of *C. amboinicus* roots treated with Cu shows piliferous layer in which cells were not clearly seen. Xylem vessels were numerous and distributed all over the medullary rays which consisted of thick-walled angular cells (Fig. 15A). Pith was narrow and irregular deposits were seen embedded (Fig. 15B).

Stem

Plants treated with Cu showed disappearance of epidermal hairs and other anatomical features were almost similar to the control (Fig. 10C). The number and shape of vessels were altered due to Cu treatment and they were unevenly distributed. The most interesting observation after the copper treatment was the removal of oil globules present in the stem tissue (Fig. 17)

The SEM images of *C. amboinicus* stem treated with Cu shows similar structure of dicot stem. Epidermis and epidermal hairs present but cannot be clearly captured in detail in the full view (Fig. 15C). Cortex cells were arranged in well-organized manner. In the magnified view, vessels contained irregular deposits (Fig. 15D). Pith region was wide and cells were compactly arranged.

Leaf

Leaf structure of *C. amboinicus* treated with Cu was almost similar to the control, except the reduced size of the lamina and midrib (Fig. 10E). Epidermal hairs in upper and lower epidermis were reduced compared to the control. Many multicellular epidermal hairs were present on both epidermis and the epidermis were damaged.

SEM image of *C. amboinicus* leaf treated with Cu showed upper and lower epidermis (Fig. 15E) and epidermal hairs were reduced in the leaves of plant treated with Cu. In the magnified view some mesophyll cells exhibited increased thickening on the entire cell wall due to the presence of some deposits (Fig. 15F). Like stem, leaf oil globules also vanished after Cu treatment. (Fig. 18).

Treatment with Mercury

Root

Roots of plants treated with Hg showed 2-3 layered rhizodermal cells which were larger than that of the control (Fig. 11A). Outer layer of rhizodermal cell showed breakage and the margin was uneven (Fig. 11A). Root hairs were totally absent. Cortical cells showed change in shape compared to the control. Cell wall thickening also observed in the cortical cells. Densely stained endodermis was very distinct but cellular details were obscure. Unlike the control, vascular region showed many large sized xylem vessels scattered in the pith region (Fig. 11B).

SEM images of root of *C. amboinicus* treated with Hg showed damaged epidermal layer (Fig. 16A). Epidermis and epidermal hairs cannot be clearly captured in detail in the full view because most of them were appeared damaged. In the

magnified view, xylem and medullary rays were also damaged or broken due to the breakage of thick cell wall. (Fig. 16B)

Stem

Stem of *C. amboinicus* plants treated with Hg showed epidermal layer with many trichomes and numerous, well-developed lenticels (Fig. 11C). Morphologically these lenticels appeared as blisters by naked eye on the stem (Fig. 5). The lenticel consisted of loosely arranged tissues exhibiting vast intercellular spaces. It appears as elevated circular, oval or elongated protrusions. Other structures of stem tissue of plant treated with Hg was found to be almost similar to the control except thick-walled cortical cells and the reduced number of vasculature (Fig. 11D).

The SEM image of *C. amboinicus* stem treated with Hg showed broken epidermal layer but epidermal hairs were clearly seen. Due to the formation of lenticels, epidermal layer in the stem appeared broken (Fig. 16D). This observation was a characteristic result of Hg treatment.

Leaf

Upper and lower epidermal layers of midrib region of leaf, treated with Hg showed the presence of large number of epidermal hairs and most of them were very large and developed as trichomes (Fig. 11E). Other structures were almost similar to the control but the vascular bundles appeared densely stained and cells showed reduced size.

SEM image of *C. amboinicus* leaf treated with Hg showed typical dicot leaf structure (Fig. 16E). The number of epidermal hairs was more compared to control. Thick-walled cell wall of mesophyll cells was completely broken resulting in an uneven distribution leaving some lacuna in between the mesophyll cells (Fig. 16F)

ANATOMY OF CONTROL PLANTS

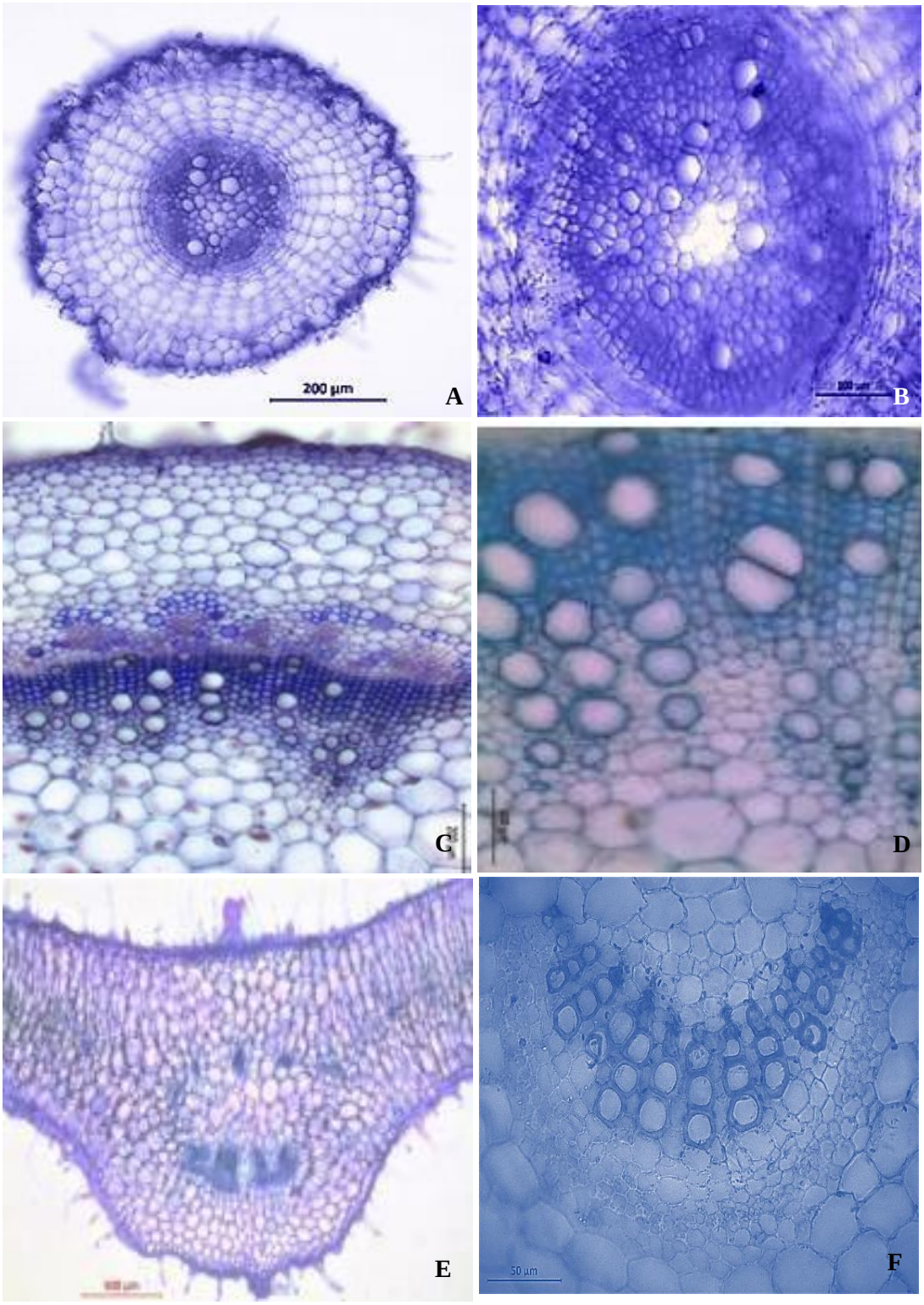


Figure: 7 A. Root T.S; **B**. Portion Enlarged (Root); **C**. Stem T.S; **D**. Portion Enlarged (Stem); **E**. Leaf T.S; **F**. Portion Enlarged (Leaf).

ANATOMY OF PLANT TREATED WITH ALUMINIUM

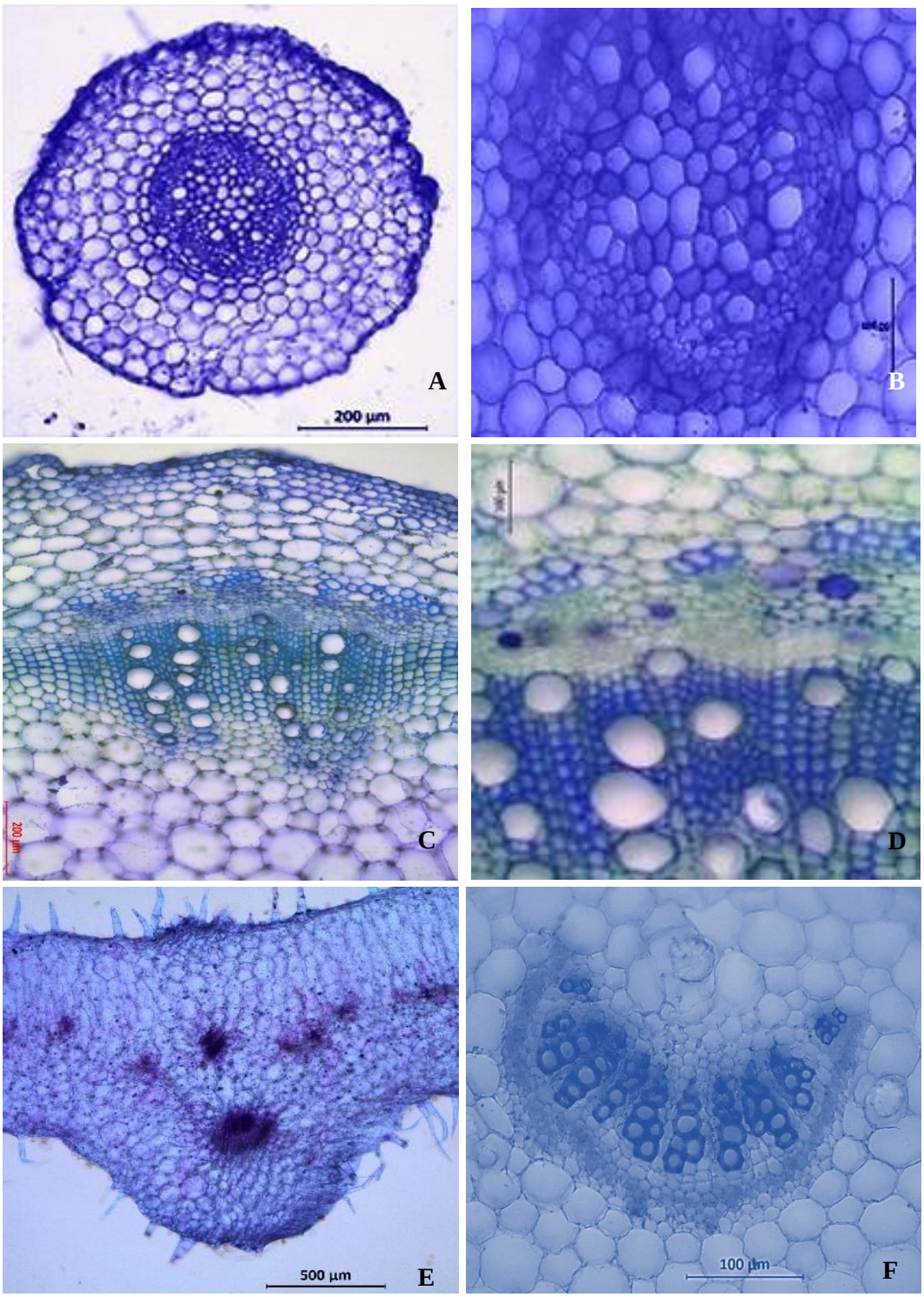


Figure 8 **A**. Root T.S; **B**. Portion Enlarged (Root); **C**. Stem T.S; **D**. Portion Enlarged (Stem); **E**. LeafT.S; **F**. Portion Enlarged (Leaf).

ANATOMY OF PLANT TREATED WITH CHROMIUM

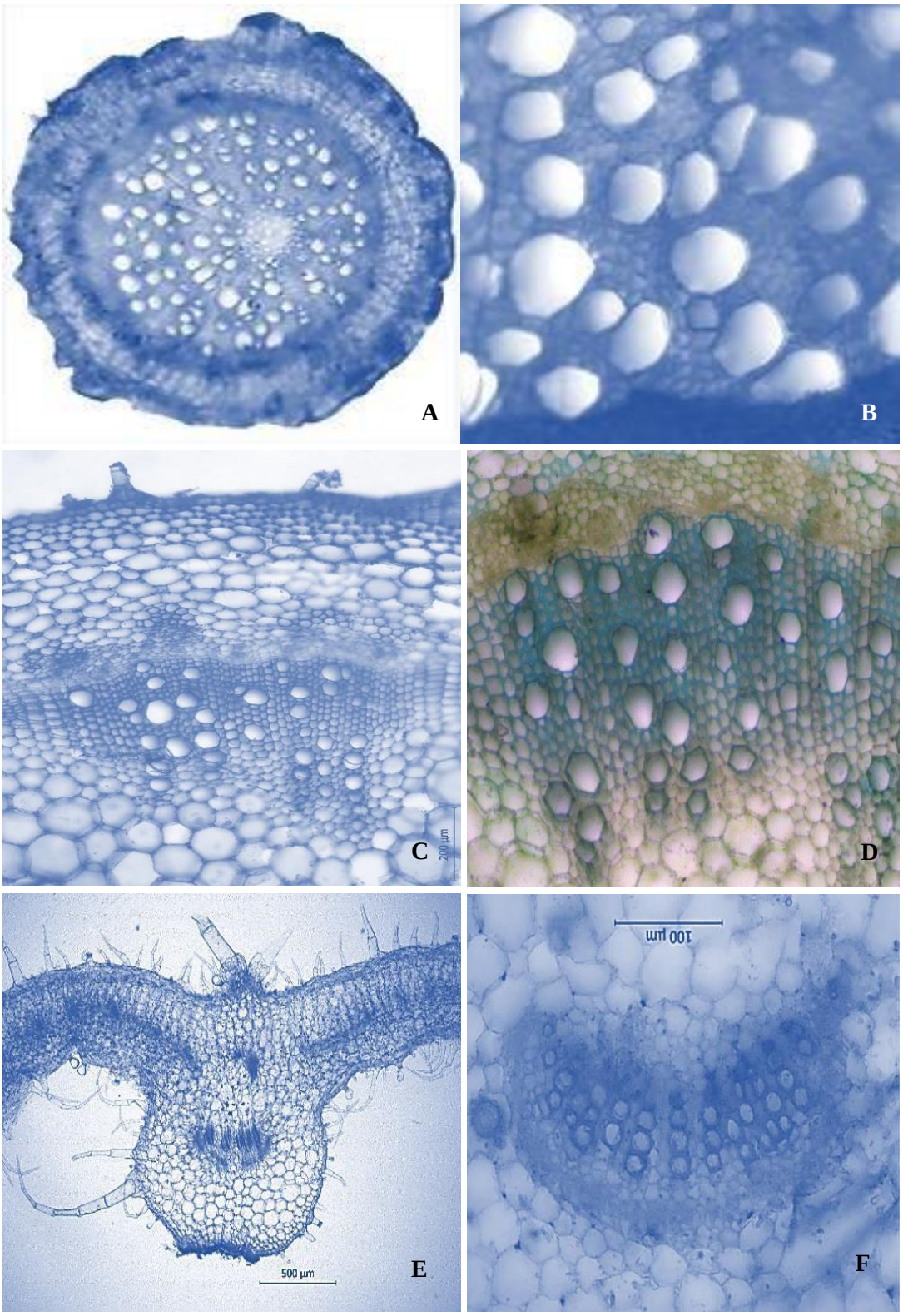


Figure 9. **A**. Root T.S; **B**. Portion Enlarged (Root); **C**. Stem T.S; **D**. Portion Enlarged (Stem); **E**. Leaf T.S; **F**. Portion Enlarged (Leaf).

ANATOMY OF PLANT TREATED WITH COPPER

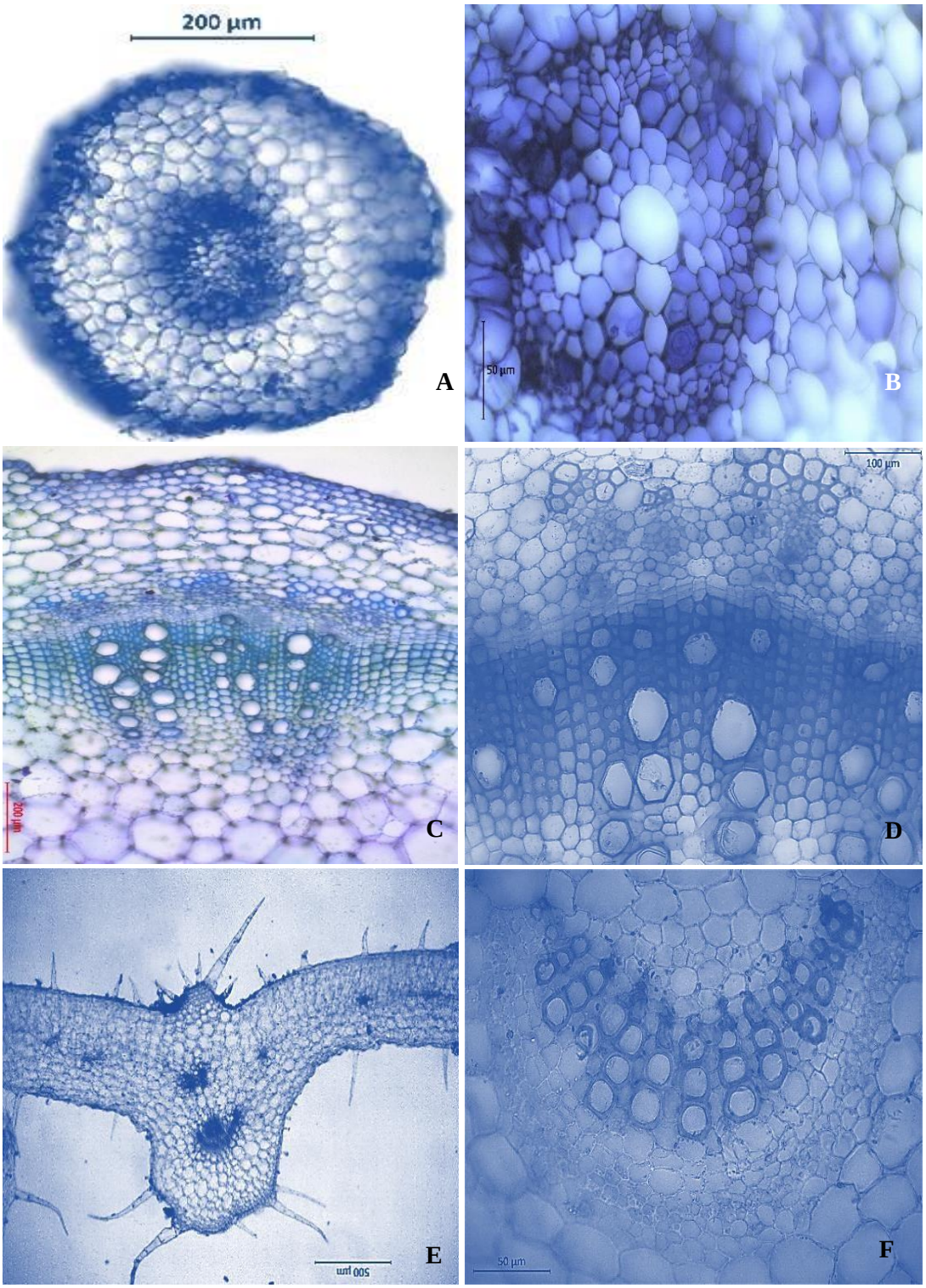


Figure 10 **A.** Root T.S; **B**. Portion Enlarged (Root); **C**. Stem T.S; **D**. Portion Enlarged (Stem); **E**. LeafT.S; **F**. Portion Enlarged (Leaf).

ANATOMY OF PLANTS TREATED WITH MERCURY

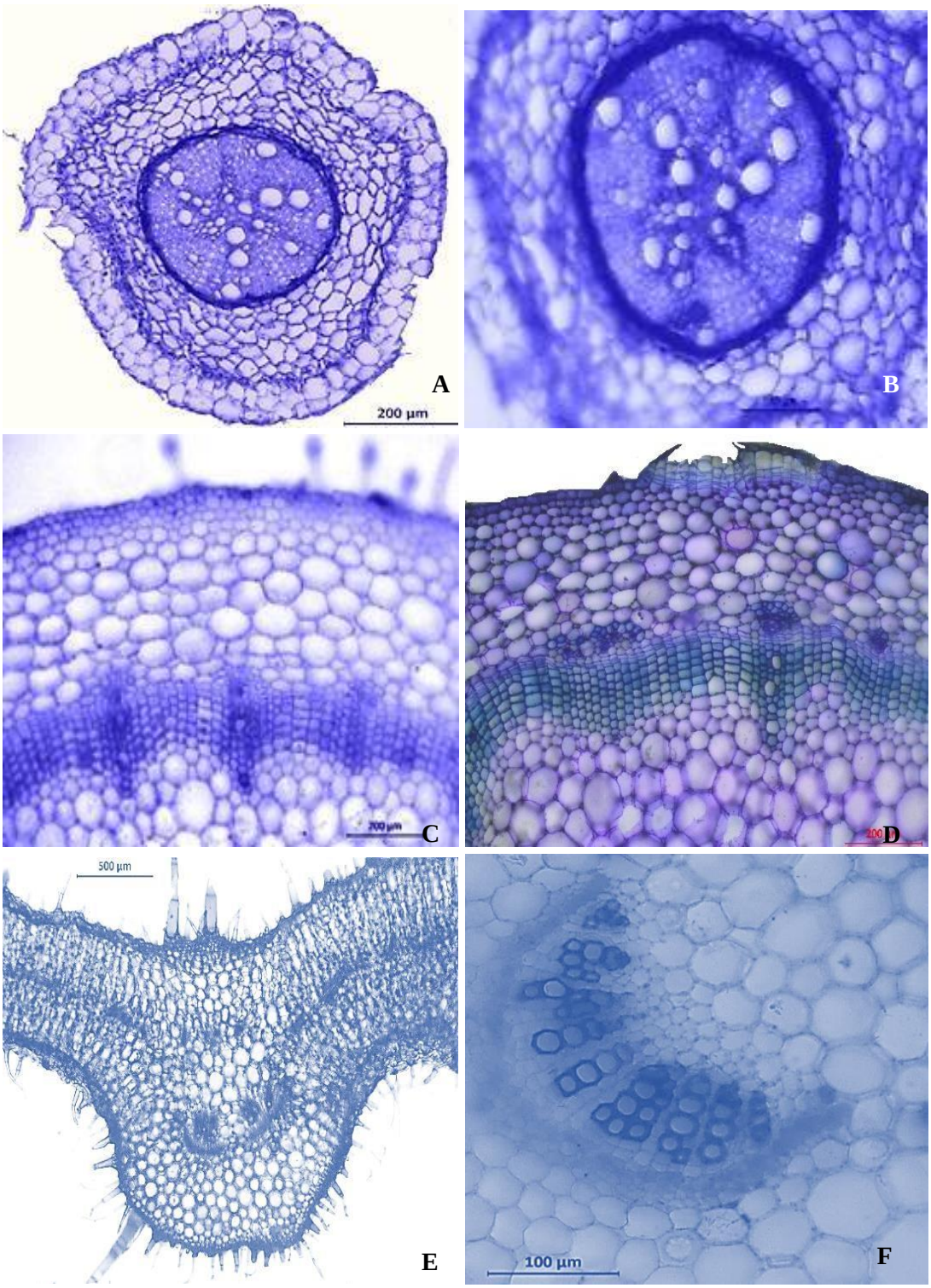


Figure: 11 **A.** Root T.S; **B**. Portion Enlarged (Root); **C**. Stem T.S; **D**. Portion Enlarged (Stem); **E**. LeafT.S; **F**. Portion Enlarged (Leaf).

SCANNING ELECTRON MICROGRAPHS OF *COLEUS AMBOINICUS*

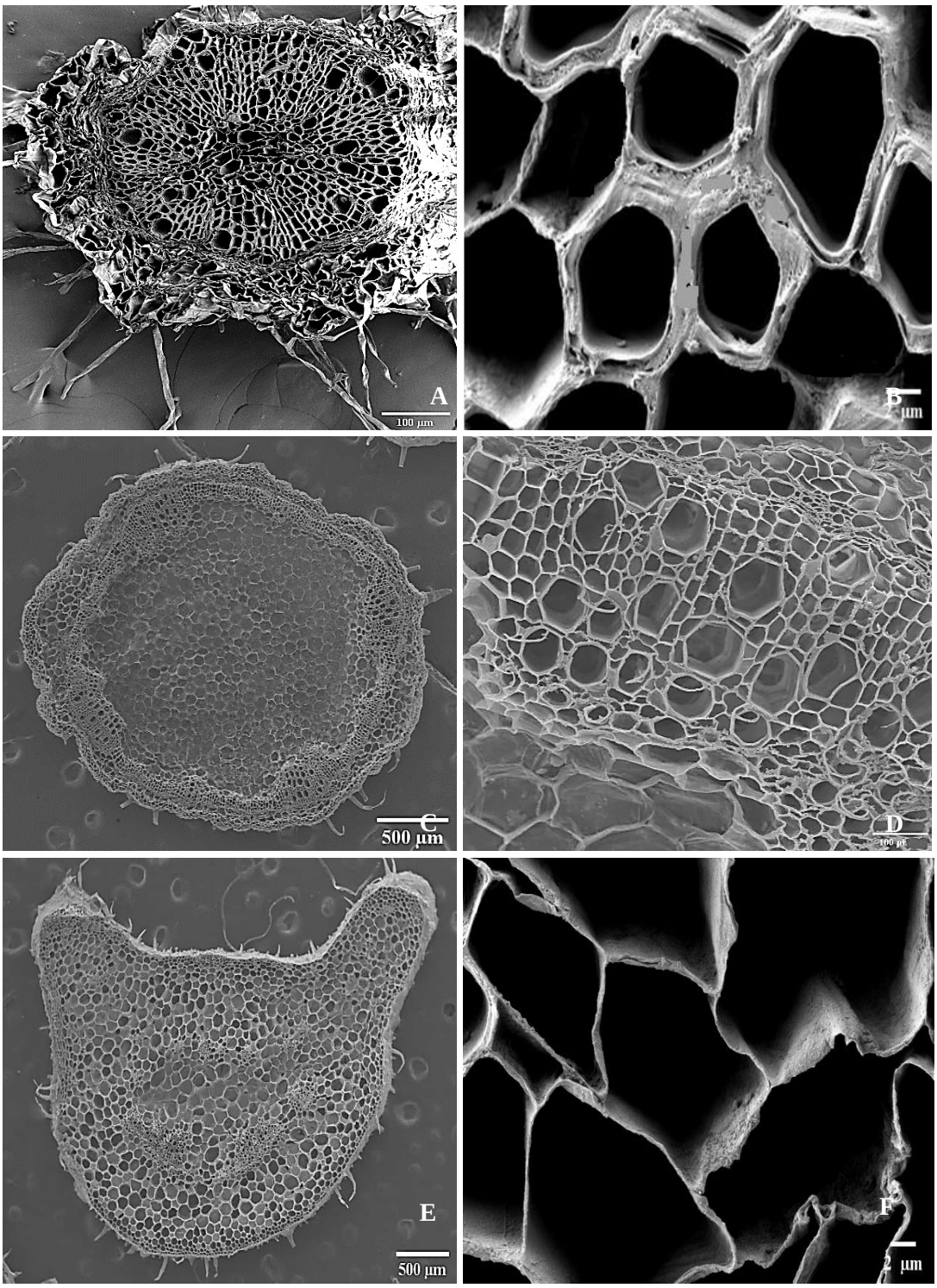


Figure:12 A. Root T.S; **B**. Portion Enlarged (Root); **C**. Stem T.S; **D**. Portion Enlarged (Stem); **E.** LeafT.S. **F.** Portion Enlarged (Leaf).

SEM OF PLANT TREATED WITH ALUMINIUM

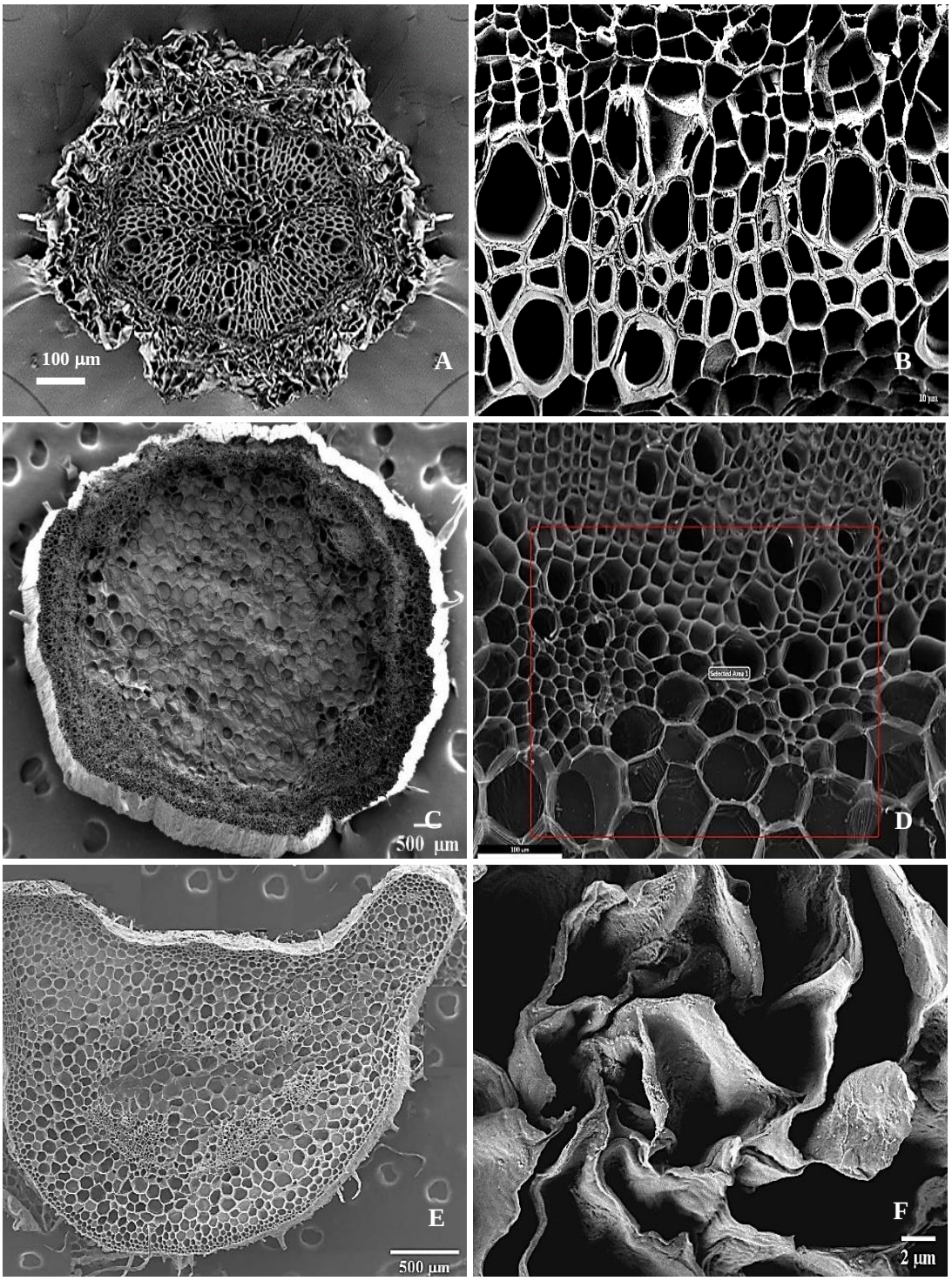


Figure: 13 **A.** Root T.S; **B**. Portion Enlarged (Root); **C.** Stem T.S, **D**. Portion Enlarged (Stem); **E**. LeafT.S; **F**. Portion Enlarged (Leaf).

SEM OF PLANT TREATED WITH CHROMIUM

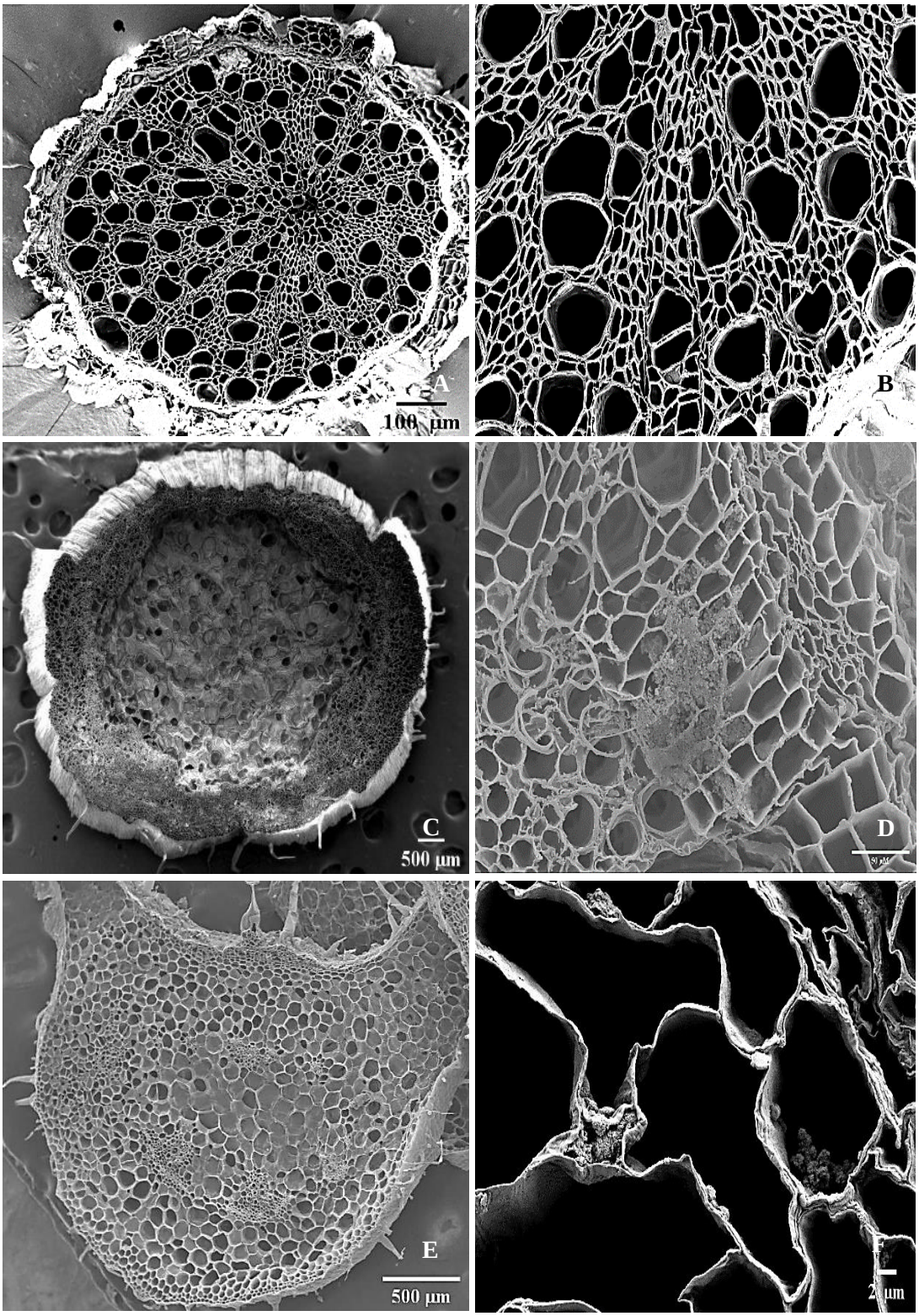


Figure:14 **A**. Root T.S; **B**. Portion Enlarged (Root); **C**. Stem T.S; **D**. Portion Enlarged (Stem); **E**. LeafT.S; **F**. Portion Enlarged (Leaf).

SEM OF PLANT TREATED WITH COPPER

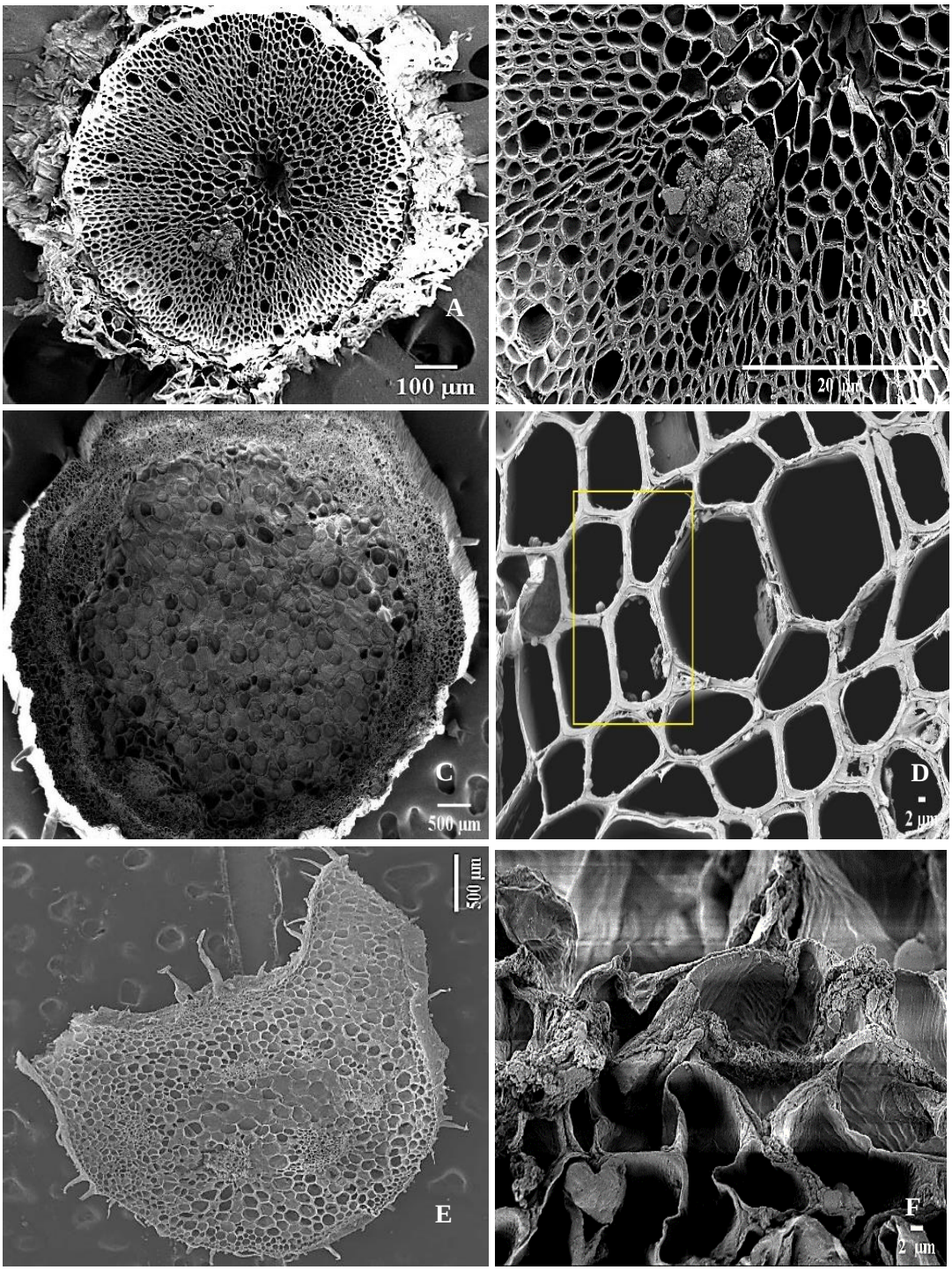


Figure:15 **A**. Root T.S; **B**. Portion Enlarged (Root); **C**. Stem T.S; **D**. Portion Enlarged (Stem); **E**. LeafT.S; **F**. Portion Enlarged (Leaf).

SEM OF PLANT TREATED WITH MERCURY

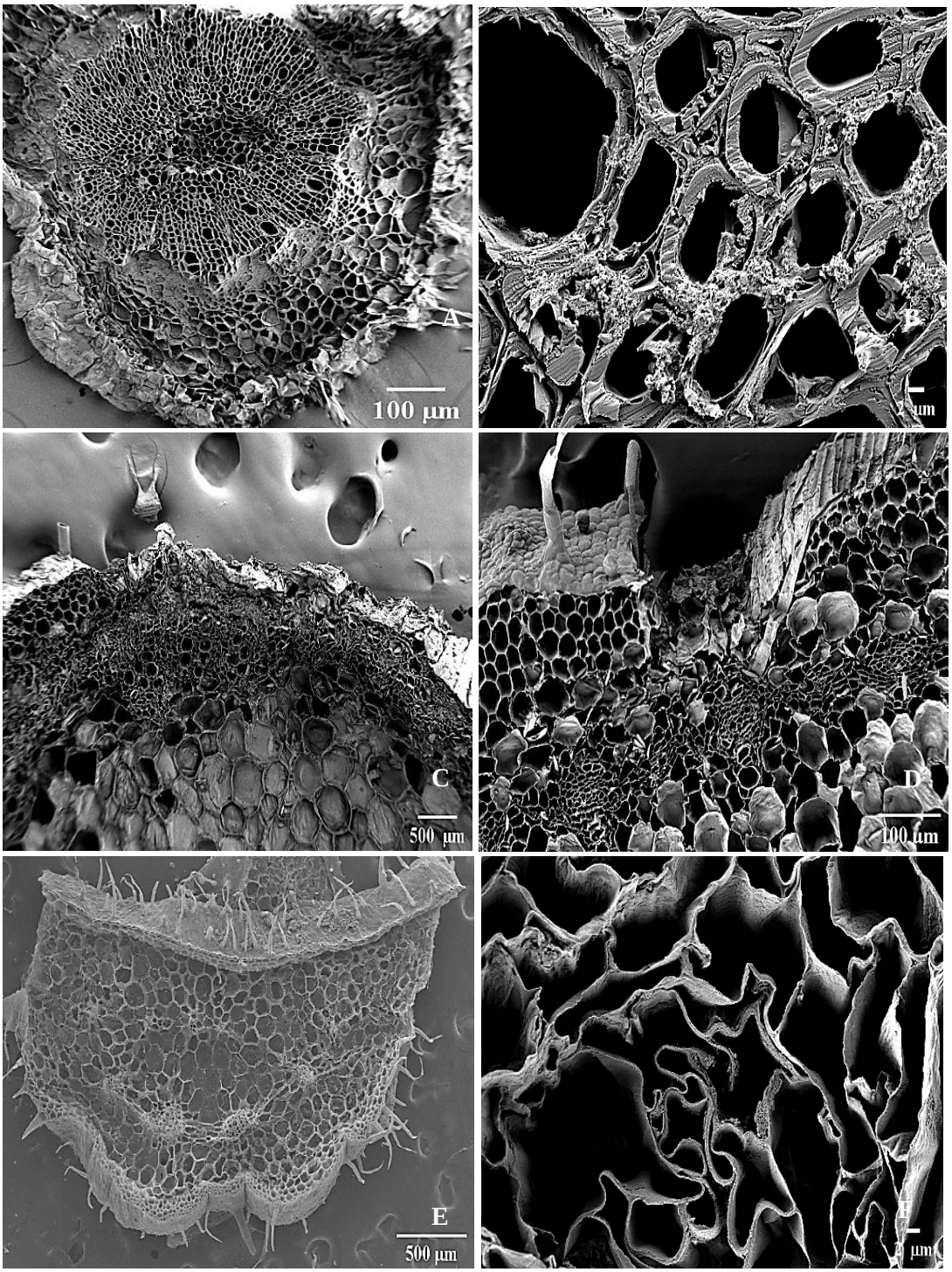
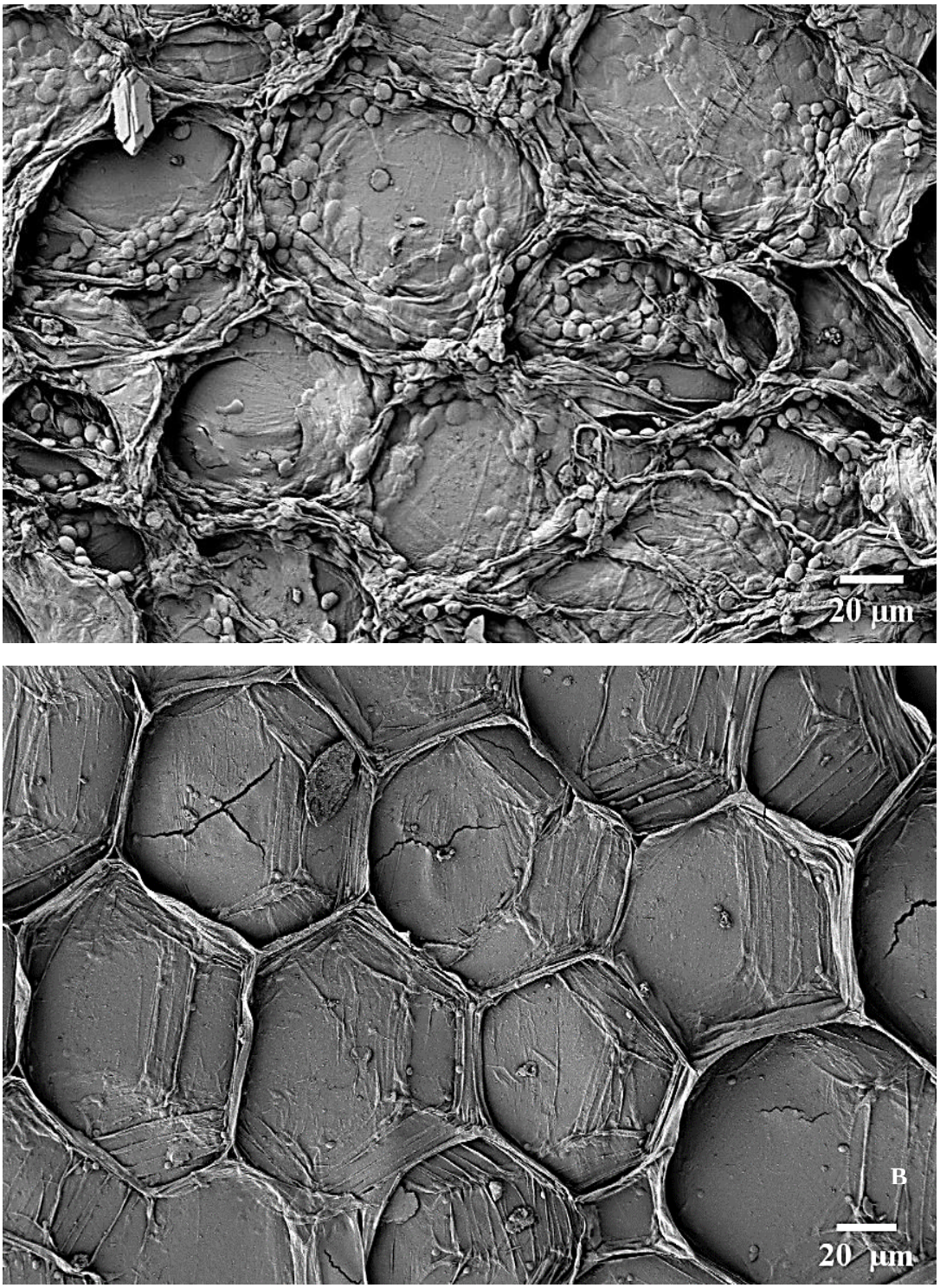


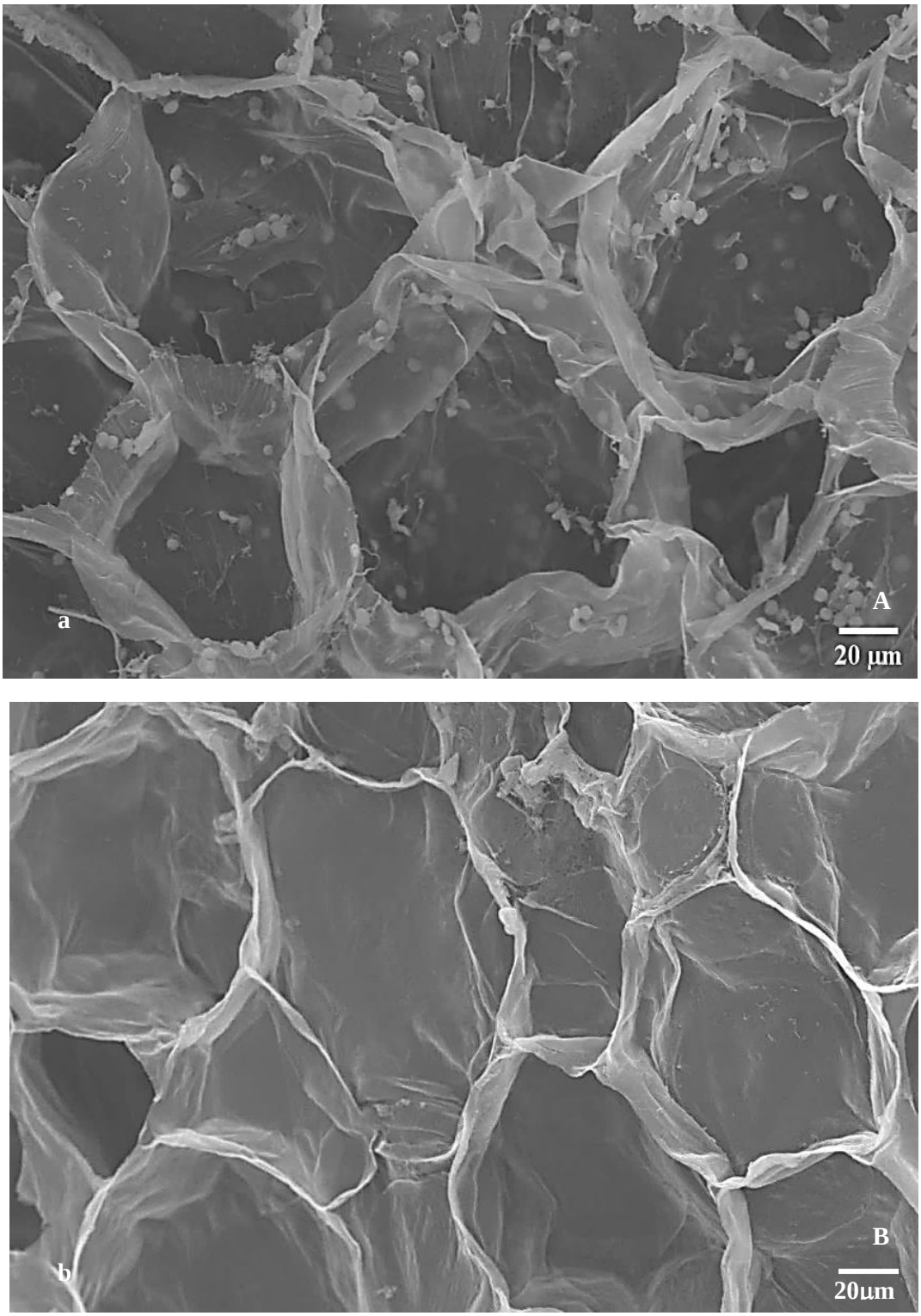
Figure:16 **A**. Root T.S; **B**. Portion Enlarged (Root); **C**. Stem T.S; **D**. Lenticels (Stem); **E**. LeafT.S; **F**. Portion Enlarged (Leaf).

Figure 17 Removal of oil globules due to Cu treatment in stem



A. Stem of *C. amboinicus*; **B**. Stem of Cu treated *C. amboinicus*

Fig. 18 Removal of oil globules due to Cu treatment in leaf



A. Leaf of *C. amboinicus;* **B**. Leaf of Cu treated *C. amboinicus*

STOMATAL INDEX

Stomatal index of *Coleus amboinicus* showed significant variations due to the treatment with Al, Cr, Cu and Hg. The increase of stomatal index was more prominent in the lower epidermis than the upper one (Table 7, Figs. 19&20). Stomatal index of control plants remained unchanged in upper and lower epidermis during 20 days of growth. In plants treated with Al, only negligible increase was observed in upper epidermis whereas in the lower epidermis gradual increase was observed. Cr treatment resulted in enhanced values of stomatal index in both upper and lower epidermis and during growth the increase was significant in the lower epidermis ($P < 0.01$). Stomatal index of plants treated with Cu was significantly increased and lower epidermis exhibited more stomatal index values than upper epidermis. Maximum stomatal index was shown by *Coleus amboinicus* subjected to Hg stress ($P < 0.01$) and there occurred significant increase during growth and the increase in stomatal index values were significant from stage to stage in lower epidermis.

Table 7 Effect of Aluminium, Chromium, Copper and Mercury on stomatal index of *Coleus amboinicus*

Treatments		Interval-days					
		0	4	8	12	16	20
Control	UE	17.01±0.12	17.02±0.32	17.05±0.25	17.08±0.11	17.13±0.23	17.25±0.39
	LE	17.2±0.09	17.3±0.11	17.89±0.21	17.94±0.09	17.99±0.11	18.5±0.5
Aluminium (500µM)	UE	17.12±0.11	18.19±0.69	18.56±0.34	20.8±0.14	23.73±0.85	23.93±0.18
	LE	17.23±0.03	18.62±0.32	19.4±0.05	22.39±0.22	24.48±0.17	25.9±0.11
Chromium (150µM)	UE	17.67±0.24	18.1±0.43	18.91±0.13	21.73±0.25	23.59±0.15	26.63±0.26
	LE	17.5±0.8	21.66±0.18	24.16±0.21	25.6±0.09	27.9±0.23	28.6±0.46
Copper (80µM)	UE	17.32±0.52	17.6±0.04	17.16±0.17	20.21±0.02	22.94±0.24	24.1±0.21
	LE	17.53±0.14	19.68±0.05	21.9±1.06	23.43±1.21	24.97±1.12	25.63±1.26
Mercury (10µM)	UE	17.1±0.56	18.6±1.02	21.3±0.13	23.13±1.04	27.48±1.09	30.41±1.57
	LE	17.73±0.49	19.21±0.11	24.56±0.19	28.96±1.01	31.4±1.04	35.13±2.03

Values given are mean of 5 replicates ±S.E

Figure : 19 Effect of Aluminium, Chromium, Copper and Mercury on stomatal index of *Coleus amboinicus*

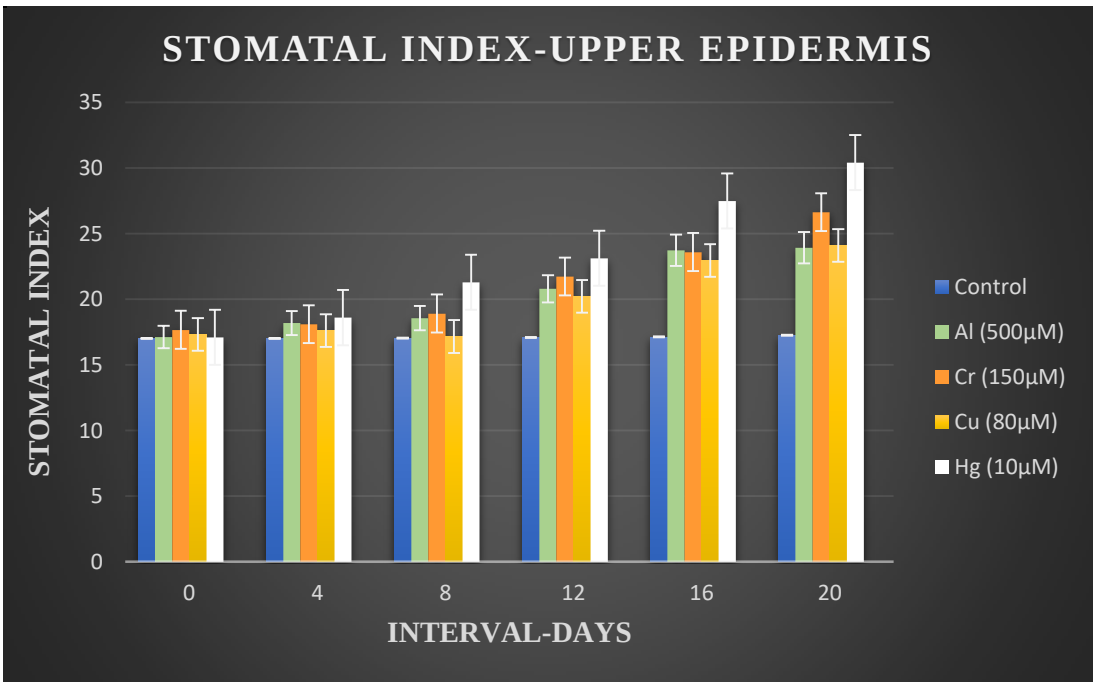
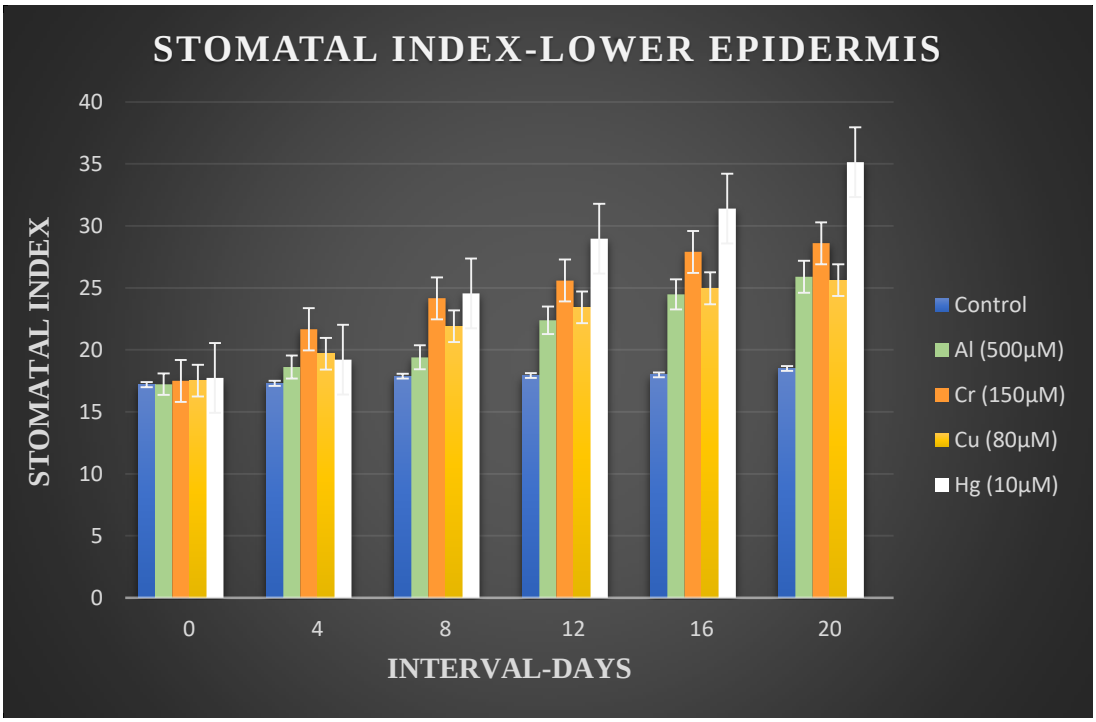


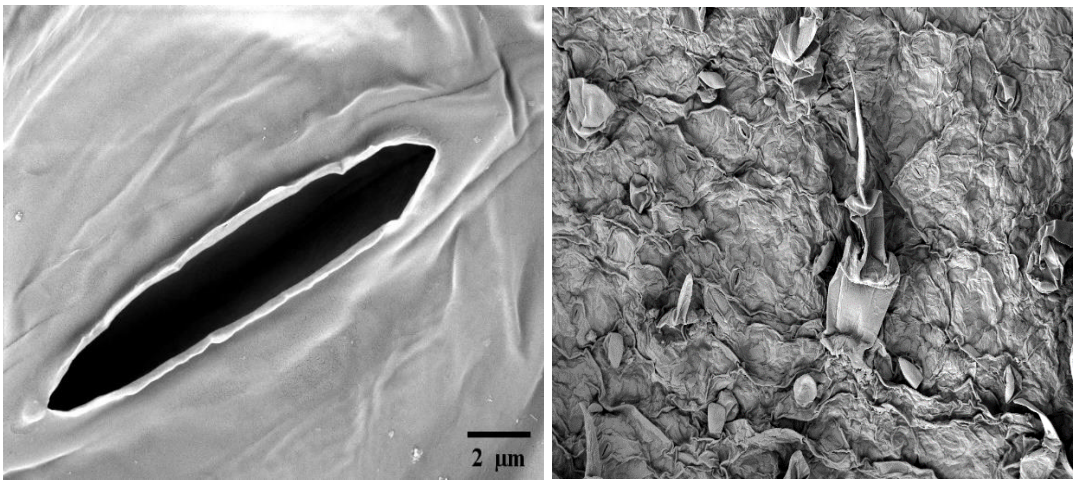
Figure : 20 Effect of Aluminium, Chromium, Copper and Mercury on stomatal index of *Coleus amboinicus*



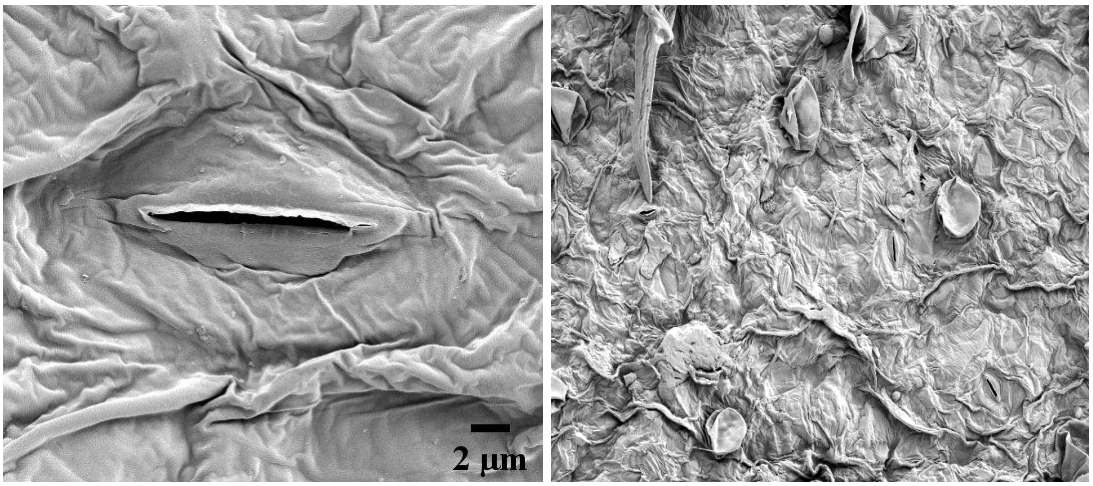
SEM study of lower epidermis showed evenly arranged epidermal cells in which stomatal cells were clearly visible. In the control, stomata appeared almost widely opened and the structure was clearly seen (Fig. 21A). In plants treated with Al, stomata of lower epidermis were found to be appeared almost closed leaving a small opening in all cells. (Fig. 21B). Stomatal aperture was almost clearly seen. Plants treated with Cr also, all stomata were found widely open with high difference in aperture size compared to Al and control (Fig. 21C). Distribution of stomatal number was slightly increased in the leaves of plants treated with Hg than thecontrol and leaf micromorphological characters of *C. amboinicus* were significantly modified upon exposure of Hg. In plant treated with Hg, structure of stomata were drastically exposed compared to the control (Fig. 21E). Cu treatment resulted in uneven distribution of stomata in the epidermal cells and stomatal aperture was almost similar to that of control(Fig. 21D)

Fig. 21 Scanning Electron Microscopic images of leaf (adaxial side) and stomata of *C. amboinicus*

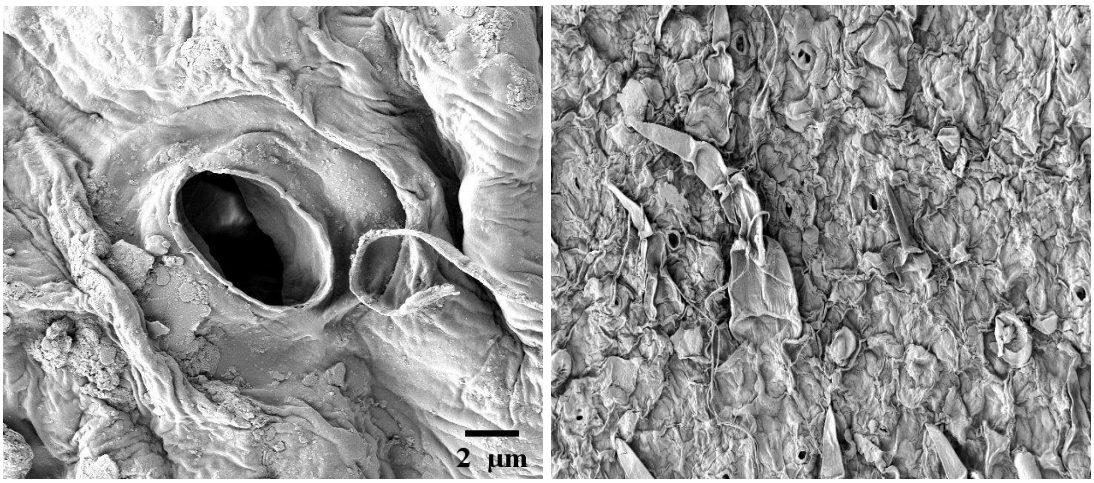
A- Control



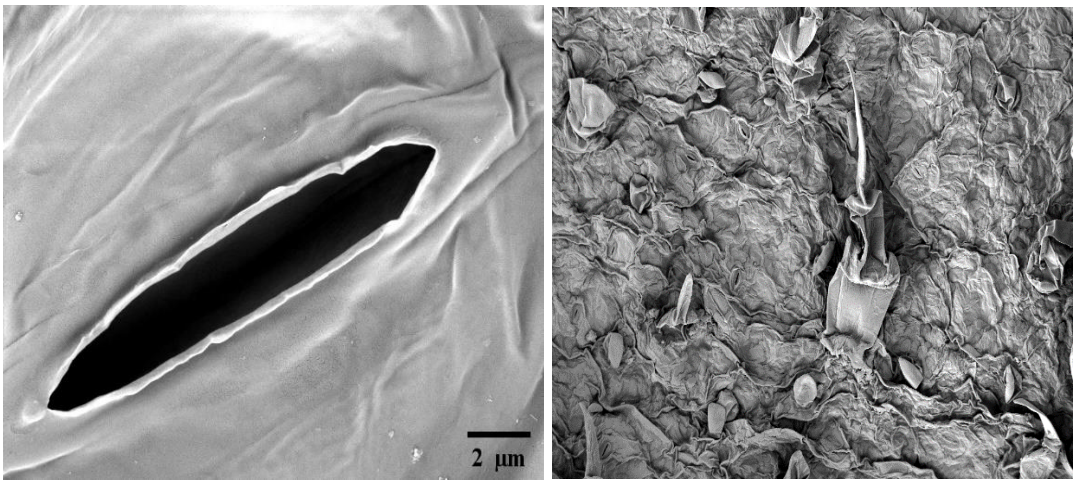
B- Al treated leaf



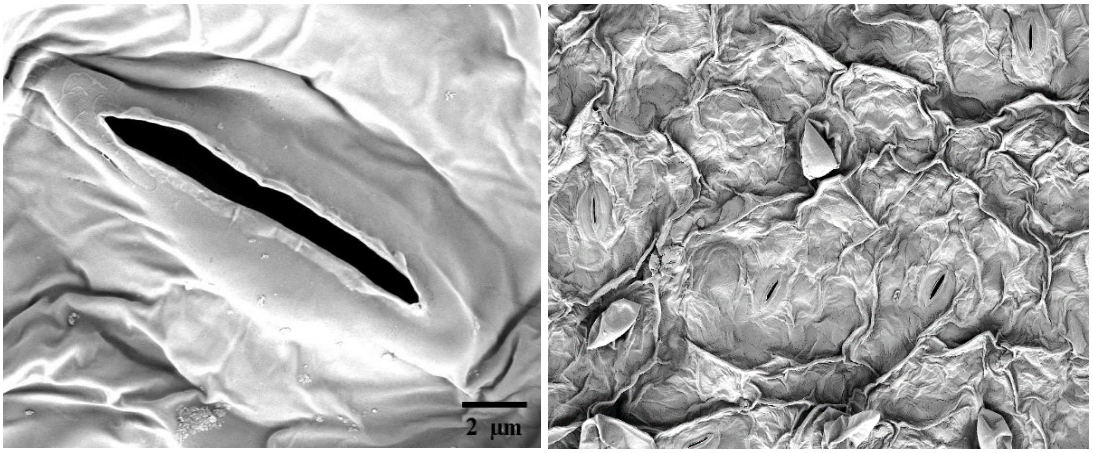
C. Cr treated leaf



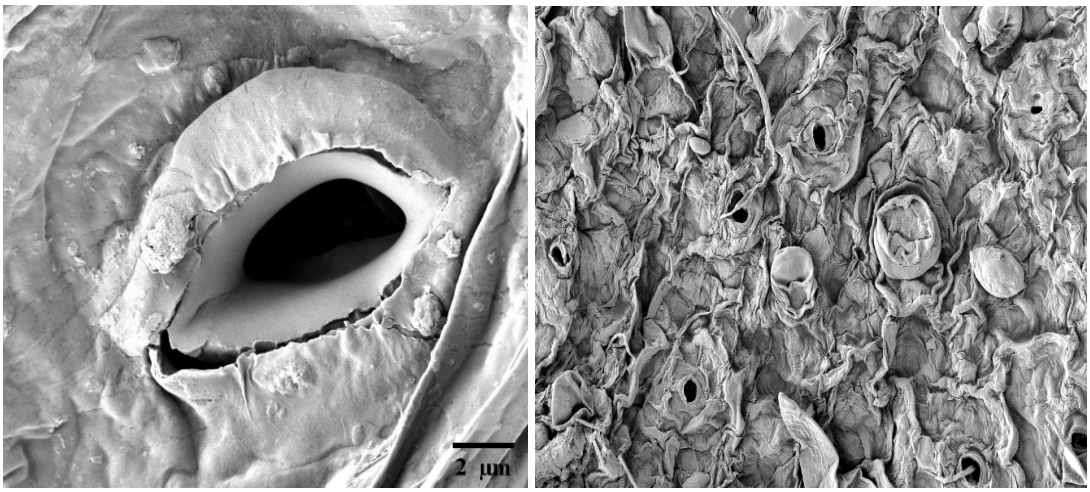
A. Control



D. Cu treated leaf



E. Hg treated leaf



EFFECT OF CU ON XYLEM WALL THICKNESS

Table 8 Xylem wall thickening in the root, stem and leaves of *C. amboinicus* subjected to CuSO4 treatments (control and 80 µM) on 20 d.

Plant sample	XYLEM WALL THICKNESS (µm)	
	Control	Cu treated
ROOT	1.123±0.125	2.643±0.113
STEM	0.874±0.174	2.983±0.185
LEAF	3.241±0.138	3.824±0.179

Values are the mean ± SE

In the case of the stelar region of root of *C. amboinicus*, the thickness of the xylem wall was enhanced from 1.123±0.125 µm in control to 2.643±0.113µm in 80 µM $CuSO_4$ treated roots. The SEM images also revealed some clotted depositions in the cells on exposure to $CuSO_4$ (Fig.15A, Table 8). Moreover, the thickness of the xylem walls of the stem was enhanced from 0.874±0.174µm in control to 2.983±0.185µm in 80 µM $CuSO_4$ treated plants. Similar to the roots, some clotted depositions in the xylem walls were observed in the stem also on exposure to $CuSO_4$ (Fig.15A). The scanning electron microscopic analysis of the xylem tissues revealed that the thickness of the xylem wall was increased in leaves upon exposure to $CuSO_4$. The xylem wall thickness was 3.241±0.138 µm in control, which enhanced to 3.824±0.179 µm when treated with 80 µM $CuSO_4$.

SCANNING ELECTRON MICROGRAPHS –ENERGY DISPERSIVE X-RAY ANALYSIS OF *COLEUS AMBOINICUS*

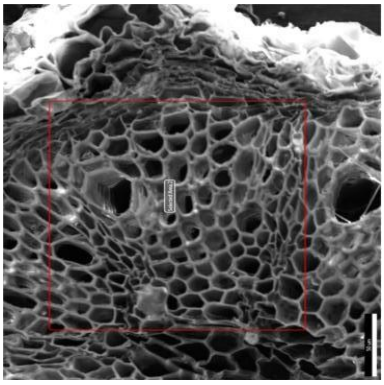
CONTROL

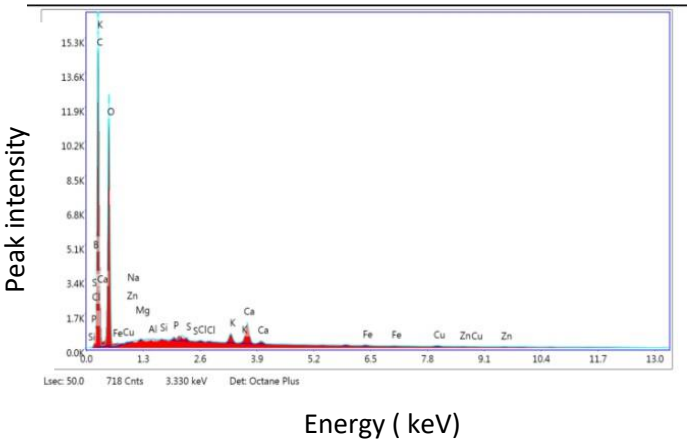
The influence of heavy metal stress on the distribution of various essential elements in the plant parts were analyzed by SEM-EDX analysis. EDX data revealed the presence and quantity of elements in the localized masses. *C. amboinicus* treated with heavy metals recorded significant variations in SEM-EDX peaks denoting various macro and micro- elemental distribution patterns in the tissues. The

variations in the peaks were varies according to the metal treated. Energy dispersive X-ray (EDX) analysis data of zoomed region of root section of *C. amboinicus* showed essential macro and micro elements such as carbon, oxygen, magnesium, aluminium, silicon, phosphorous, sulphur, chlorine, potassium, calcium, boron, iron, copper and zinc present in the tissues (Fig. 22A). In the stem and leaf tissues, also showed the same essential macro and microelements as that of root (Figs. 22 B&C). Among the essential elements identified, the major portion constitutes C and O in all the plant tissues.

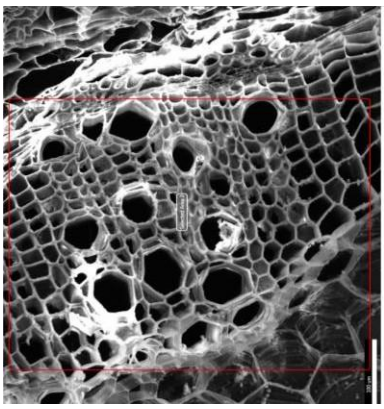
Fig. 22 Scanning Electron Micrographs and EDX spectrum of *Coleus amboinicus*

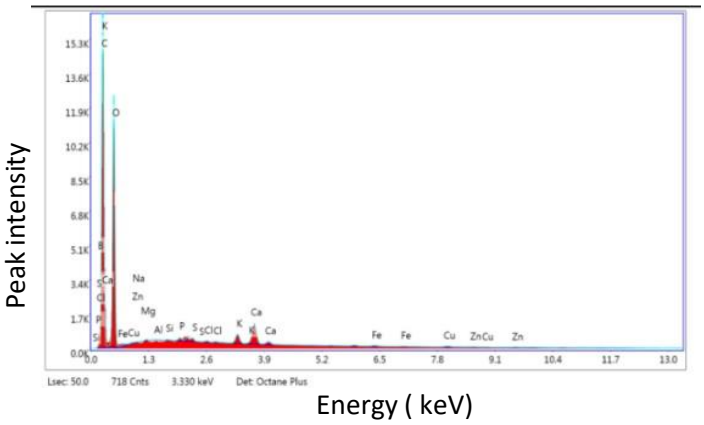
A-Root



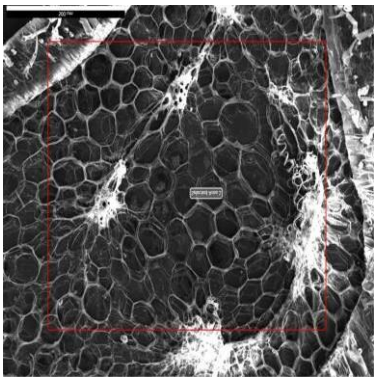


B- Stem





C- Leaf



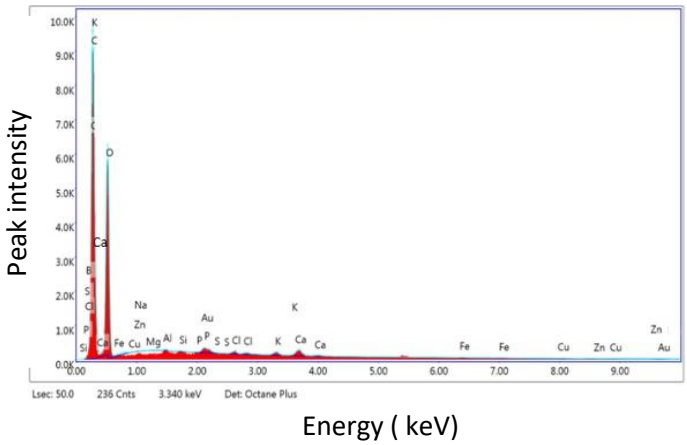


Table 9 SEM-EDX data showing the distribution of various macro and micro-elements (weight %) in the root tissues of *C. amboinicus* on 20 d of treatments

Essential Elements		**Distribution of Elements (Weight %)**				
		Control	**Aluminium**	**Chromium**	**Copper**	**Mercury**
Macro-Elements	C	54.8	52.67	46.78	48.42	50.24
	O	43.22	42.62	47.21	44.1	44.88
	Ca	1.45	1.93	1.65	23.65	1.31
	P	0.35	0.43	0.45	0.46	0.48
	Mg	0.08	0.08	0.07	0.09	0.06
	K	0.15	0.16	0.25	0.17	0.15
	S	0.25	0.32	0.39	0.45	0.36
	Si	0.1	0.28	0.23	0.26	0.24
Micro-Elements	Cu	0.13	0.18	0.16	0.93	0.12
	Zn	0.12	0.13	0.12	0.13	0.12
	Fe	0.07	0.16	0.13	0.18	0.17
	Cl	0.06	0.14	0.15	0.18	0.16
	B	1.03	1.06	1.01	1.08	1.05
Treated Metals	Hg	0	0	0	0	0.87
	Cr	0	0	1.92	0	0
	Al	0	1.98	0	0	0

Table 10 SEM-EDX data showing the distribution of various macro and micro-elements (weight %) in the stem tissues of *C. amboinicus* on 20 d of treatments

Essential Elements		**Distribution of Elements (Weight %)**				
		Control	**Aluminium**	**Chromium**	**Copper**	**Mercury**
Macro-Elements	C	53.15	49.84	52.25	53.1	52.55
	O	43.77	43.48	42.77	43.22	41.83
	Ca	0.96	0.93	0.92	0.97	0.97
	P	0.25	0.27	0.24	0.26	0.25
	Mg	0.21	0.24	0.29	0.27	0.29
	K	0.13	0.13	0.14	0.13	0.12
	S	0.21	0.22	0.23	0.22	0.23
	Si	0.19	0.23	0.27	0.24	0.26
Micro-Elements	Cu	0.36	0.42	0.45	1.07	0.49
	Zn	0.05	0.05	0.08	0.04	0.09
	Fe	0.07	0.04	0.03	0.05	0.44
	Cl	0.13	0.18	0.16	0.18	0.13
	B	1.1	1.08	1.02	1.07	1.03
TREATED METALS	Hg	0	0	0	0	0.32
	Cr	0	0	0.85	0	0
	Al	0	1.12	0	0	0

Table 11 SEM-EDX data showing the distribution of various macro and micro-elements (weight %) in the leaf tissues of *C. amboinicus* on 20 d of treatments

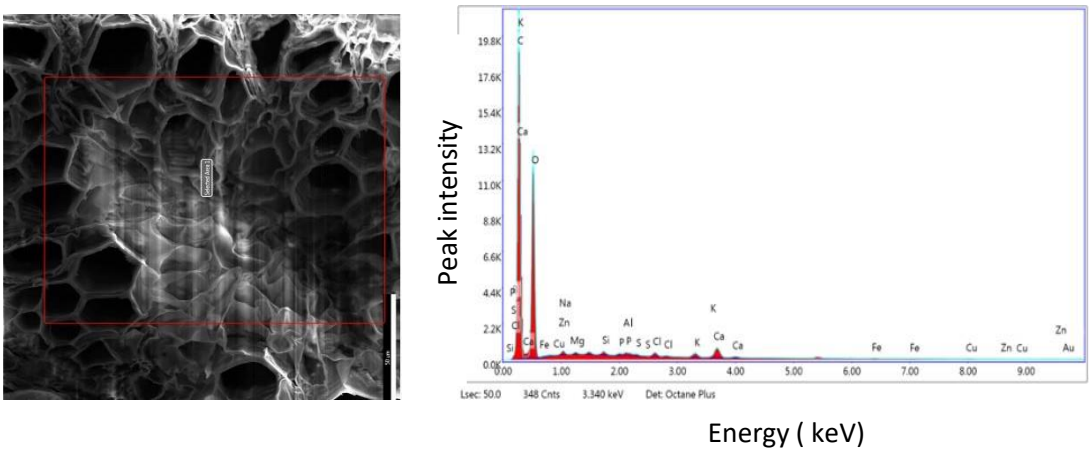
Essential Elements		**Distribution of Elements (Weight %)**				
		Control	**Aluminium**	**Chromium**	**Copper**	**Mercury**
Macro-Elements	C	49.23	50.25	47.29	45.65	47.29
	O	43.21	41.77	42.02	42.67	41.02
	Ca	4.87	4.92	4.86	4.76	4.86
	P	0.31	0.33	0.32	0.19	0.32
	Mg	1.1	0.82	0.46	0.49	0.3
	K	0.16	0.15	0.17	0.16	0.18
	S	0.32	0.39	0.35	0.39	0.35
	Si	0.14	0.15	0.12	0.11	0.17
Micro-Elements	Cu	0.57	0.5	0.52	1.12	0.51
	Zn	0.09	0.18	0.19	0.18	0.17
	Fe	0.18	0.16	0.18	0.16	0.18
	Cl	0.25	0.26	0.24	0.29	0.24
	B	0.86	0.89	0.89	0.91	0.87
Treated Metals	Hg	0	0	0	0	0.37
	Cr	0	0	0.92	0	0
	Al	0	1.12	0	0	0

EFFECT OF ALUMINIUM

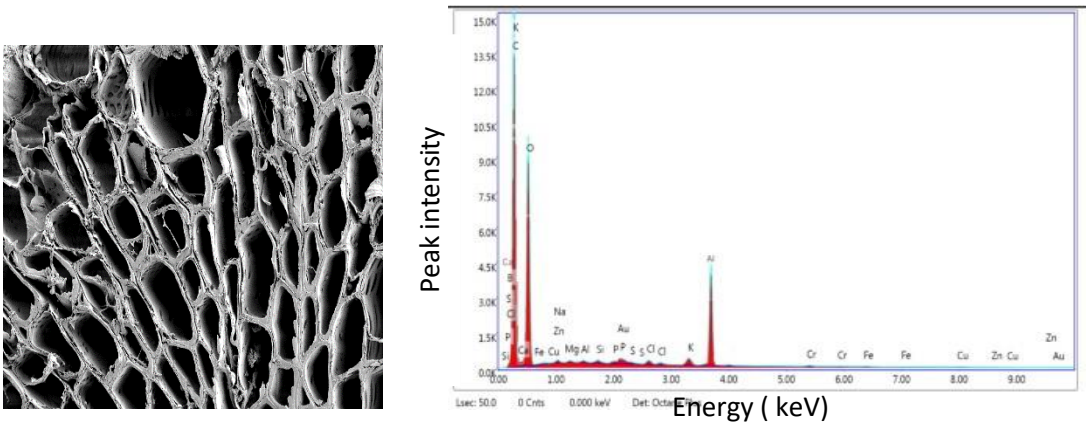
Comparative study using energy dispersive X-ray analysis on the effect of Al treatment on *C. amboinicus* showed maximum occurrence of aluminium compared to control (Fig. 23). The root tissue of plant treated with Al showed a marginal increase of potassium, copper and chlorine (Fig.23A, Table 9). All other elements remained unchanged in the root tissues. Decrease of calcium, potassium, iron and increase of magnesium and chlorine was observed in the stem tissues (Fig. 23B, Table 10). Leaves of plants treated with Al showed maximum aluminium content compared to other tissues (Fig. 23C). Potassium and copper contents were decreased in the leaf tissues due to Al treatment. Other elements such as silicon, phosphorous, sulphur, chlorine, iron, and boron remained same as control.

Fig. 23 Scanning Electron Micrographs and EDX spectrum of *Coleus amboinicus* treated with Aluminium

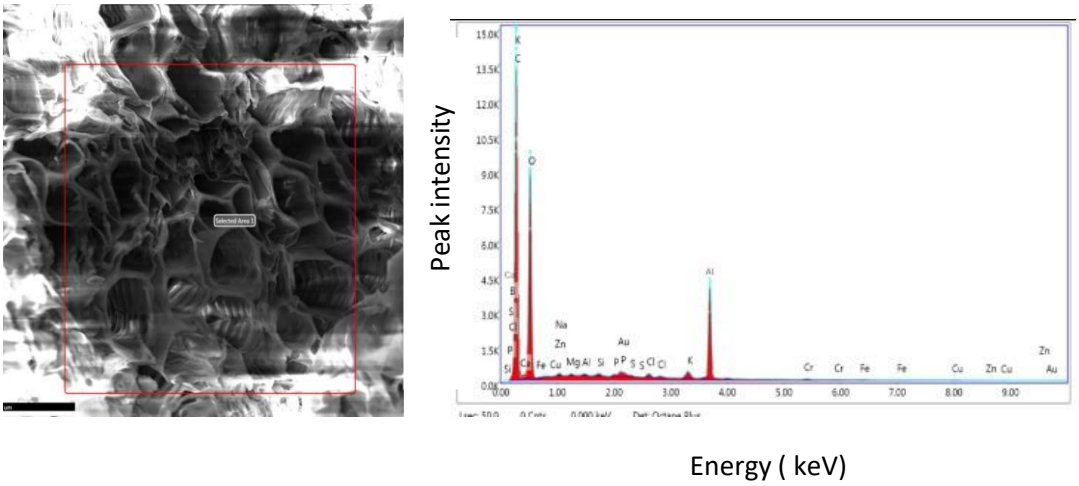
A-Root



B-Stem



C-Leaf

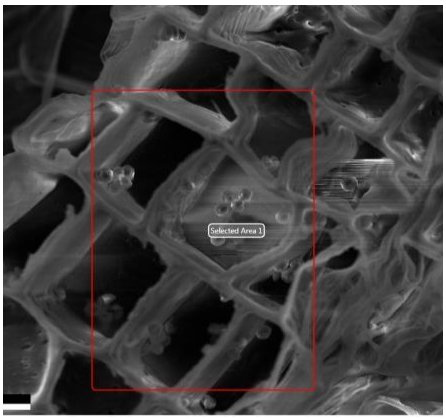


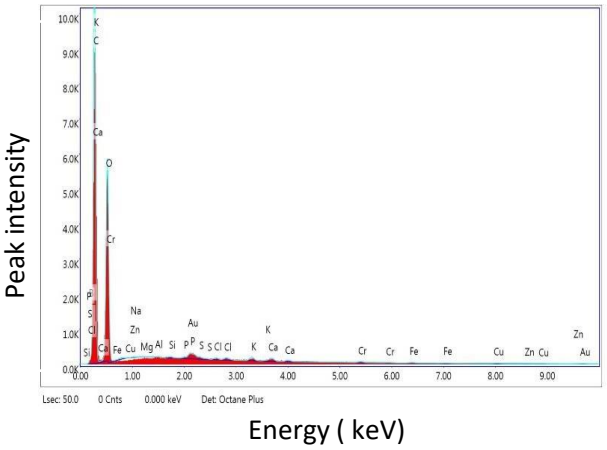
EFFECT OF CHROMIUM

Root tissues of plants treated with chromium showed maximum chromium atoms compared to other tissues (Fig. 24). Chromium treatment resulted in remarkable variation in the distribution of atoms in the root tissues of *C. amboinicus* (Fig. 24A, Table 9). In the root tissues, due to chromium treatment there is increase in the distribution of elements such as oxygen, potassium and iron whereas carbon decreased significantly. The other elements sodium, magnesium, silicon, sulfur, calcium, copper and zinc remained the same as control. In stem tissues, Cr treatment showed increase of calcium, potassium, copper and zinc compared to control (Fig. 22B,Table 9). Distribution of magnesium atoms has decreased in the leaf tissues (Fig. 22C, Table:10). Leaf tissues treated with Cr showed high chromium content (Fig. 22C,Table 11). Phosphorous, sulfur and zinc increased in leaf tissues whereas copper, magnesium and oxygen were decreased.

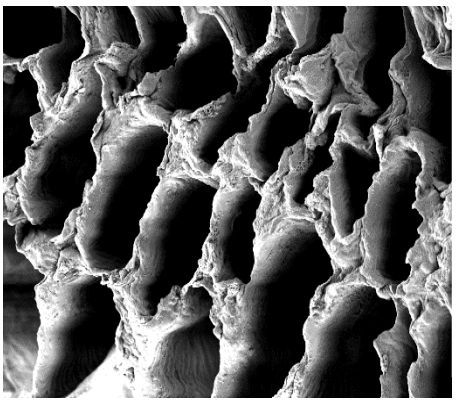
Fig.24 Scanning Electron Micrographs and EDX spectrum of *Coleus amboinicus* treated with Chromium

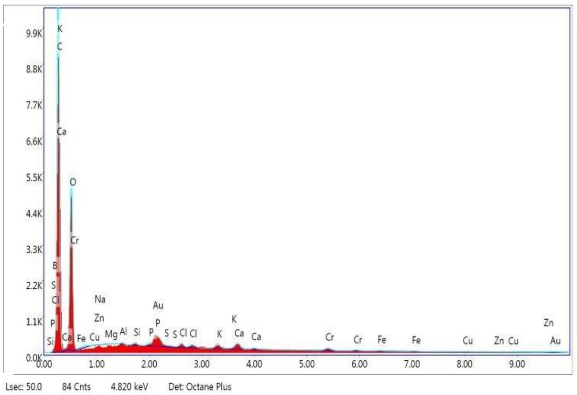
A-Root



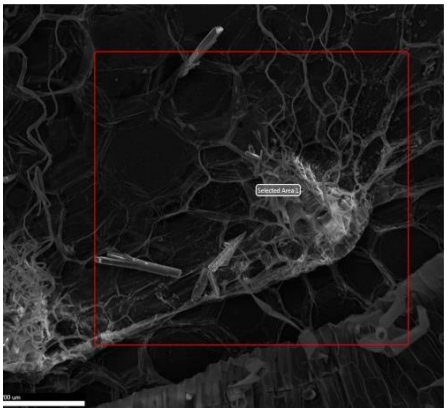


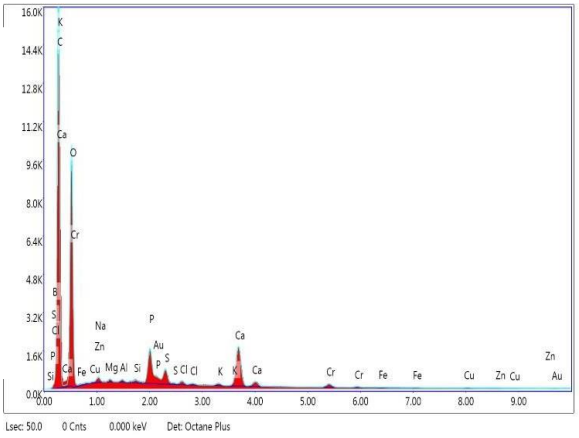
B-Stem





C-Leaf



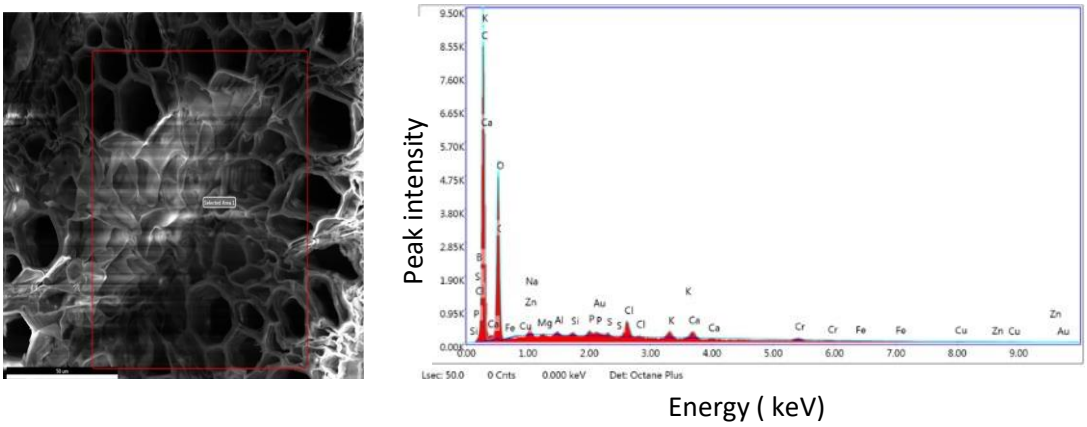


EFFECT OF COPPER

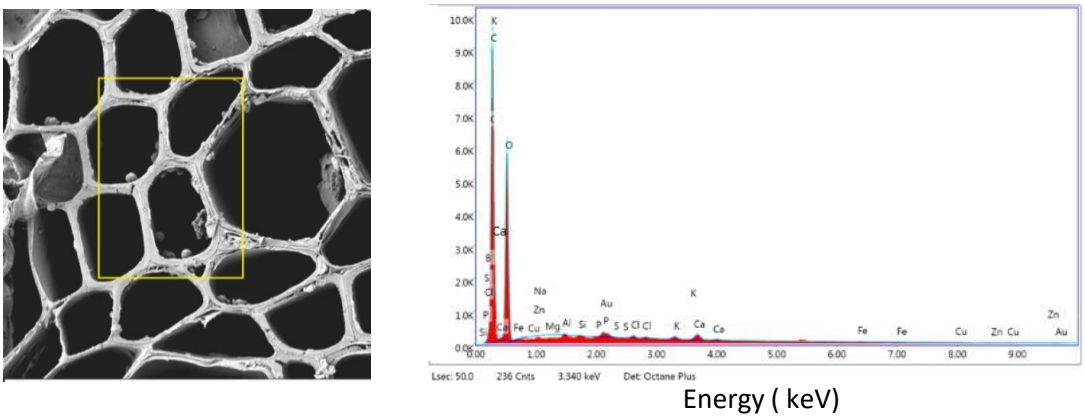
Copper treatment resulted in maximum calcium and copper atoms in the root compared to the control (Table 9). Increase of phosphorous, magnesium, pottassium and iron atoms were observed in the root tissue of *C. amboinicus* due to Cu treatment (Fig. 25A, Table 9). Stem tissues also showed high copper atoms compared to the control (Fig. 25B). In the stem tissues, magnesium, was increased. Zinc and iron decreased in stem tissues (Table 10). Leaf tissues showed maximum copper compared to control (Fig. 25C). Decrease of magnesium and phosphorous were seen and the distribution of other atoms remained unaltered.

Fig. 25 Scanning Electron Micrographs and EDX spectrum of *Coleus amboinicus* treated with Copper

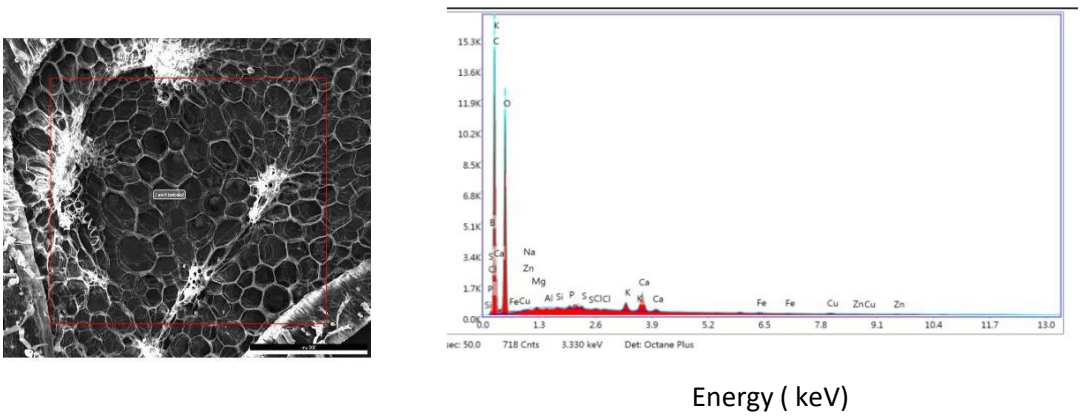
A –Root



B- Stem



C-Leaf

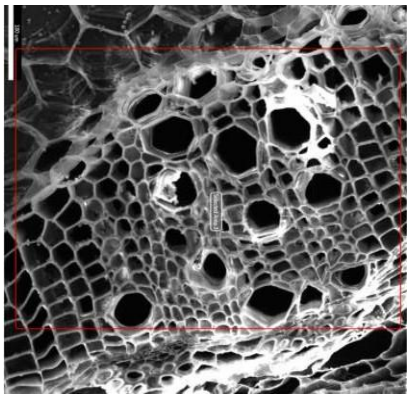


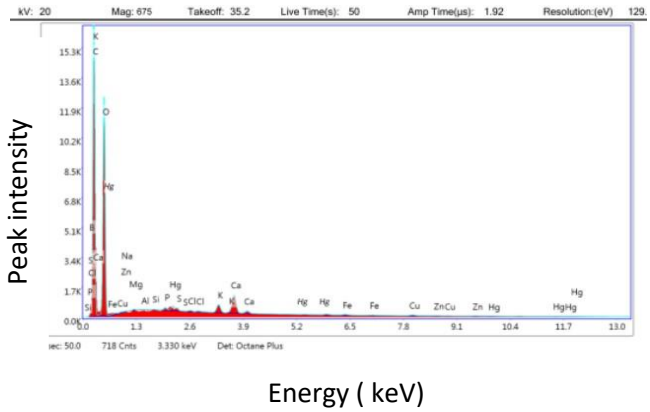
EFFECT OF MERCURY

In *C. amboinicus* treated with Hg, maximum Hg atoms were present in the root tissues (Fig. 26, Table:9). Significant increase of iron, sulfur, boron, phosphorous and chlorine and also the decrease of magnesium were observed after the treatment. Distribution of mercury atoms were seen in the stem tissues of Hg treated plant (Fig. 26, Table 9). There is an increase in the distribution of silicon, calcium, copper and iron but oxygen and potassium get reduced. Phosphorous and chlorine remained unchanged. Maximum mercury atoms were present in the root tissue treated with Hg and there is reduction in oxygen atoms. Calcium, sulfur and coppershowed significant increase when compared to the control.

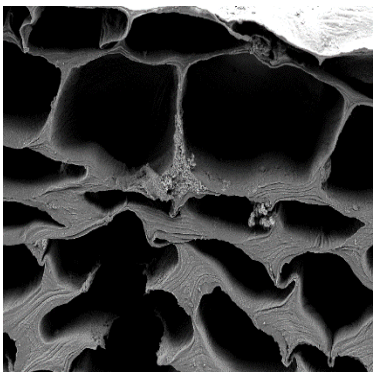
Fig. 26 Scanning Electron Micrographs and EDX spectrum of *Coleus amboinicus* treated with Mercury

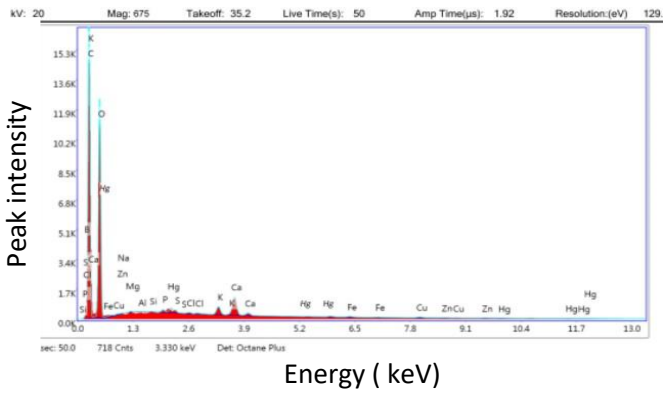
A –Root



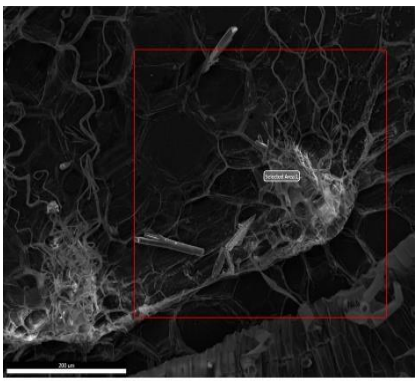


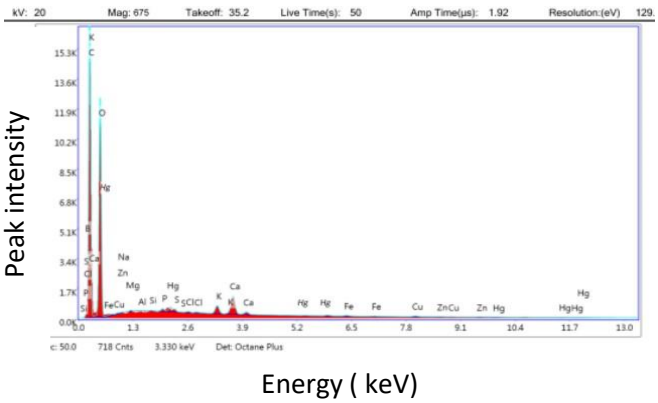
B-Stem





C-Leaf





DRY WEIGHT PERCENTAGE

Dry weight percentage of plant parts of *Coleus amboinicus* varied significantly among different treatments with metals. Dry weight of roots of Al treated plant registered a reduction during first three intervals and thereafter a steady increase was observed (Table 12). A gradual increase in dry weight of stem tissue was observed in all intervals (Fig. 28). But leaf of plants treated with Al showed a gradual but insignificant increase (Fig. 29). Roots of plants treated with Cr showed a decline in dry weight during the initial three intervals and thereafter a gradual increase was exhibited (Fig. 27). Stem of plants treated with Cr showed only negligible increase during growth (Fig. 28). Gradual increase in dry weight was shown by the leaves of plants treated with chromium (Fig. 29). Compared to the control, dry weight of leaves of plants treated with chromium was significantly reduced during all intervals. Dry matter of roots of plants treated with copper resulted in decline up to 12^{th} day and thereafter a gradual increase was observed.

Stem tissues of plants treated with copper resulted in a gradual increase of dry matter (Table 12). The dry matter of leaves of plants treated with copper remained almost unaltered up to 12^{th} day followed by gradual and significant increase in dry weight. Dry matter of roots of plants treated with mercury resulted in a gradual and significant increase of dry weight throughout the growth period. In case of stem tissues, the dry matter gradually increased but not significant. Compared to the control, dry weight of stem of plants treated with mercury was significantly reduced during all intervals. Dry weight of leaves of plants treated with mercury resulted in a negligible increase up to 16^{th} day and thereafter a gradual but significant increase was observed (Fig. 29)

Table 12 Effect of Aluminium, Chromium, Copper and Mercury on dry weight percentage in *Coleus amboinicus*

Treatments	Tissues	INTERVAL – DAYS					
		0	4	8	12	16	20
Control	Root	11.9±0.92	13.3±0.15	16.4±0.13	19.9±0.11	20.8±0.13	24.1±0.11
	Stem	4.1±0.14	5.3±0.45	7.9±0.99	13.1±0.22	14.9±0.12	16.1±0.12
	Leaf	3.9±0.23	5.1±0.88	6.2±0.92	9.4±0.98	11.8±0.09	14.3±0.08
Aluminium (500µM)	Root	11.3±0.12	8.9±0.98	10.4±0.08	10.3±0.14	13.4±0.06	15.6±0.11
	Stem	4.4±0.69	6.4±0.74	7.3±0.21	8.9±0.98	9.4±0.96	10.3±0.16
	Leaf	3.2±0.34	4.9±0.89	5.6±0.24	6.1±0.72	8.3±0.81	8.9±0.92
Chromium (150µM)	Root	11±0.96	9.9±0.72	8.1±0.92	8.9±0.44	10.5±0.23	10.9±0.22
	Stem	4.3±0.84	7.1±0.24	6.3±0.88	5.8±0.68	6.9±0.82	7.5±0.98
	Leaf	3.2±0.93	4.2±0.62	4.9±0.15	6.3±0.72	7±0.93	8.9±0.99
Copper (80µM)	Root	11.6±0.18	10.5±0.22	10.8±0.19	13.6±0.21	14.9±0.16	17.6±0.27
	Stem	4.9±0.98	7.4±0.74	8.3±0.73	9.4±0.33	10.3±0.38	10.9±0.21
	Leaf	3.3±0.42	5.9±0.91	6.1±0.98	8.9±0.51	9.3±0.03	10.3±0.43
Mercury (10µM)	Root	11.8±0.14	14.5±0.08	15.9±0.12	16.3±0.42	17.8±0.30	18.9±1.36
	Stem	4.3±0.64	6.4±0.91	7.3±0.88	8.9±0.93	9.9±0.95	10.3±0.48
	Leaf	3.1±0.24	5.4±0.87	8.3±0.91	10.2±0.14	15.6±0.32	19.3±1.18

Values given are mean of 5 replicates ±S.E

Fig. 27 Effect of Aluminium, Chromium, Copper and Mercury on dry weight percentage of root in *Coleus amboinicus*

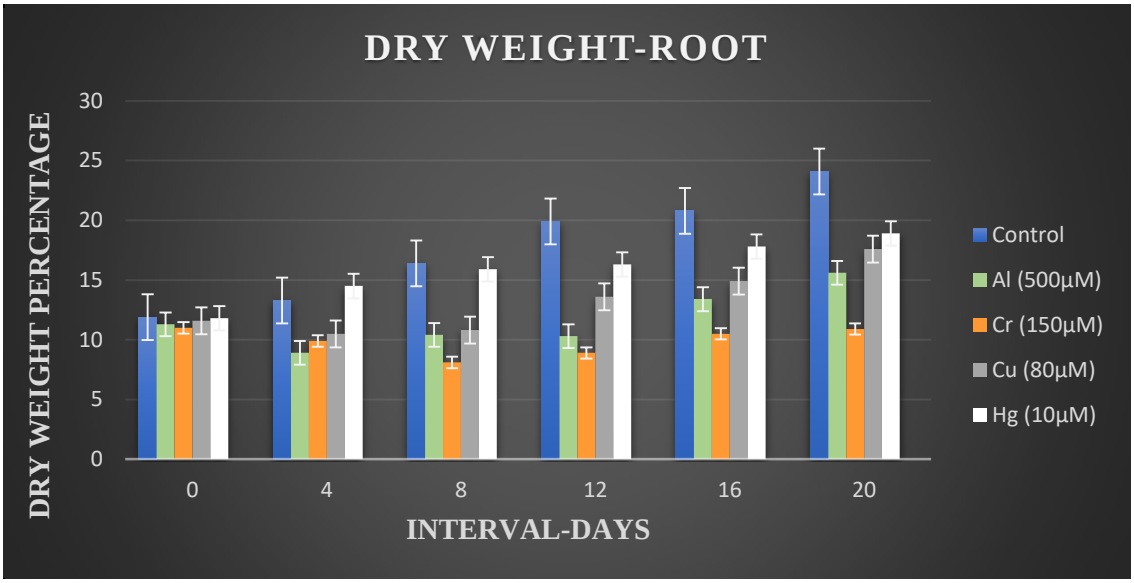


Fig. 28 Effect of Aluminium, Chromium, Copper and Mercury on dry weight percentage of stem in *Coleus amboinicus*

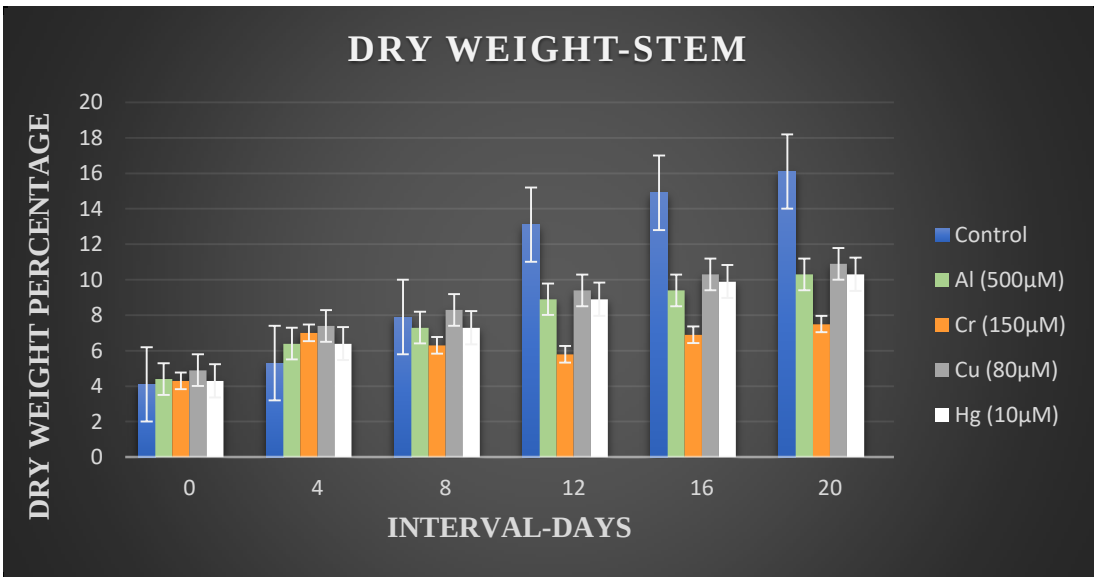
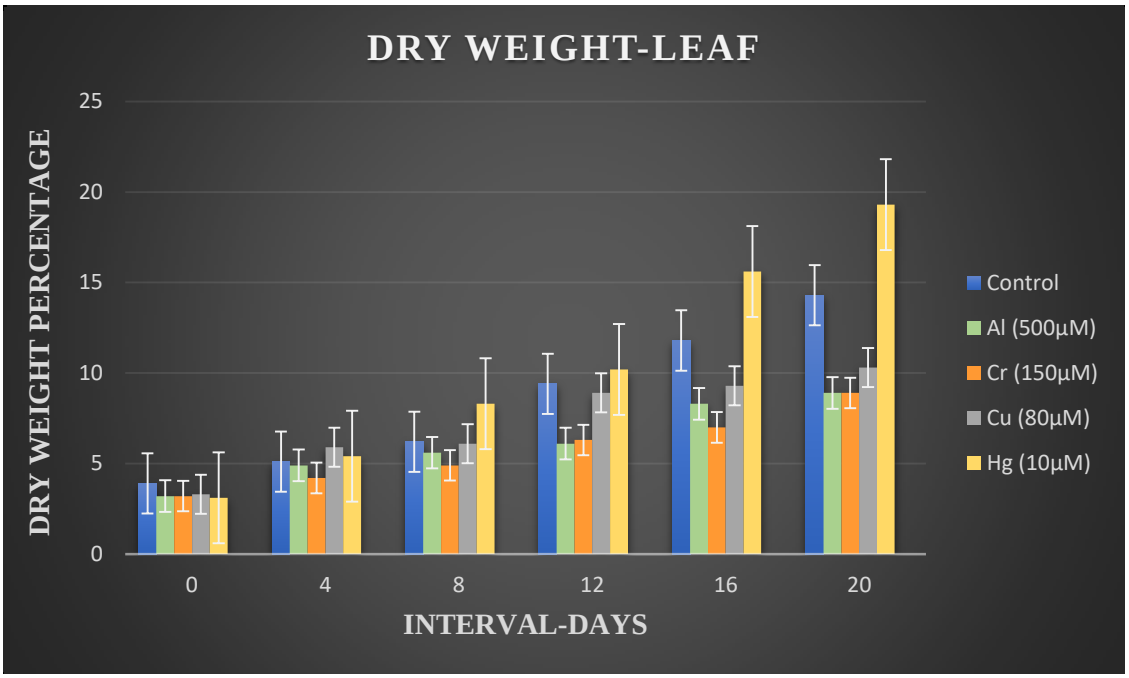


Fig. 29 Effect of Aluminium, Chromium, Copper and Mercury on dry weight percentage of leaf in *Coleus amboinicus*



PIGMENT DISTRIBUTION

Chlorophyll a, chlorophyll b, total chlorophyll and carotenoid content of control leaves of *C. amboinicus* registered significant and linear increase during all stages of growth (Table 13, Figs. 30-33).

Leaves of plants treated with Al resulted in gradual increase of chl a content throughout the growth period. Chl b also was increased in comparison with the control in the earlier stages up-to 12^{th} stage and decreased insignificantly during final stages of growth (Fig. 30). Carotenoid content of plants treated with Al was reduced in initial stages of growth and after 8^{th} day, insignificant gradual increase was seen (Fig. 33).

Chlorophyll a, chlorophyll b as well as total chlorophyll of leaves of plants treated with chromium was increased in all stages of growth up-to 16^{th} day followed by a gradual reduction compared to the control. Carotenoid content also was reduced in first stage and significantly increased during all other stages of growth.

Copper treatment resulted in increased chlorophyll a, chlorophyll b and total chlorophyll content during stages of growth up-to 12^{th} day compared to the control and other treatments thereafter a gradual reduction occurred. Carotenoid content was least in final stages of copper treated plant compared to other treatment and control.

Plants treated with mercury showed an increase in chlorophyll a content up to 16^{th} day of treatment followed by an insignificant reduction till 20^{th} day of treatment. Chlorophyll b, showed an gradual increase up-to 16^{th} day followed by gradual reduction, was maintained the same trend in total chlorophyll also throughout the interval. Plants treated with mercury showed only insignificant changes in carotenoid content compared to the control and in 12^{th} day and in 16^{th} day, the result showed a small increase

Fig. 30 Effect of Aluminium, Chromium, Copper and Mercury on *Chl a* of leaf in *Coleus amboinicus*

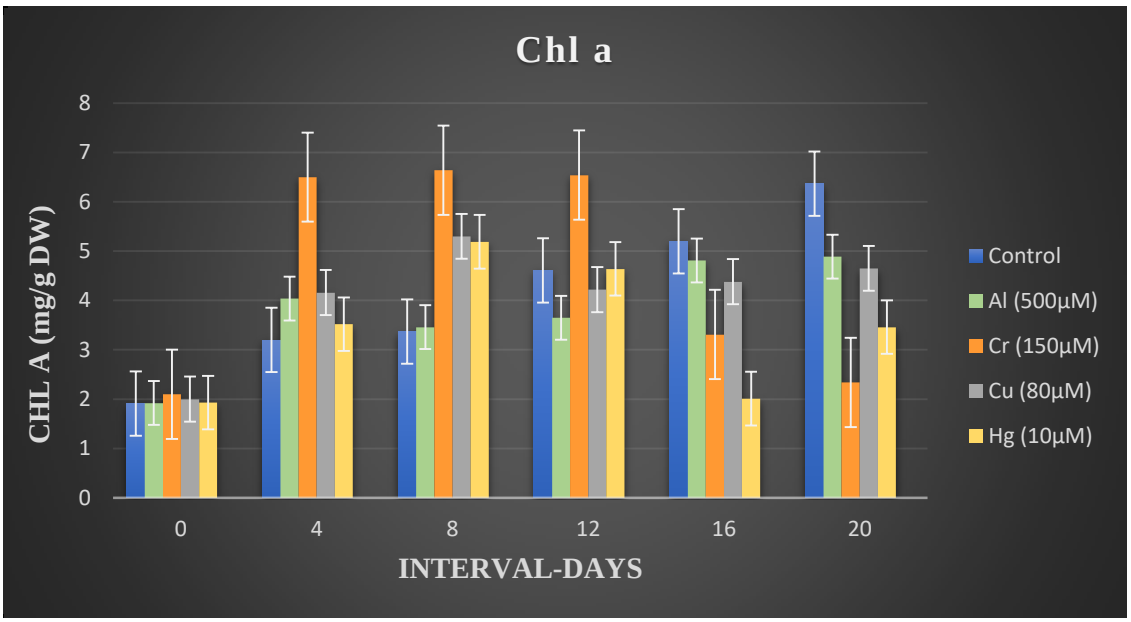


Fig. 31 Effect of Aluminium, Chromium, Copper and Mercury on Chl b of leaf in *Coleus amboinicus*

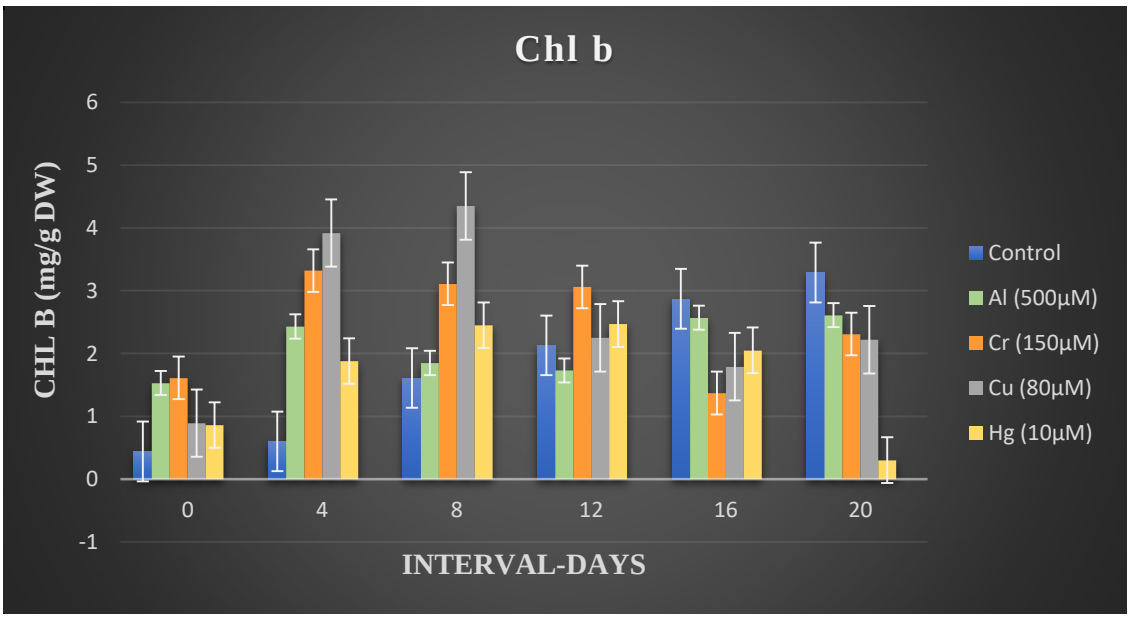


Fig. 32 Effect of Aluminium, Chromium, Copper and Mercury on Total Chlorophyll of leaf in *Coleus amboinicus*

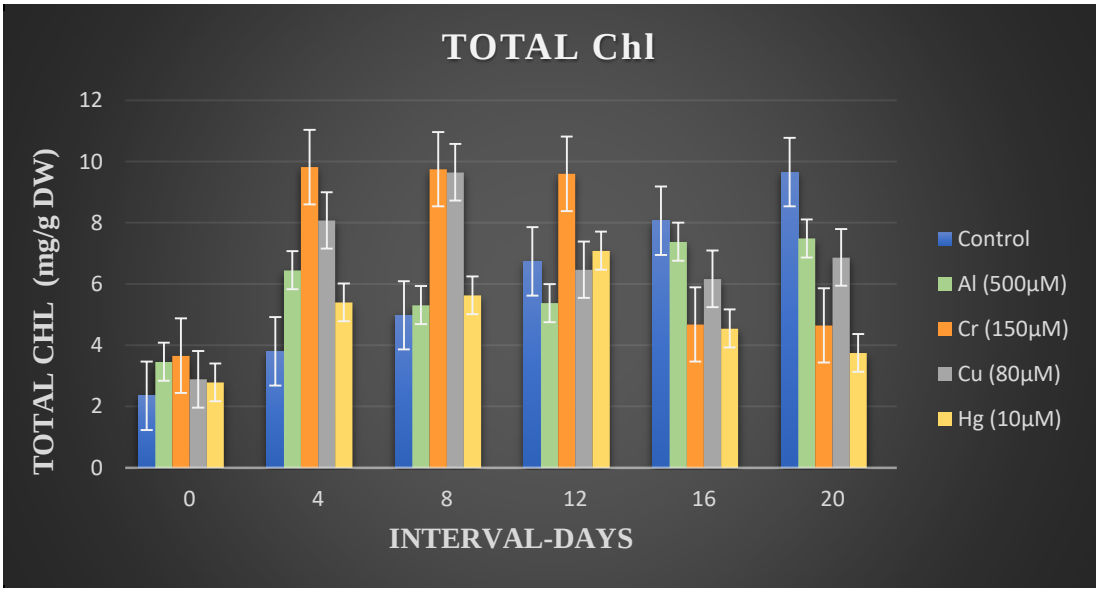


Fig. 33 Effect of Aluminium, Chromium, Copper and Mercury on Carotenoids of leaf in *Coleus amboinicus*

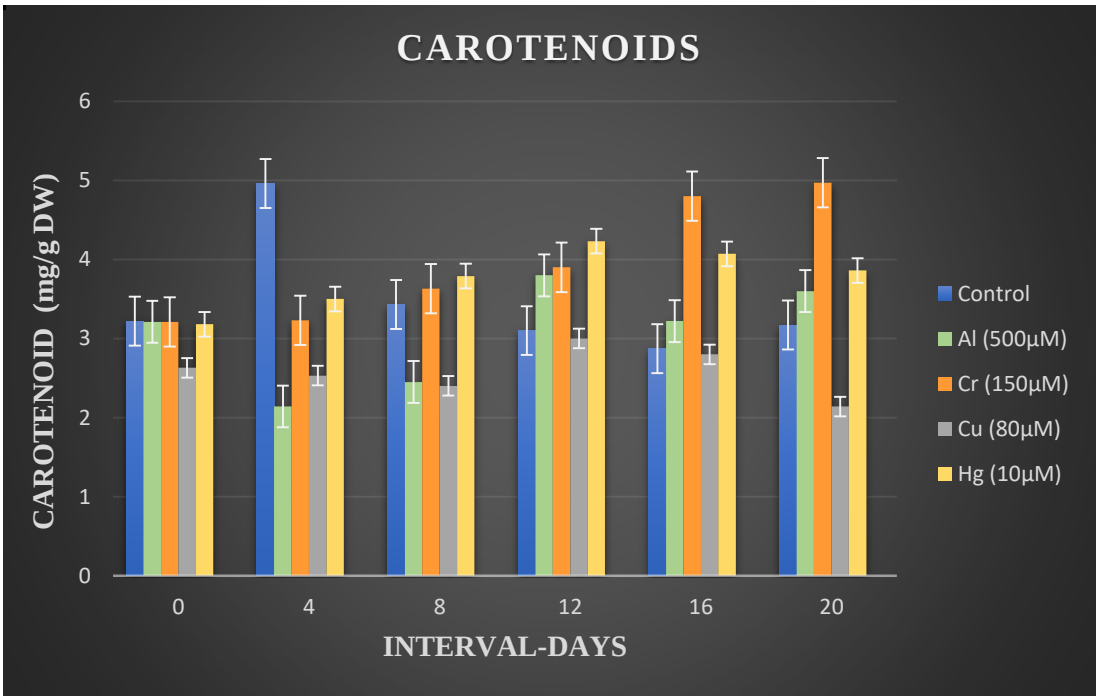


Table 13 Effect of Aluminium, Chromium, Copper And Mercury On Pigment Distribution Of Leaf In *Coleus amboinicus*

Chlorophyll content mg/g DW

Treatments		Intervals- Days					
		0	**4**	**8**	**12**	**16**	**20**
Control	Chl a	1.91± 0.12	3.20±0.019	3.37±0.06	4.61±0.04	5.20±0.02	6.37±0.03
	Chl b	0.44±0.011	0.60±0.003	1.61±0.012	2.13±0.002	2.87±0.012	3.29±0.012
	Chl a/b	4.34±0.013	5.33±0.023	2.09±0.005	2.16±0.003	1.81±0.013	1.93±0.023
	Total Chl	2.35±0.021	3.8±0.012	4.98±0.03	6.74±0.021	8.07±0.004	9.66±0.09
	Carotenoid	3.22±0.003	4.96±0.002	3.43±0.002	3.10±0.013	2.87±0.012	3.17±0.023
Aluminium (500µM)	Chl a	1.92±0.023	4.04±0.028	3.46±0.004	3.65±0.016	4.81±0.021	4.89±0.012
	Chl b	1.53±0.013	2.43±0.022	1.85±0.012	1.73±0.007	2.57±0.023	2.61±0.08
	Chl a/b	1.25±0.003	1.66±0.015	1.87±0.012	2.1±0.015	1.87±0.025	1.8±0.013
	Total Chl	3.46±0.001	6.45±0.014	5.31±0.009	5.38±0.004	7.38±0.09	7.49±0.09
	Carotenoid	3.21±0.002	2.14±0.018	2.45±0.004	3.80±0.003	3.22±0.01	3.60±0.012
Chromium (150µM)	Chl a	2.1±0.012	6.5±0.003	6.64±0.16	6.54±0.05	3.31±0.023	2.34±0.022
	Chl b	1.61±0.022	3.32±0.012	3.11±0.006	3.06±0.002	1.37±0.025	2.31±0.03
	Chl a/b	1.3±0.03	1.95±0.05	2.13±0.016	2.14±0.04	2.41±0.008	1.01±0.08
	Total Chl	3.66±0.012	9.82±0.004	9.75±0.005	9.6±0.012	4.68±0.016	4.65±0.06
	Carotenoid	3.21±0.024	3.23±0.021	3.63±0.017	3.90±0.023	4.80±0.012	4.97±0.013
Copper (80µM)	Chl a	2±0.012	4.16±0.002	5.30±0.007	4.22±0.004	4.38±0.013	4.65±0.012
	Chl b	0.89±0.022	3.92±0.001	4.35±0.003	2.25±0.03	1.79±0.008	2.22±0.07
	Chl a/b	2.24±0.03	1.06±0.014	1.21±0.05	1.87±0.08	2.44±0.013	2.09±0.09
	Total Chl	2.89±0.003	8.08±0.23	9.65±0.15	6.47±0.17	6.17±0.001	6.87±0.004
	Carotenoid	2.63±0.022	2.53±0.032	2.4±0.013	3±0.013	2.80±0.012	2.14±0.021
Mercury (10µM)	Chl a	1.93±0.017	3.53±0.017	5.19±0.005	4.63±0.16	2.01±0.023	3.46±0.025
	Chl b	0.86±0.014	1.88±0.09	2.45±0.04	2.47±0.06	2.05±0.021	0.30±0.004
	Chl a/b	2.24±0.09	1.87±0.026	2.11±0.03	1.82±0.09	0.98±0.03	11.5±0.03
	Total Chl	2.79±0.015	5.40±0.021	5.63±0.015	7.09±0.001	4.55±0.022	3.75±0.02
	Carotenoid	3.18±0.017	3.50±0.001	3.79±0.021	4.23±0.015	4.07±0.009	3.86±0.12

Values given are mean of 5 replicates ±S.E

TOTAL SOLUBLE SUGAR

Soluble sugar content of tissues of *C. amboinicus* root, stem and leafshowed significant variation among treatments with aluminium, chromium, copper and mercury (Table 14, Figs. 34-36).

Total soluble sugars of roots of plants treated with Al were increased continuously during all intervals (Fig. 34). Total soluble sugars of the stem showed gradual and insignificant increase throughout the growth period. The sugar content in stem was comparatively higher than that of the root. Leaves of plants treated with Al showed continuous increase of sugar content in the entire growth period and the increase from stage to stage was significant ($P<0.01$). When compared to the control, the increase was negligible.

Treatment with chromium resulted in gradual and negligible increase of sugar content in root tissues of *C. amboinicus*. when compared to the control, the increase was insignificant. Total sugar content of stem tissue exhibited unaltered values up to 8^{th} day of growth thereafter sugar content was increased compared to the control (Fig. 35). Total soluble sugar content in leaf tissues showed continuous increase during growth and compared to the control, the increase was significant during final stages.

Sugar content in root tissues of *C. amboinicus* due to copper treatment remained unchanged during all stages of growth. During the growth period, stem tissues exhibited a gradual increase of sugar content. When compared to the control, the changes were insignificant. But the leaf tissues showed significant increase of sugar content ($P < 0.01$) in all stages of growth. When compared to the control the increase was gradual and insignificant. Root tissues of plants treated with Hg showed gradual increase during the growth and changes were insignificant compared to the control. Gradual increase was observed during growth in stem tissues of mercury treated plants (Fig. 35). When compared to the control the increase was significant. After mercury treatment the leaf tissues exhibited increased sugar content and the increase was gradual and significant ($P <0.01$).

Table 14 Effect of Aluminium, Chromium, Copper and Mercury on total soluble Sugar content of root, stem and leaf in *Coleus amboinicus*

Total soluble Sugar content mg/g DW

Treatments	Tissues	Interval days					
		0	4	8	12	16	20
Control	Root	6.04±0.81	7.9±0.95	8.5±0.85	9.1±0.97	9.6±0.95	10.1±0.03
	Stem	10.42±0.09	10.79±0.21	11.1±0.12	11.63±0.03	11.92±0.14	12.2±0.02
	Leaf	16.03±0.03	21.62±1.13	23.5±1.07	28.81±1.12	34.47±1.07	36.31±1.08
Aluminium (500µM)	Root	6.1±0.96	7.2±0.99	8.9±0.86	9.7±0.97	10.5±0.04	12.3±0.05
	Stem	10.7±0.91	11.5±0.23	12.3±0.17	13.9±0.23	14.5±0.13	15.6±0.09
	Leaf	16.5±1.14	24.2±1.12	26.9±1.07	30.7±1.18	36.9±1.18	39.31±1.12
Chromium (150µM)	Root	6.01±0.88	7.9±0.98	9.2±0.03	10.1±0.02	11.8±0.04	13.7±0.03
	Stem	10.38±0.21	12.3±0.18	12.9±0.16	14.3±0.04	15.9±0.12	17.4±0.05
	Leaf	16.7±1.12	27.3±1.23	29.45±1.02	33.2±1.12	38.5±1.02	41.3±1.08
Copper (80µM)	Root	6.2±0.91	7.5±0.95	8.4±0.13	9.9±0.01	10.9±0.06	12.1±0.12
	Stem	10.52±0.17	11.3±0.09	12±0.09	13.6±0.02	14.7±0.16	15.9±0.14
	Leaf	16.28±1.03	25.1±1.18	27.3 ±1.23	30.9±1.01	36±1.02	39.9±1.13
Mercury (10µM)	Root	6.4± 0.71	8.2±0.98	9.9±0.94	11.1±0.12	12.9±0.09	14.3±0.17
	Stem	10.43±0.21	13.9±0.12	14.6±0.26	16.3±0.16	18.2±0.17	19.9±0.06
	Leaf	16.39±1.06	28.24±1.15	31.23±1.06	35.68±1.21	40.62±2.23	47.93±1.24

Values given are mean of 5 replicates ±S.E

Fig. 34 Effect of Aluminium, Chromium, Copper and Mercury on total Sugar content in root of *Coleus amboinicus*

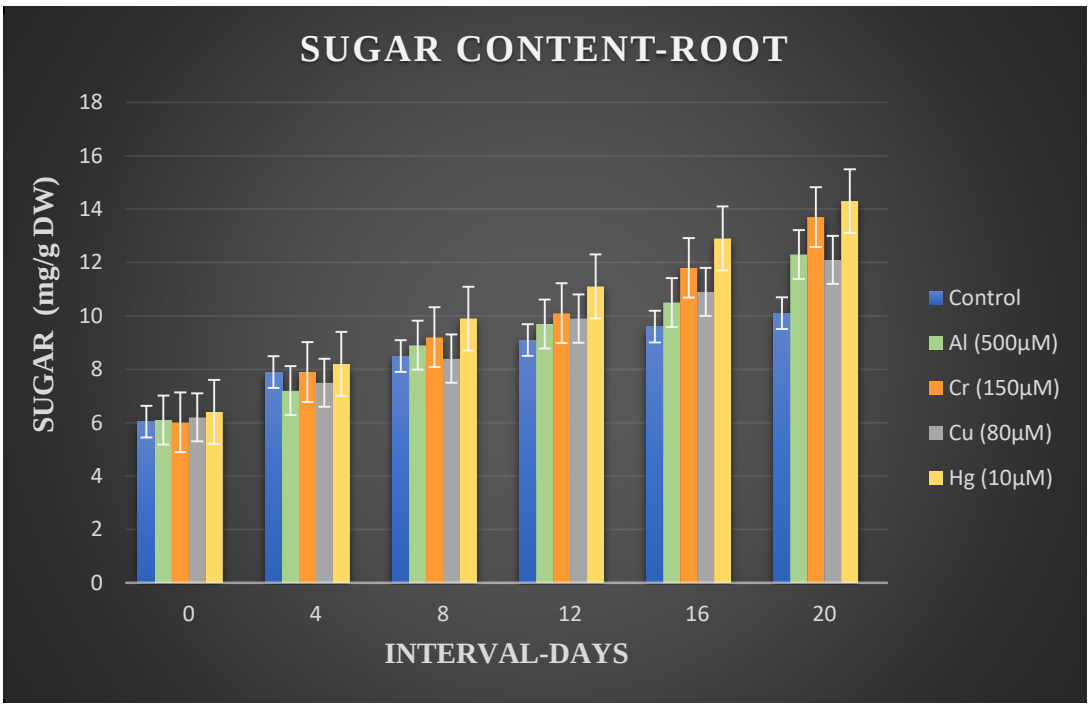


Fig. 35 Effect of Aluminium, Chromium, Copper and Mercury on total Sugar content in stem of *Coleus amboinicus*

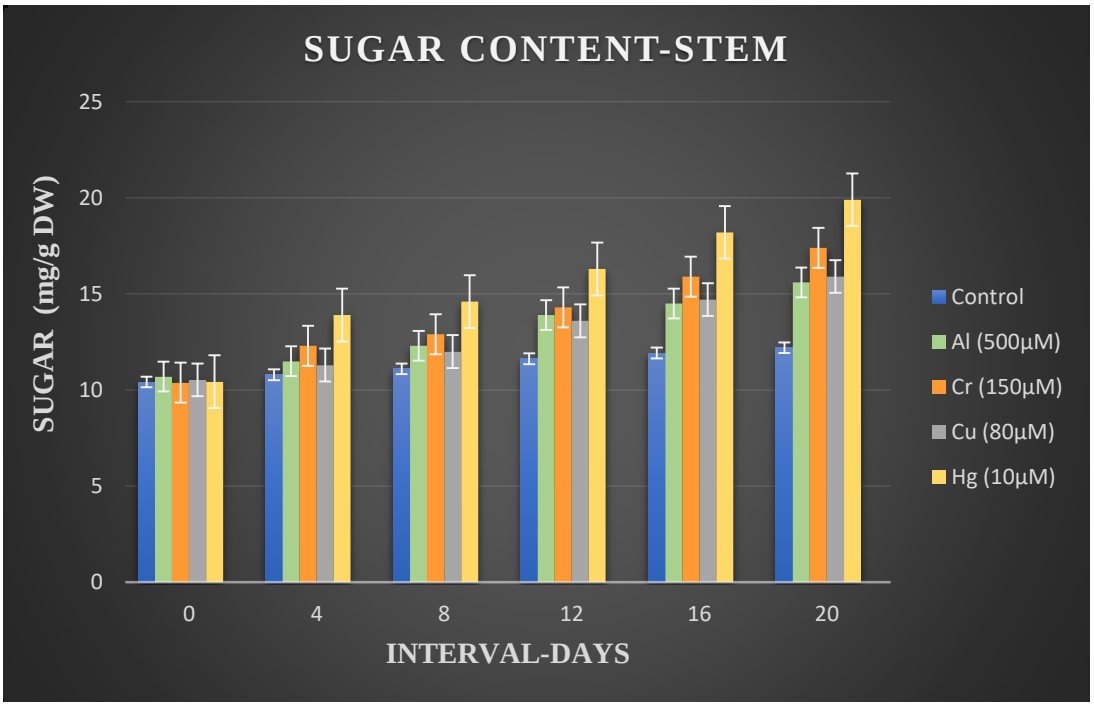
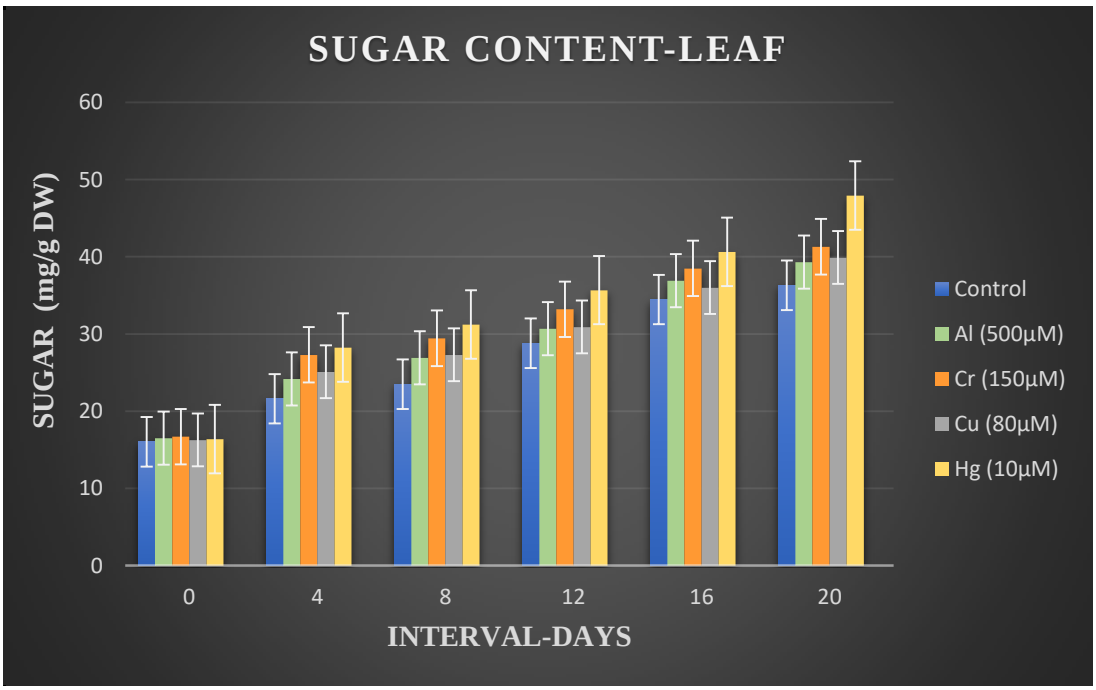


Fig. 36 Effect of Aluminium, Chromium, Copper and Mercury on total Sugar content in leaf of *Coleus amboinicus*



STARCH CONTENT

Starch content of tissues of *C. amboinicus* showed significant variation among treatments with aluminium, chromium, copper and mercury (Table 15, Figs. 37-39).

During all stages of growth, starch content of root, stem and leaf of plants treated with Al was increased compared to the control continuously during all intervals (Table 15). Comparatively low starch content was present in the roots than stem and leaf and during growth, only marginal increase was observed. Only gradual increase was observed in the distribution of starch content in stem, and the increase was almost insignificant in between the stages. In the leaf tissues only marginal increase of starch content was observed (Fig. 39).

Treatment with chromium exhibited increased starch content of all parts of the plant during all stages of growth compared to control. The root tissues showed a gradual and steady increase of starch content due to chromium treatment (Fig. 37).

Stem tissues of plants treated with chromium also showed a gradual increase and in between the intervals the increase of starch content was significant. Starch content in leaf tissues showed maximum increase of content in chromium treated plants and then gradualdecline happened in final stages of growth (Fig. 39).

Copper treatment resulted in marginal increase of starch content in the initial stages and then the content remained unchanged. The starch content in stem tissue was higher than root and there occurred only a marginal increase during growth. But the leaf tissues showed significant increase ($P< 0.01$) of starch content in all stages of growth except 20^{th} day when compared to the control.

Treatment with Hg showed maximum increase of starch content in all tissues compared to the control and other treatments (Table:15). Starch content of root tissues showed a marginal increase throughout the growth period. Stem of plants treated with mercury resulted only a marginal increase of starch content during growth (Fig. 38). An exorbitant increase was observed in the leaf tissues of plants treated with mercury up to 20^{th} day followed by a significant decline.

Table 15 Effect of Aluminium, Chromium, Copper and Mercury on total starch content of root, stem and leaf in *Coleus amboinicus*

Total starch content mg/g DW

Treatments	Tissues	0	4	8	12	16	20
Control	Root	2.2±0.11	4.8±0.05	5.16±0.55	7.9±0.07	8.12±0.05	9.34±0.03
	Stem	11.8±0.09	13.41±0.21	15.84±0.12	16.94±0.93	17.83±0.84	19.45±0.92
	Leaf	29.5±1.03	34.71±1.13	39.5±1.07	42.23±1.12	46.83±1.07	50.92±1.08
Aluminium (500µM)	Root	2.4±0.16	5.3±0.19	6.8±0.16	8.3±0.07	8.82±0.04	9.92±0.05
	Stem	11.5±0.11	14.9±0.23	16.74±0.17	17.45±0.23	18.4±0.13	20.85±0.09
	Leaf	30±0.14	36.2±0.12	40.9±1.07	44.2±0.98	48.38±1.18	56.82±1.12
Chromium (150µM)	Root	2.1±0.48	5.9±0.18	7.12±0.03	8.3±0.02	8.9±0.04	10.1±0.03
	Stem	11.3±0.21	15.2±0.18	17.34±0.16	18.89±0.04	19.65±0.12	21.2±0.05
	Leaf	28.92±1.12	49.5±2.23	72.89±2.02	84.34±3.12	57.87±3.02	52.92±1.08
Copper (80µM)	Root	2.4±0.11	5.1±0.15	5.76±0.13	6.87±0.01	8.93±0.06	9.87±0.12
	Stem	11.5±0.17	14±0.09	16.23±0.09	17.21±0.02	17.98±0.16	18.54±0.14
	Leaf	27.76±0.03	36.8±1.18	49.98±2.23	51.56±2.01	54.74±2.02	48.54±2.13
Mercury (10µM)	Root	2.5± 0.11	5.8±0.08	6.72±0.14	8.23±0.12	8.94±0.09	9.84±0.17
	Stem	11.2±0.21	15.7±0.12	16.78±1.26	18.93±1.16	19.94±1.17	21.95±1.06
	Leaf	29.1±0.06	58.8±2.15	67.98±2.06	74.92±2.21	79.26±2.23	53.14±2.24

Values given are mean of 5 replicates ±S.E

Fig. 37 Effect of Aluminium, Chromium, Copper and Mercury on total starch content in root of *Coleus amboinicus*

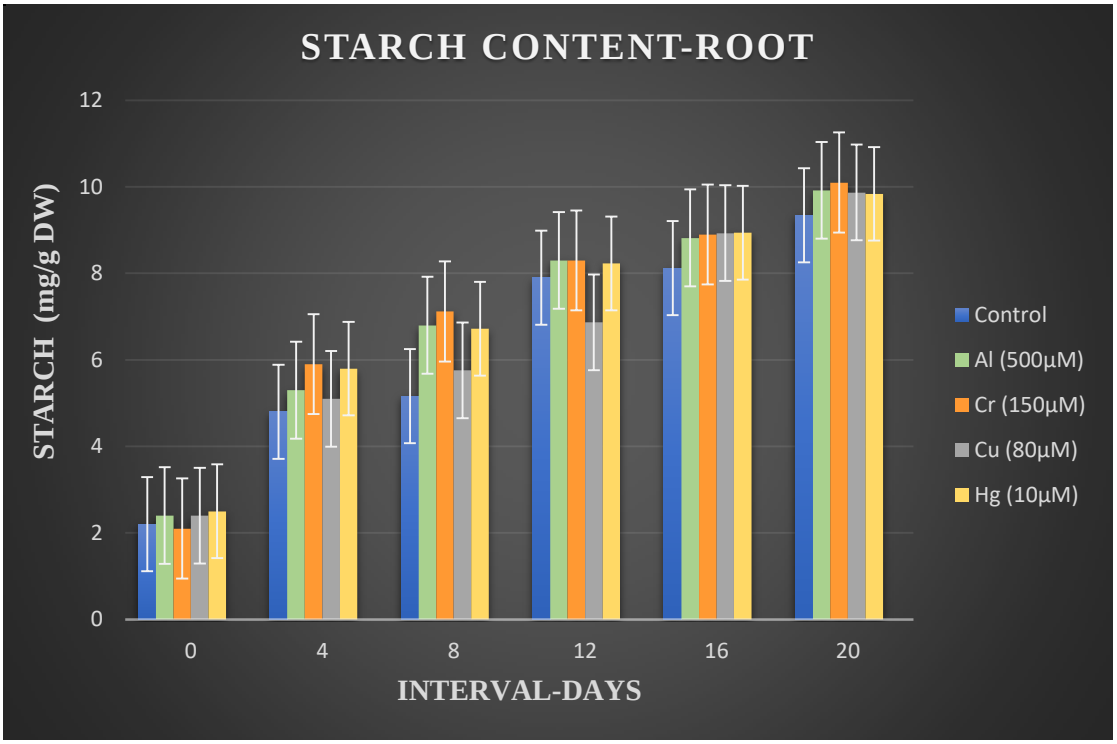


Fig. 38 Effect of Aluminium, Chromium, Copper and Mercury on total starch content in stem of *Coleus amboinicus*

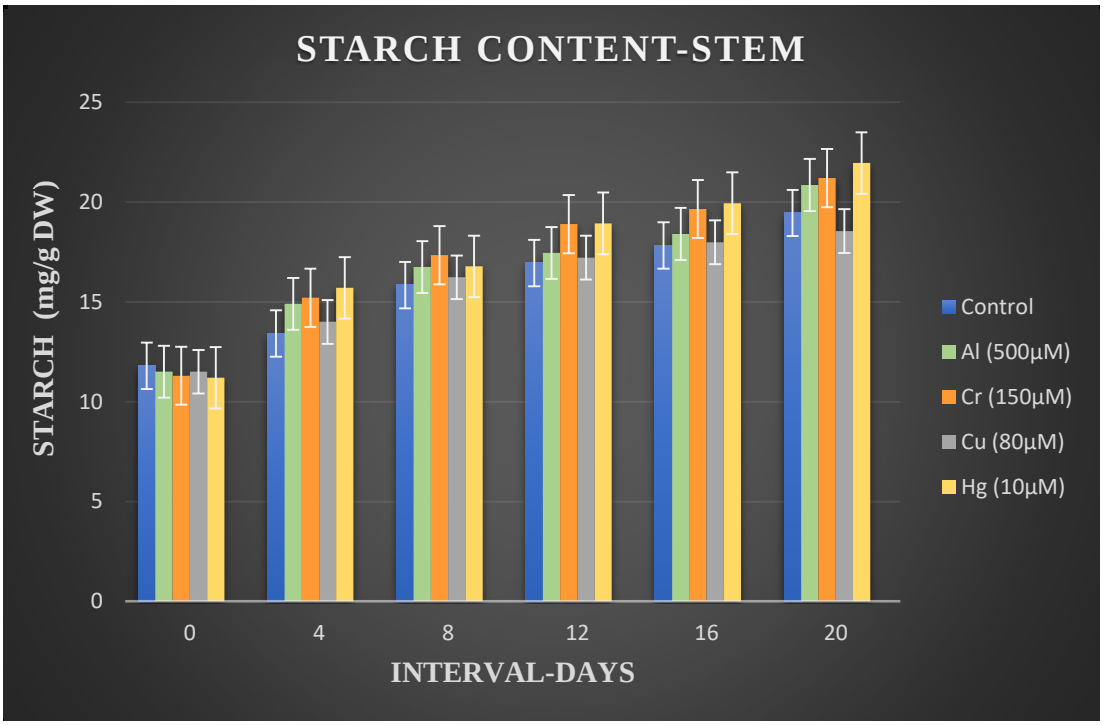
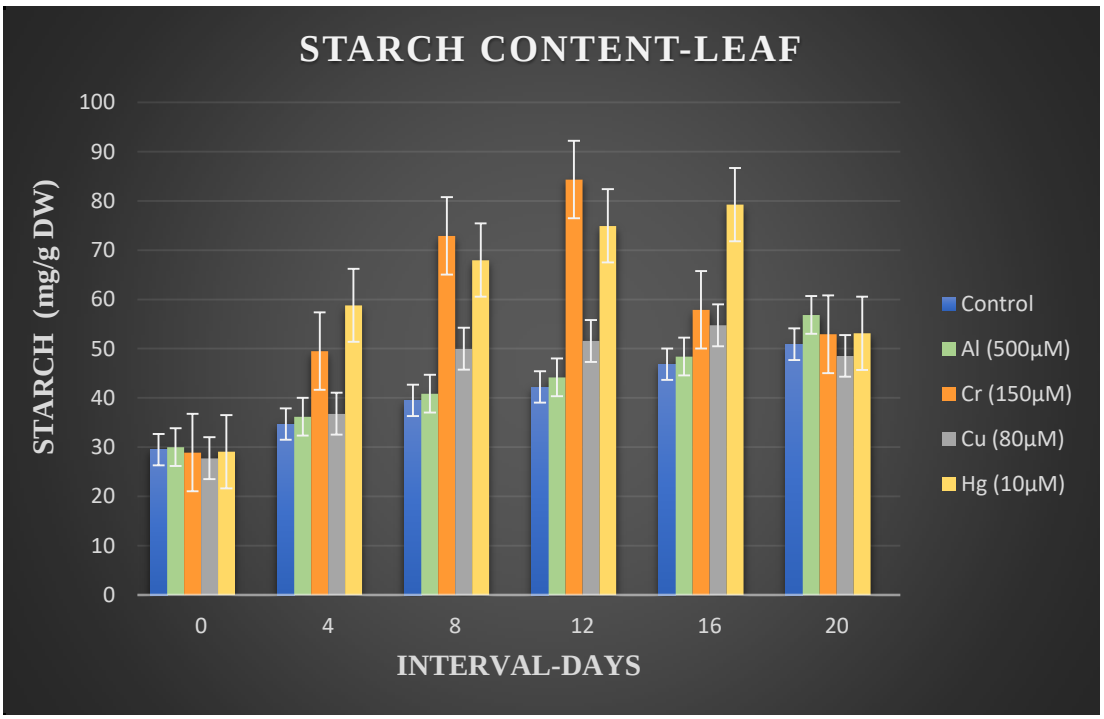


Fig. 39 Effect of Aluminium, Chromium, Copper and Mercury on total starch content in leaf of *Coleus amboinicus*



Total Free Amino Acids

Free amino acid content of tissues of *C. amboinicus* showed significant variation among treatments with aluminium, chromium, copper and mercury (Table:16, Figs. 40-42).

Free amino acid content roots of plants treated with Al showed a marginal decrease compared to the control during all stages of growth. Content in stem also exhibited a negligible decrease throughout the growth period and when compared to the control, stem showed more free amino acids. Free amino acid content of leaf tissues of Al treated plants exhibited decrease of amino-acids during the entire growth period.

Free amino-acid content of roots of plants treated with Cr exhibited gradual decrease up to 16th day and thereafter the decrease was significant. Stem tissues showed continuous and significant decrease throughout growth period and in case of leaf, continuous decrease of aminoacid content was observed up to 12th day followed

by highly significant decrease during the final stages.

Free amino acid content of roots of plants treated with Cu decreased throughout the period. During all stages of growth stem tissues exhibited gradual decrease of amino acid content and when compared to the control, the decrease was highly significant. But the leaf tissues almost decreased but not significantly when compared to the control.

Treatment with Hg, root showed gradual decrease of free amino acids up to 12^{th} day followed by significant decrease. Free amino acid content of stem tissues of Hg treated plants exhibited significant decrease in all stages of growth. In leaf tissues gradual and significant decrease ($P< 0.01$) was observed.

Table 16 Effect of Aluminium, Chromium, Copper and Mercury on free amino acid content of root, stem and leaf in *Coleus amboinicus*

Free amino acid content mg/g DW

Treatments	**Tissues**	**Interval days**					
		0	**4**	**8**	**12**	**16**	**20**
CONTROL	Root	48±1.04	52±2.05	57±2.05	60±2.07	69±3.05	77±3.01
	Stem	70±2.03	80±1.21	95±1.12	110±2.03	130±1.14	170±3.02
	Leaf	110±3.05	250±2.13	340±4.07	440±4.12	550±3.07	640±4.08
ALUMINIUM (500µM)	Root	47±2.06	51±2.19	56±3.16	60±3.07	62±4.01	75±5.05
	Stem	69±2.03	78±3.23	86±4.17	101±3.23	116±3.01	158±4.09
	Leaf	114±2.01	220±4.12	320±3.07	410±3.18	470±2.18	580±4.12
CHROMIUM (150µM)	Root	48±2.08	50±2.08	54±2.03	59±2.02	60±1.04	62±2.03
	Stem	68±1.04	76±2.18	82±3.16	94±2.04	115±3.12	126±3.05
	Leaf	112±2.11	205±3.23	270±3.02	390±3.12	460±3.02	580±4.08
COPPER (80µM)	Root	49±1.14	52±1.15	56±1.13	59±1.01	63±1.01	66±1.12
	Stem	69±1.03	74±2.09	84±2.09	97±7.02	110±1.16	150±2.14
	Leaf	110±2.02	215±2.18	280 ±2.23	370±2.01	460±2.02	580±2.13
MERCURY (10µM)	Root	47± 1.01	49±1.08	51±1.14	53±2.12	55±1.09	57±1.17
	Stem	70±1.21	72±1.12	78±1.26	85±2.16	99±2.17	124±3.06
	Leaf	110±2.05	190±1.15	250±2.06	370±3.21	430±3.23	526±3.24

Values given are mean of 5 replicates ±S.E

Fig. 40 Effect of Aluminium, Chromium, Copper and Mercury on total free amino acids content in root of *Coleus amboinicus*

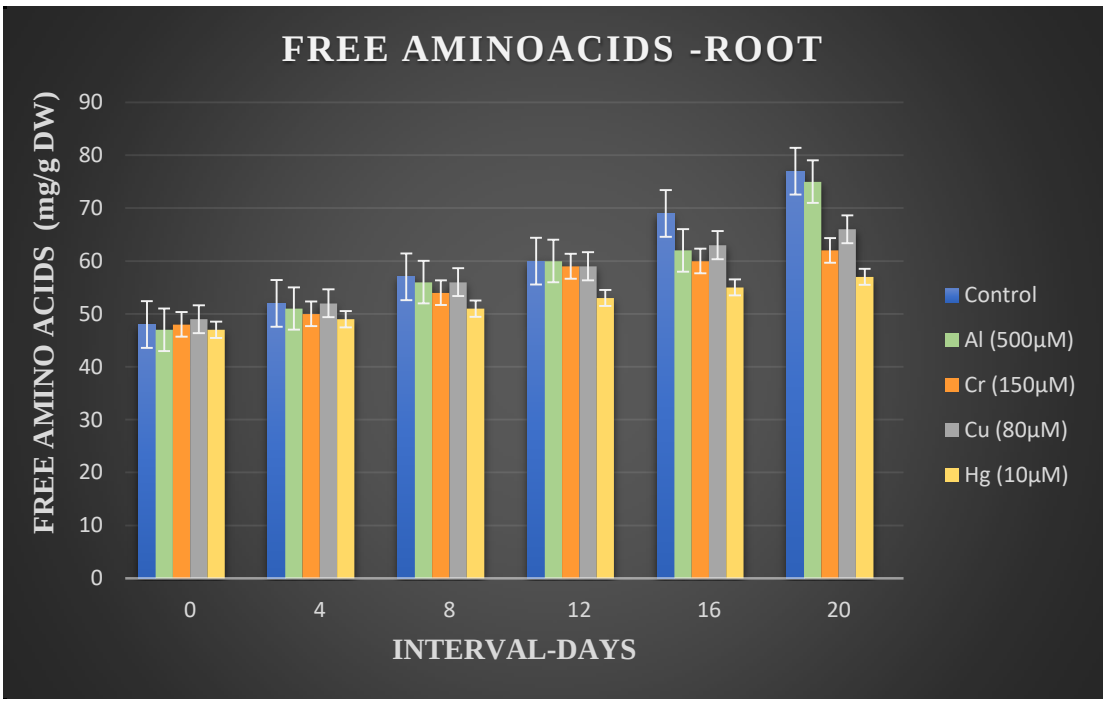


Fig. 41 Effect of Aluminium, Chromium, Copper and Mercury on total free amino acids content in Stem of *Coleus amboinicus*

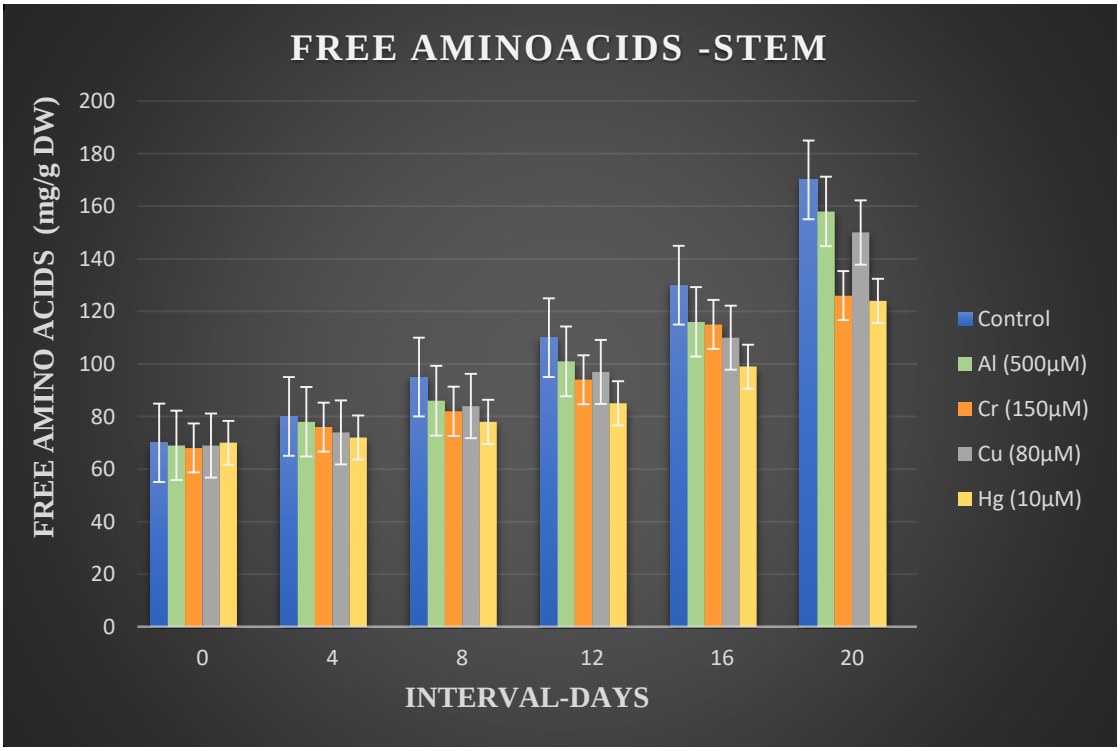
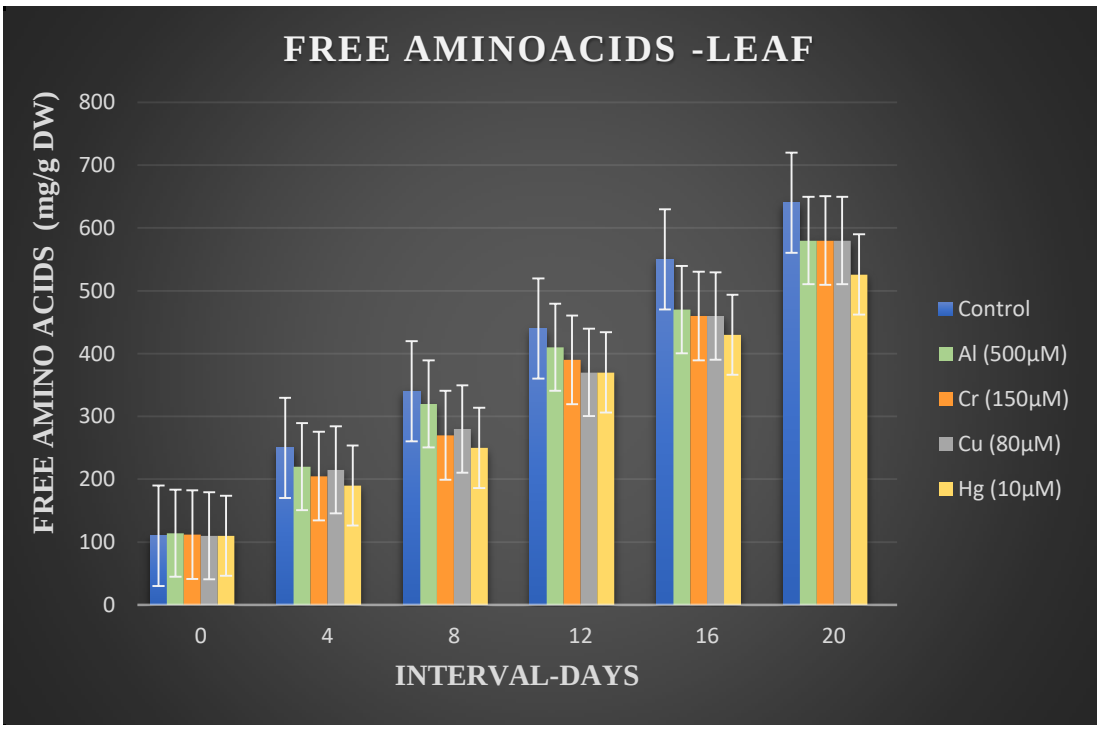


Fig. 42 Effect of Aluminium, Chromium, Copper and Mercury on total free amino acids content in leaf of *Coleus amboinicus*



TOTAL PROTEIN

Protein content of tissues of *C. amboinicus* showed significant variation among treatments with aluminium, chromium, copper and mercury (Table:17, Figs. 43-45).

Protein content of roots of plants treated with Al was significantly increased during all intervals except 20th day. Aluminium- treated plant stem showed significant increase ($P< 0.01$) up to 16th day followed by gradual reduction. In case of leaf tissues, Al treatment resulted in negligible changes during initial stages and the content was significantly high at final stages of growth compared to control.

Treatment with Cr exhibited gradual but marginal increase of protein content in the earlier stages of growth of root tissues thereafter the increase was significant ($P< 0.01$). Stem tissues of plants treated with chromium remained unchanged

throughout the growth period, but the values were lower than the control. Leaf tissues showed significant increase up to 16th day followed by a significant reduction of protein content compared to the control.

Copper treatment resulted in significant reduction of protein content throughout the growth period in root tissues. Unlike that of the control the protein content were increased in treated plants. During the initial stages of growth, stem tissues showed gradual and significant increase of protein content ($P< 0.01$) followed by a significant reduction was observed during the final stages of growth. Leaf tissues showed almost remained unchanged protein content in all stages of growth compared to the control except 4th day in which the increase was significant (Fig.45).

Roots of plant treated with mercury showed only a significant increase on 4th day followed by continuous reduction and the changes afterwards were not significant. Compared to the control, protein content of roots of mercury treated plants were negligibly reduced. Stem of plants treated with mercury showed insignificant changes of protein content in between stages also during growth. Treatment with mercury showed insignificant increase of protein content in leaf tissues compared to the control. Protein content of the leaf was very high during 4-12 days and thereafter reduced gradually compared to the control leaf.

Table 17 Effect of Aluminium, Chromium, Copper and Mercury on total protein content of root, stem and leaf in *Coleus amboinicus*
Protein mg / g DW

Treatments	Tissues	Interval- days					
		0	**4**	**8**	**12**	**16**	**20**
CONTROL	Root	14.23±0.93	18.76±0.82	23.91±1.03	27.48±1.06	29.11±0.94	34.5±0.85
	Stem	7.93±0.12	9.91±0.82	11.23±0.73	14.72±0.96	17.21±0.09	21.85±0.34
	Leaf	18.67±0.82	22.53±0.44	26.9±0.32	28.14±0.12	29.78±0.45	37.36±0.65
ALUMINIUM (500µM)	Root	16.77±0.73	28.62±0.46	31.64±0.85	39.24±0.32	41.63±0.39	39.61±0.11
	Stem	9.19±0.23	15.23±0.73	16.35±0.45	18.26±0.99	22.77±0.17	20.78±0.65
	Leaf	18.42±0.15	24.35±0.62	28.91±0.19	36.43±0.83	39.54±0.48	42.31±0.34
CHROMIUM (150µM)	Root	16.97±0.93	19.71±0.37	29.62±0.23	36.93±0.16	47.32±0.23	47.32±0.69
	Stem	8.92±0.23	13.43±0.17	29.32±0.82	31.73±0.67	36.5±0.66	29±0.17
	Leaf	17.33±0.84	33.5±0.27	43.61±0.36	53.54±0.63	43.42±0.56	39.2±0.22
COPPER (80µM)	Root	15.93±0.34	38.74±0.38	42.43±0.23	43.36±0.23	47.2±0.12	39.31±0.65
	Stem	9.75±0.56	22.42±0.81	25.68±0.57	27.56±0.85	34.93±0.88	31.36±0.34
	Leaf	16.64±0.58	30.63±0.32	37.51±0.32	36±0.64	33.28±0.36	29.53±0.62
MERCURY (10µM)	Root	15.64±0.43	38.46±0.82	43.31±0.25	46.1±0.92	50.12±0.28	48.25±0.45
	Stem	9.11±0.26	22.63±0.32	29.02±0.43	35.3±0.85	32.23±0.91	28.22±0.12
	Leaf	17.69±0.54	42.6±0.73	45.34±0.54	51.72±0.23	45.92±0.45	43.56±0.91

Values given are mean of 5 replicates ±S.E

Fig. 43 Effect of Aluminium, Chromium, Copper and Mercury on total protein content in root of *Coleus amboinicus*

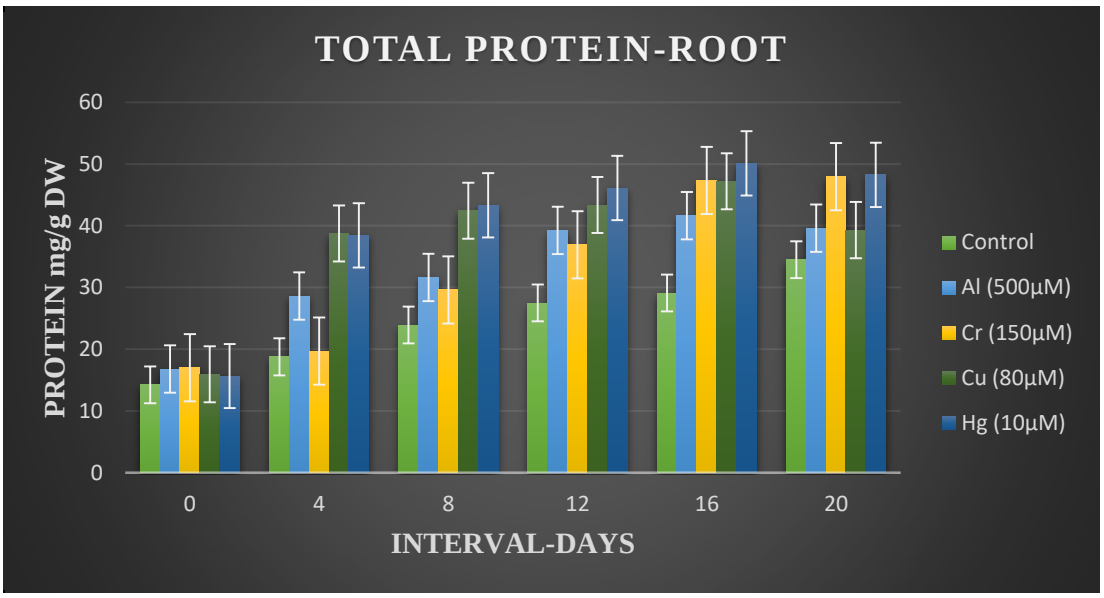


Fig. 44 Effect of Aluminium, Chromium, Copper and Mercury on total protein content in stem of *Coleus amboinicus*

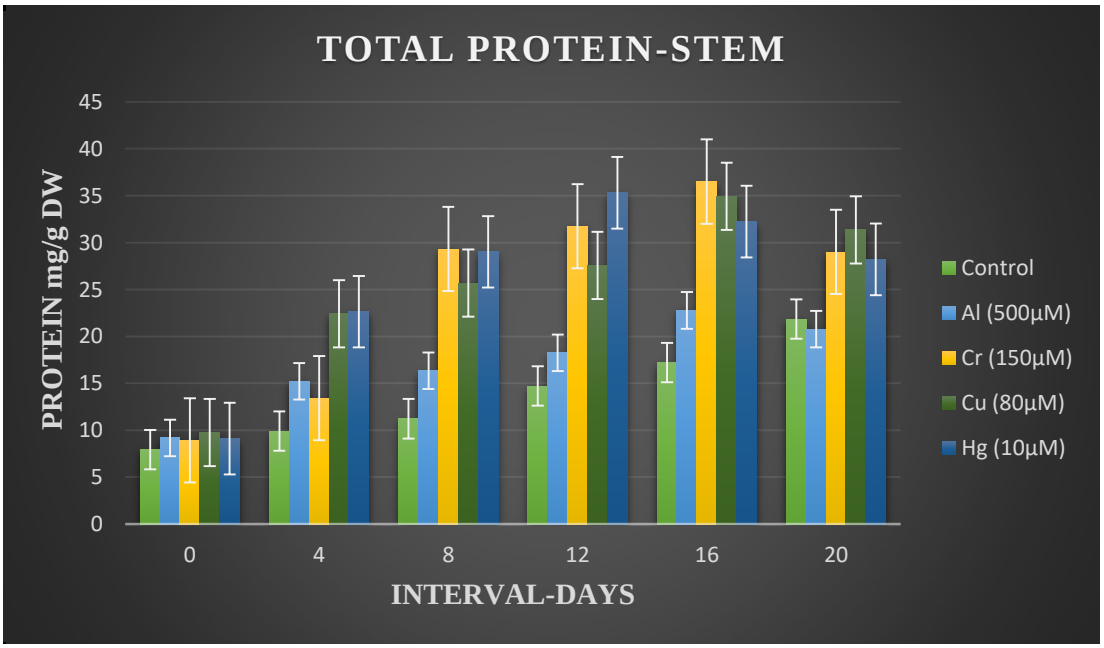
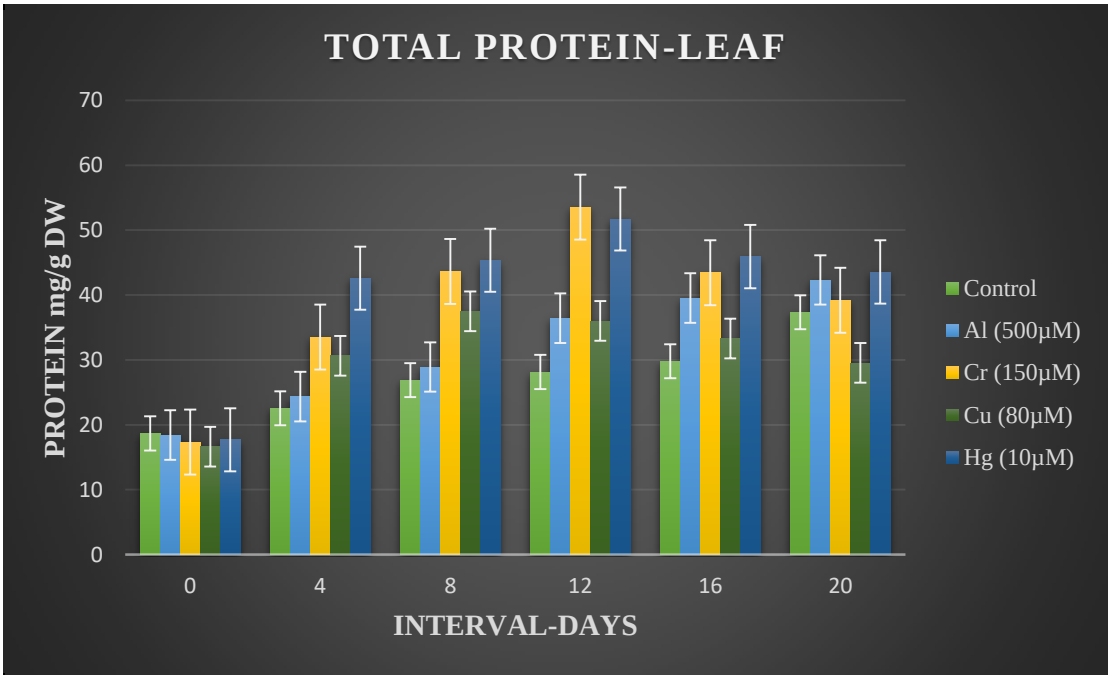


Fig. 45 Effect of Aluminium, Chromium, Copper and Mercury on total protein content in leaf of *Coleus amboinicus*



PROLINE

C. amboinicus plants subjected to treatment with aluminium resulted in a gradual and significant increase (2-3 fold) of proline in the roots compared to the control during all intervals. Proline content was significantly and continuously increased in the stem tissues of plants treated with aluminium at all intervals. Leaf of plants treated with aluminium exhibited significant increase of proline content during the entire period of growth.

Roots contained significantly increased proline in plants treated with chromium compared with control plants. During the growth there occurred a significant reduction of proline content during final stages of growth. In case of stem tissues, the proline content was comparatively high than the control and during growth insignificant fluctuations was observed. Proline content of leaves of plants treated with chromium was very high and significantly increased during initial stages of growth and declined drastically during final stages.

Copper treatment resulted in an enormous decrease of proline content and remained unchanged throughout the growth period in the roots and was comparatively lower than the control. The proline content of the stem tissues was remained unchanged during all stages of growth. Proline content of leaf insignificantlychanged due to copper treatment during the growth period.

Treatment with mercury resulted in an exorbitant increase of proline content in the roots up to 12th day and there after gradually reduced. Stem of mercury treated plants showed unchanged proline values up to 20th day where significant reduction was observed. The proline content of leaf tissues showed negligible changes during growth period.

Table 18 Effect of Aluminium, Chromium, Copper and Mercury on total proline content of root, stem and leaf in *Coleus amboinicus*

Proline mg/g dry weight

Treatments	Tissues	Interval days					
		0	4	8	12	16	20
CONTROL	Root	0.38±0.02	0.31±0.03	0.27±0.03	0.21±0.01	0.16±0.03	0.13±0.06
	Stem	0.27±0.01	0.23±0.02	0.18±0.04	0.12±0.02	0.09±0.01	0.09±0.02
	Leaf	0.21±0.01	0.17±0.02	0.19±0.01	0.23±0.03	0.3±0.04	0.34±0.03
ALUMINIUM (500µM)	Root	0.40±0.09	0.33±0.01	0.30±0.06	0.36±0.04	0.39±0.03	0.46±0.07
	Stem	0.28±0.02	0.35±0.04	0.40±0.05	0.44±0.06	0.51±0.02	0.56±0.01
	Leaf	0.19±0.03	0.28±0.06	0.35±0.02	0.39±0.05	0.44±0.02	0.64±0.04
CHROMIUM (150µM)	Root	0.45±0.05	0.65±0.05	0.73±0.01	0.51±0.03	0.3±0.03	0.11±0.04
	Stem	0.30±0.08	0.39±0.04	0.44±0.06	0.49±0.01	0.53±0.01	0.44±0.02
	Leaf	0.22±0.03	0.62±0.03	0.58±0.02	0.64±0.05	0.68±0.02	0.52±0.01
COPPER (80µM)	Root	0.40±0.08	0.46±0.01	0.51±0.01	0.54±0.02	0.62±0.04	0.57±0.05
	Stem	0.28±0.01	0.32±0.03	0.37±0.02	0.43±0.03	0.48±0.01	0.52±0.01
	Leaf	0.28±0.03	0.31±0.05	0.45±0.03	0.47±0.03	0.51±0.03	0.53±0.02
MERCURY (10µM)	Root	0.40±0.09	0.60±0.01	0.63±0.06	0.68±0.04	0.9±0.03	0.86±0.07
	Stem	0.28±0.02	0.35±0.04	0.40±0.05	0.44±0.06	0.51±0.02	0.69±0.01
	Leaf	0.19±0.03	0.48±0.06	0.55±0.02	0.69±0.05	0.74±0.02	0.68±0.04

Values given are mean of 5 replicates ± S.E

Fig. 46 Effect of Aluminium, Chromium, Copper and Mercury on total proline content in root of *Coleus amboinicus*

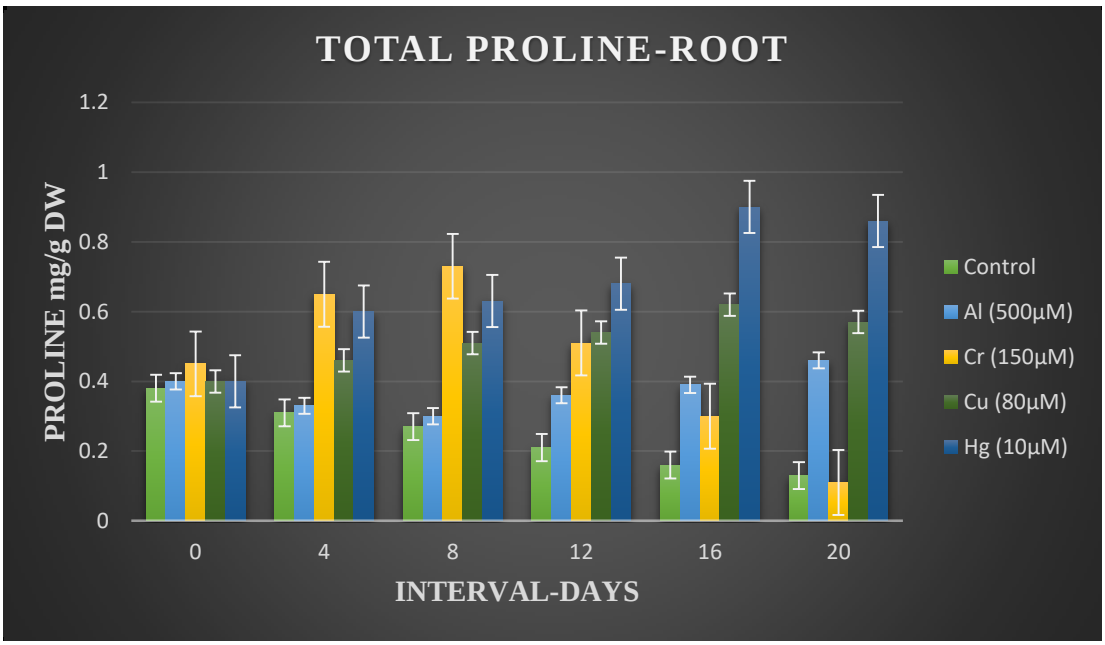


Fig. 47 Effect of Aluminium, Chromium, Copper and Mercury on total proline content in stem of *Coleus amboinicus*

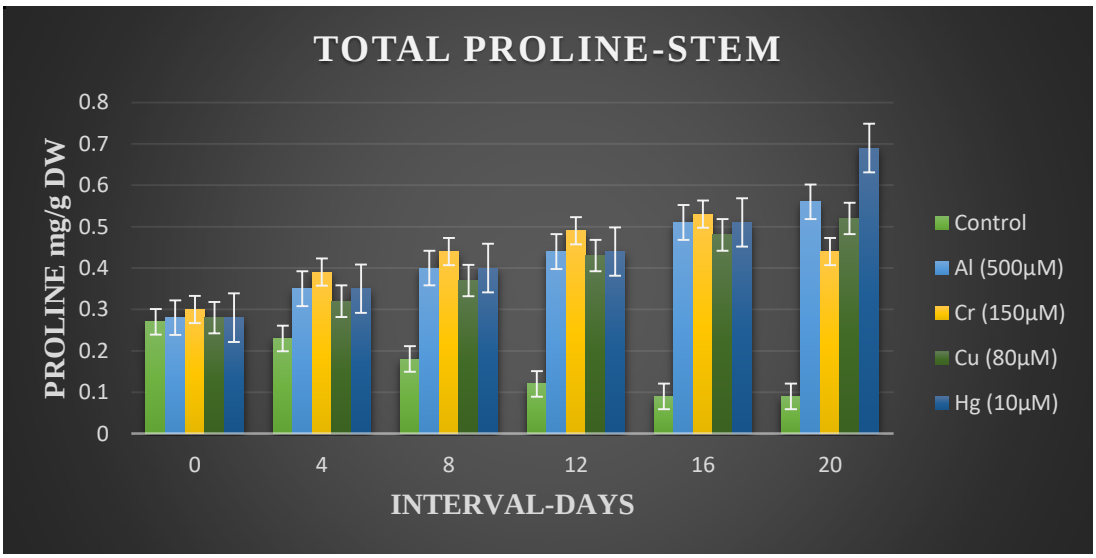
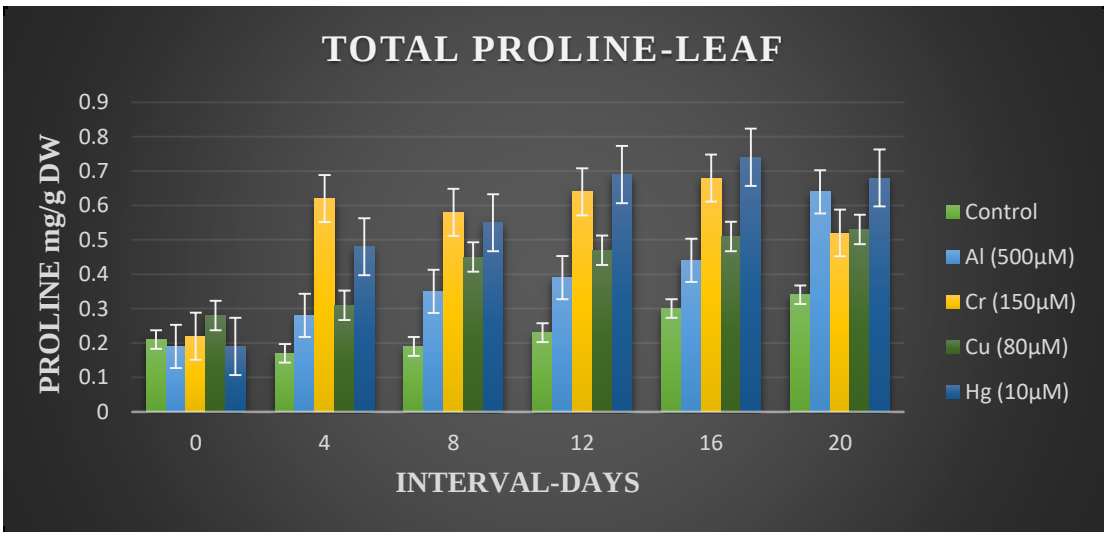


Fig. 48 Effect of Aluminium, Chromium, Copper and Mercury on total proline content in leaf of *Coleus amboinicus*



PHENOLICS

Treatment with aluminium resulted in the insignificant increase of phenolic content in the root tissue compared to the control. The stem tissues showed a two-fold increase in the first interval (4th day) and the increase was significant during all intervals. Leaf tissue also exhibited significant and gradual increase in phenolics in all stages.

Plants treated with chromium showed significantly high phenolics content in the initial stages of growth compared to the control root. But during growth, an insignificant reduction occurred during final stages. Distribution of phenolics in the stemtissue of Cr treated plants was very high compared to that of control, whereas leaf tissues showed more phenolic content up to16th day followed by a reduction.

The plants treated with copper shows the phenolic content of root tissues was more compared to the control, in all stages. Instem tissues, the phenolics content was doubled on 4th day and significantly higher than the control. Phenolics content in stem tissue increased in all stages compared to control whereas leaf tissue showed very high phenolics content in all stages comparedto other tissues on one hand and other treatments.

Mercury treatment revealed a gradual increase of phenolic content in the roots. Phenolic content of stem in plants treated with Hg showed insignificant growth or increase. Leaf tissue exhibited significant increase of phenolic content in almost allstages compared to stem and root.

Table 19 Effect of Aluminium, Chromium, Copper and Mercury on total phenolics content of root, stem and leaf in *Coleus amboinicus*

Total phenolics mg/g dry weight

Treatments	Tissue	0	4	8	12	16	20
Control	Root	9.31±0.21	10.19±0.08	11.65±0.1	12.53±0.05	14.1±0.16	16±0.09
	Stem	5.12±0.12	6.98±0.15	8.8±0.23	9.98±0.12	11.94±0.07	12.36±0.12
	Leaf	14.68±0.09	17.35±0.17	19.78±0.03	21.9±0.14	23±0.12	25.87±0.23
Aluminium (500µM)	Root	10.86±0.02	12.72±0.09	13.56±0.17	15.76±0.21	17.6±0.17	18.56±0.09
	Stem	6.35±0.15	14.32±0.04	21.74±0.14	23.15±0.17	26.76±0.06	22.76±0.26
	Leaf	17.61±0.18	25.98±0.12	36.52±0.23	38.26±0.28	45.37±0.15	37.61±0.32
Chromium (150µM)	Root	11.13±0.05	21.54±0.21	22.89±0.32	19.78±0.31	18.79±0.02	15.72±0.06
	Stem	7.98±0.04	16.91±0.17	17.5±0.27	18.1±0.02	23.45±0.03	22.34±0.28
	Leaf	16.84±0.17	35.46±0.06	39.87±0.09	54.6±0.34	31.9±0.15	24.98±0.05
Copper (80µM)	Root	10.35±0.09	12.11±0.01	13.15±0.16	18.33±0.28	17.86±0.08	23.96±0.17
	Stem	7.12±0.04	16.34±0.03	21.8±0.08	23.76±0.09	21.57±0.16	24.85±0.24
	Leaf	15.98±0.12	34.64±0.12	41.75±0.19	48.59±0.26	54.23±0.04	53.78±0.18
Mercury (10µM)	Root	9.75±0.14	12.70±0.01	16.74±0.25	16.45±0.37	17.65±0.16	19.88±0.25
	Stem	7.9±0.08	11.56±0.15	12.84±0.35	14.2±0.06	14.87±0.23	14.92±0.28
	Leaf	17.1±0.04	31.33±0.06	35.8±0.24	36.98±0.18	38.12±0.36	40.12±0.12

Values given are mean of 5 replicates ±S.E

Fig. 49 Effect of Aluminium, Chromium, Copper and Mercury on total phenolics content in root of *Coleus amboinicus*

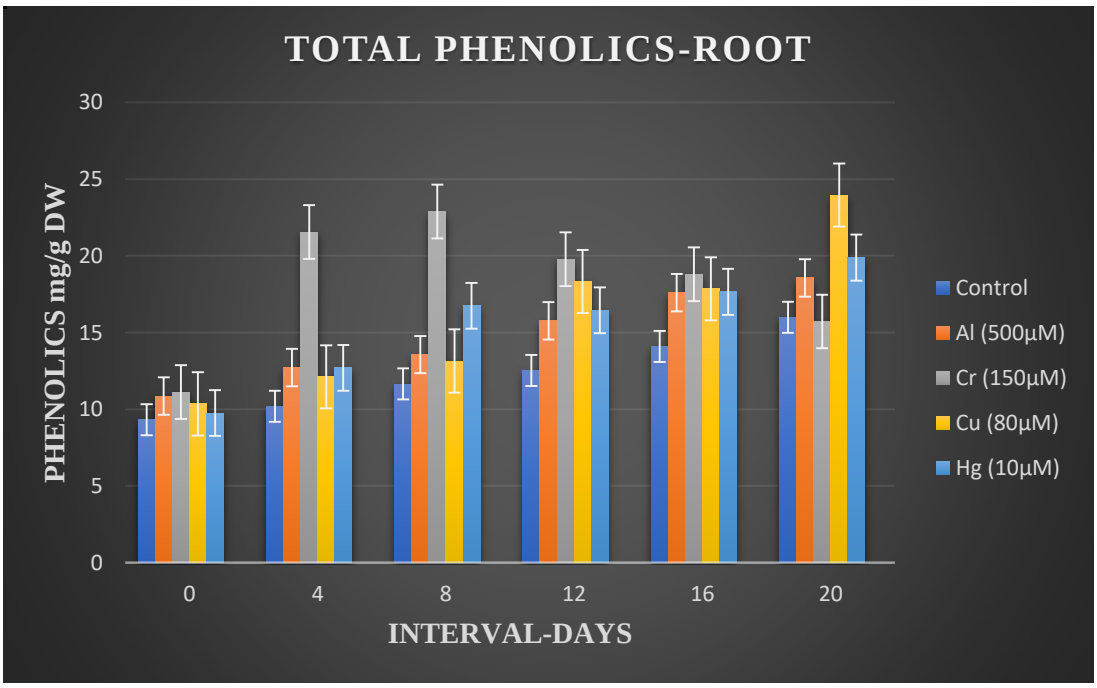


Fig. 50 Effect of Aluminium, Chromium, Copper and Mercury on total phenolics content in stem of *Coleus amboinicus*

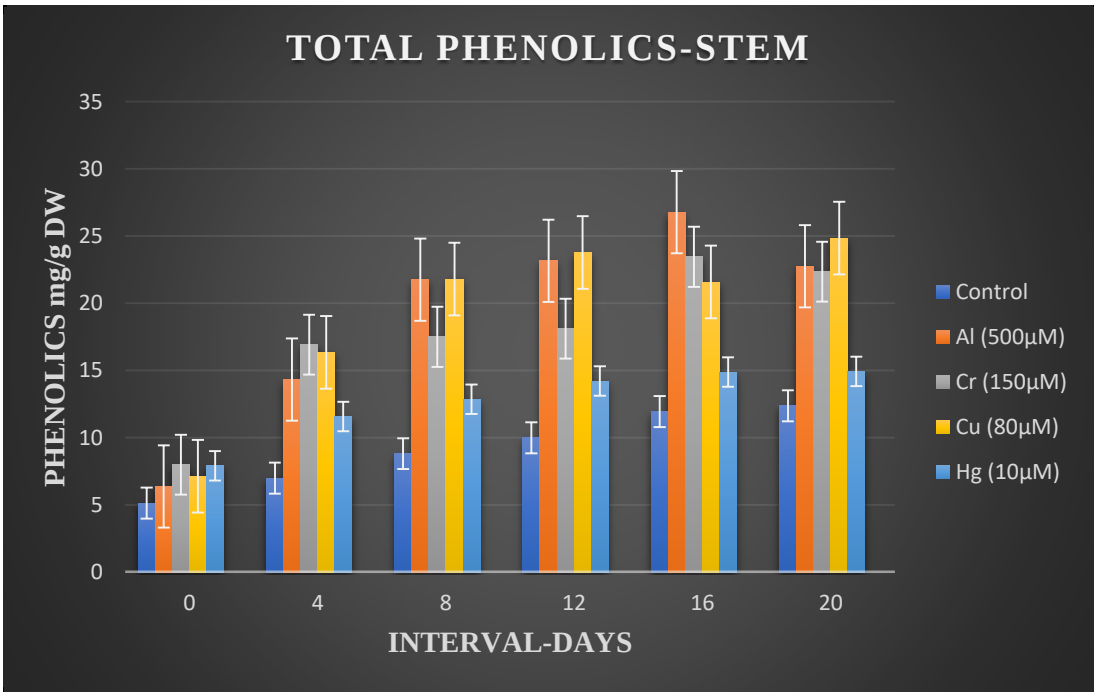
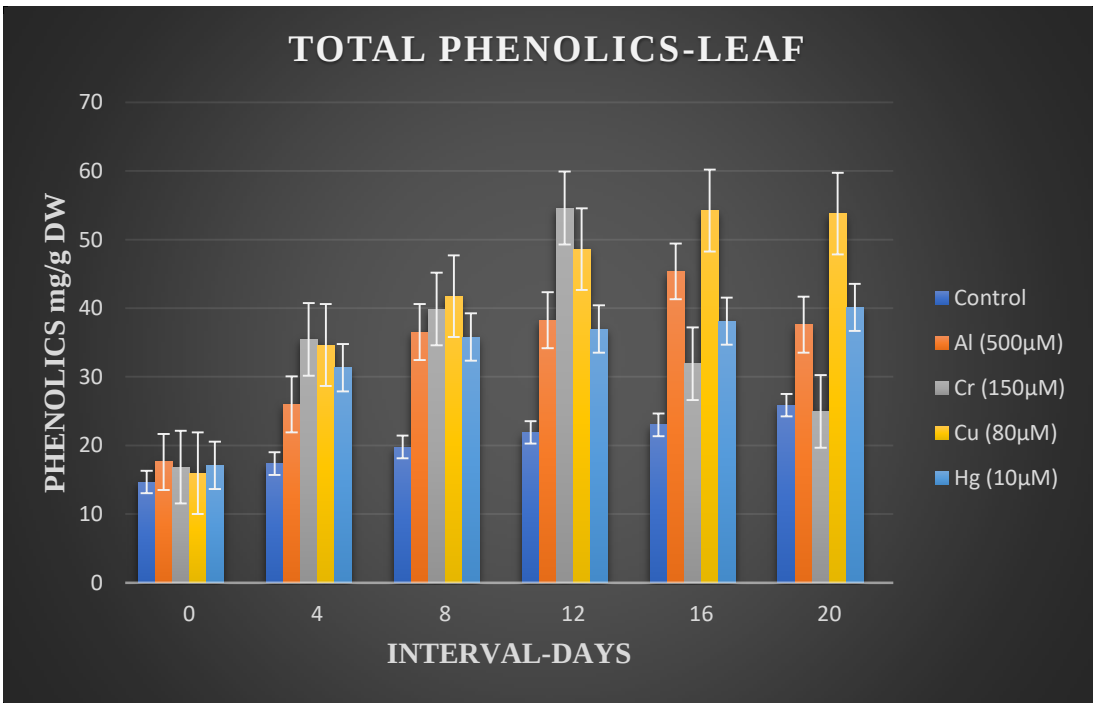


Fig. 51 Effect of Aluminium, Chromium, Copper and Mercury on total phenolics content in leaf of *Coleus amboinicus*



MDA CONTENT

During each stage of growth (20 days), the MDA content of control plants showed a linear increase in the root, stem, and leaves (Table 20: Figs. 52-54).

Root tissue of plants treated with aluminium showed continuous and significant increase in MDA content compared to the control root. MDA content of stem tissues of aluminium treated plant was also very high compared to the control and increased significantly at each interval. MDA content of leaf tissues in the plant treated with aluminium exhibited continuous increase throughout the growth period.

Chromium treatment showed several fold increases of MDA content in the root tissues compared to the control. MDA content of stem tissue of chromium treated plants showed a gradual increase up to 16th day followed by a gradual reduction. MDA content of leaves in the chromium treated plants was very high when compared to the control and it exhibited a continuous increase during growth up to 12th day followed by significant reduction.

MDA content of the roots of plants treated with copper was very high compared to control and continuous and significant increase when compared to the control and only slight fluctuations were observed between the stages up to 20th day. Copper treatment showed significantly high MDA content in the stem compared to the control and the increase was gradual. Leaf of copper treated plants showed very high MDA contentcompared to control in all stages of growth.

MDA content of the roots of plants treated with mercury was significantly higher than the control showing a continuous increase up to 20th day followed by a reduction in the final stage. MDA content of stem tissue of mercury was higher than the control and during growth the values showed considerable fluctuations and values remained unchanged up to 16th day followed by a significant increase. In leaves, the MDA content significantly increasing in each interval.

Table 20 Effect of Aluminium, Chromium, Copper and Mercury on total MDA content of root, stem and leaf in *Coleus amboinicus*

MDA content µg/g dry weight

Treatments	Tissues	0	4	8	12	16	20
Control	ROOT	6.41±0.12	7.32±0.19	9.18±0.19	11.24±0.17	12.87±0.25	13.56±0.19
	STEM	10.32±0.36	11.56±0.12	12.17±0.11	15.23±0.12	20.63±0.16	21.37±0.12
	LEAF	14.31±0.33	17.22±0.32	20.32±0.23	24.45±0.39	26.82±0.27	28.45±0.32
Aluminium (500µM)	ROOT	6.92±0.52	17.34±0.26	24.56±0.32	32.45±0.23	39.63±0.36	45.82±0.46
	STEM	10.45±0.45	19.62±0.16	27.18±0.08	33.64±0.32	41.82±0.28	55.43±0.69
	LEAF	14.01±0.12	28.92±0.48	38.12±0.51	49.32±0.47	53.83±0.22	50.23±1.52
Chromium (150µM)	ROOT	6.78±0.23	26.61±0.18	56.68±0.64	61.31±1.72	59.74±0.36	39.45±0.36
	STEM	10.95±0.19	31.33±0.57	38.36±0.34	46.64±0.35	29.43±0.43	26.48±0.19
	LEAF	14.54±0.66	20.74±0.32	23.43±0.24	53.37±1.11	31.38±0.66	21.53±0.15
Copper (80µM)	ROOT	6.23±0.32	15.64±0.45	29.61±0.29	35.91±0.17	30.45±0.42	34.78±0.52
	STEM	10.82±0.12	19.35±0.26	21.84±0.67	46.1±0.32	53.34±0.34	55.61±1.16
	LEAF	14.45±0.79	31.65±0.23	59.31±1.01	47.35±1.5	58.52±1.2	39.43±1.19
Mercury (10µM)	ROOT	6.82±0.42	18.05±0.16	25.53±0.47	38.27±0.51	57.43±0.21	29.62±0.21
	STEM	10.25±0.57	12.64±0.25	21.3±0.13	25.16±0.49	25.92±0.62	44.34±0.32
	LEAF	14.36±0.23	23.46±0.28	35.64±0.32	41.34±0.23	49.36±0.47	47.38±0.54

Values given are mean of 5 replicates ±S.E

Fig. 52 Effect of Aluminium, Chromium, Copper and Mercury on total MDA content in root of *Coleus amboinicus*

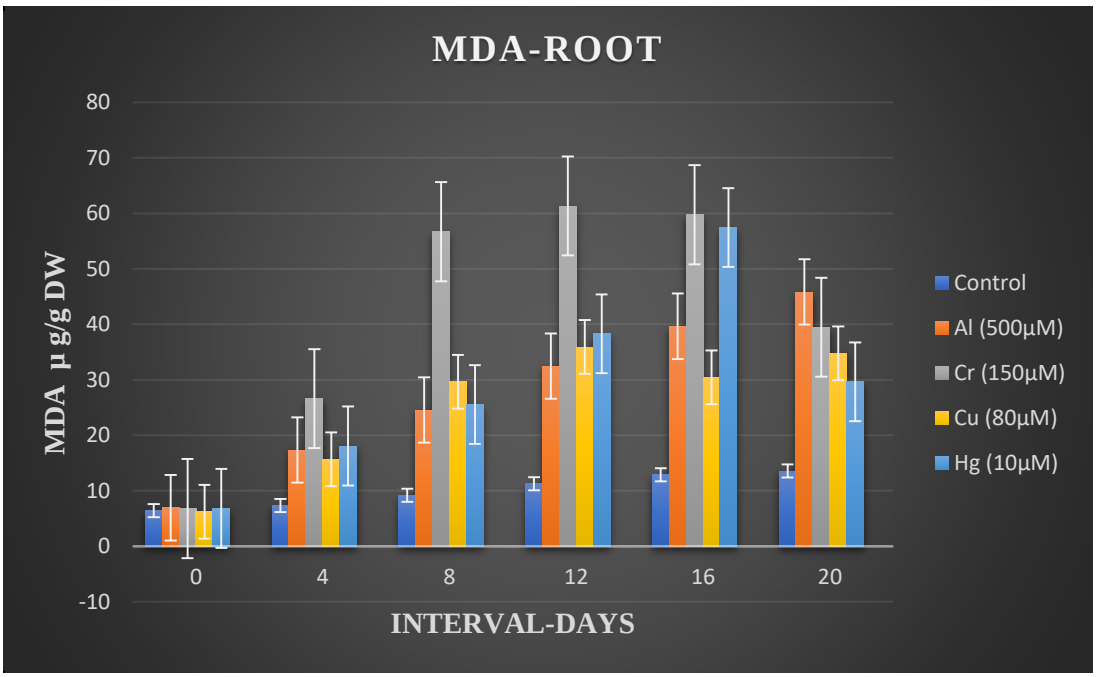


Fig. 53 Effect of Aluminium, Chromium, Copper and Mercury on total MDA content in Stem of *Coleus amboinicus*

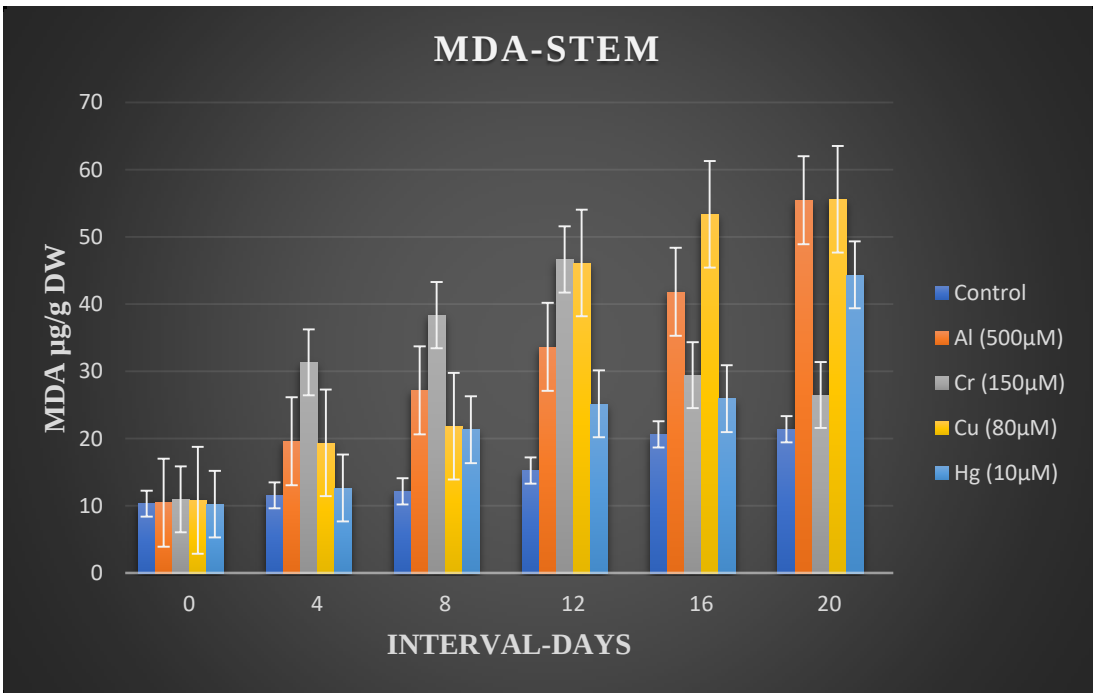
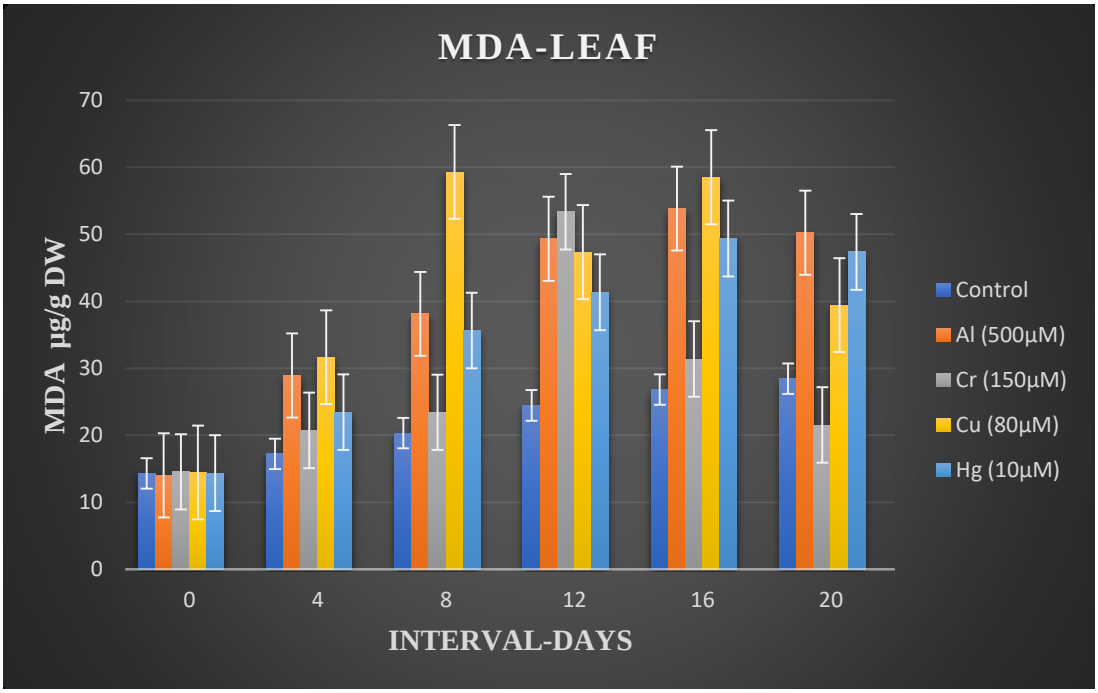


Fig. 54 Effect of Aluminium, Chromium, Copper and Mercury on total MDA content in leaf of *Coleus amboinicus*



SOD

Superoxide dismutase (SOD) activity showed an increasing trend of activity in all tissues of control plants during all intervals (Table 21, Figs. 55-57). In aluminium treated plants the activity of SOD was more or less similar to that of control in case of all tissues.

Root tissues of plants treated with chromium exhibited significant increase of SOD activity during initial phase compared to the control. Stem tissue showed more or less similar to that of root in initial days. Leaf tissues exhibited more or less same trend as that of root and stem and the activity was comparatively showed a gradual reduction.

SOD activity of all tissues of copper treatment was significantly higher than respective controls. In case of root tissues, significant increase (2 fold) on 4^{th} day followed by gradual reduction. Gradual reduction was very low when compared to the control. During early stages, the SOD activity of leaf tissue was comparatively higher than the control.

In the plants subjected to mercury, the activity of SOD was decreased gradually compared to control. In all tissues the highest SOD activity was observed during the initial stages of treatments. In case of roottissues, the activity was more in initial stages but during growth the changes were negligible. Stem tissues showed increased SOD activity in initial stages and then decreased significantly during further growth but leaf showed very high SOD activity in early period which reduced gradually during further growth.

Table 21 Effect of Aluminium, Chromium, Copper and Mercury on SOD activity of root, stem and leaf in *Coleus amboinicus*

SOD Unit activity/mg protein (Specific activity)

	Tissues	**Interval Days**		
		4	**12**	**20**
CONTROL	ROOT	8.14 ± 0.92	12.87 ±0.92	15.68 ±1.21
	STEM	7.23 ± 0.91	10.34 ±0.98	14.56 ±0.92
	LEAF	7.03 ±0.82	11.98 ±1.03	18.1 ±1.08
ALUMINIUM (500µM)	ROOT	6.76 ± 0.74	8.31 ± 0.92	16.23 ±1.03
	STEM	7.81 ±0.89	12 ± 0.98	14.34 ±0.97
	LEAF	6.87 ± 0.97	10.78 ±0.92	16.32 ±1.42
CHROMIUM (150µM)	ROOT	18.65 ±1.12	12.98 ±0.83	9.45 ±0.93
	STEM	17.98 ±1.32	12.92 ±0.94	16.76 ±1.12
	LEAF	19.43 ± 1.21	15.98 ± 1.2	12.83 ±1.05
COPPER (80µM)	ROOT	15.43 ±1.26	20.6 ± 1.12	25.91 ± 1.15
	STEM	11.31 ±1.09	17 ±1.07	22.23 ±1.03
	LEAF	12.9 ±1.32	16.72 ±1.04	19.31 ± 2.25
MERCURY (10µM)	ROOT	12.01 ±1.12	11.41 ±0.92	9.87 ± 0.93
	STEM	13.52 ±1.18	8.18 ± 0.81	7.56 ±0.97
	LEAF	21.92 ±1.32	16.62 ±1.05	12.72 ± 0.92

Values given are mean of 5 replicates ± S.E

Fig. 55 Effect of Aluminium, Chromium, Copper and Mercury on SOD activity in root of *Coleus amboinicus*

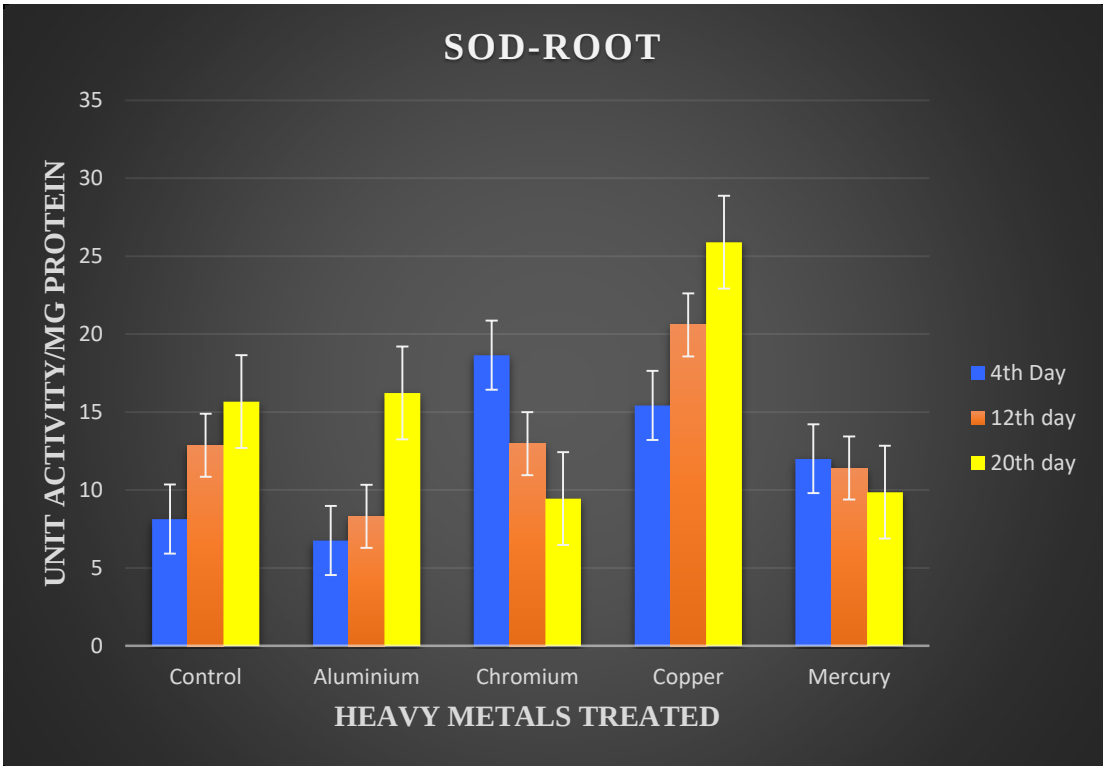


Fig. 56 Effect of Aluminium, Chromium, Copper and Mercury on SOD activity in stem of *Coleus amboinicus*

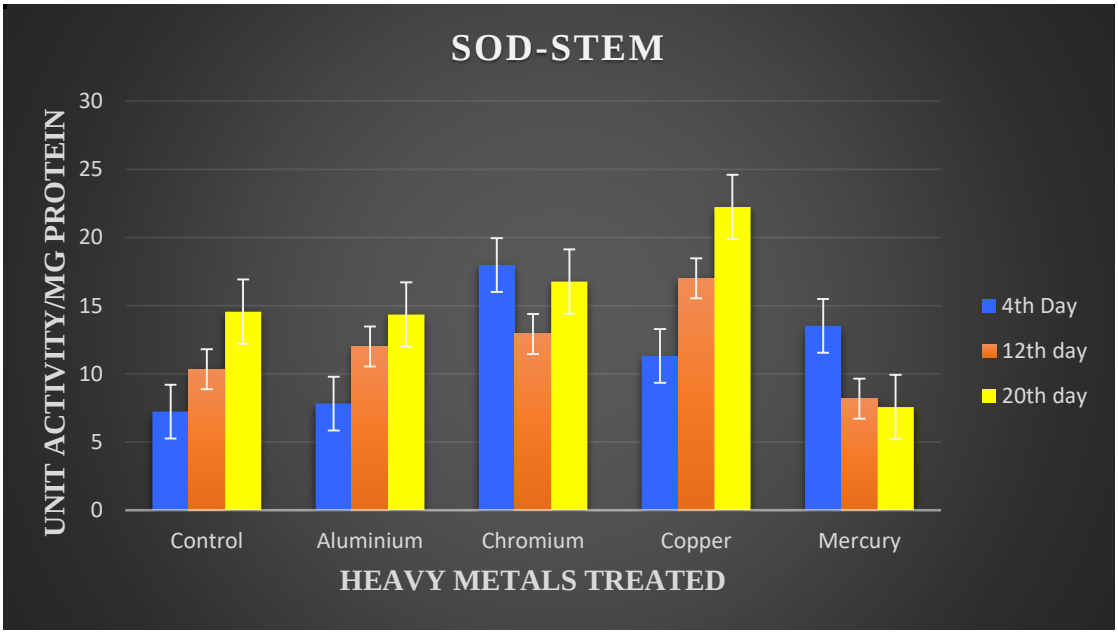
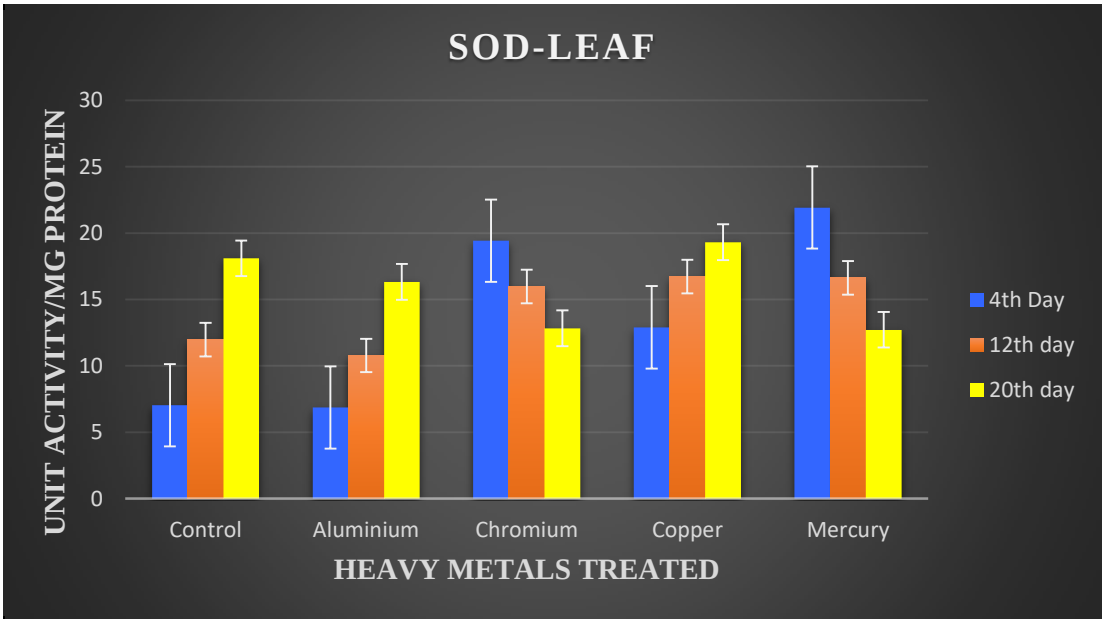


Fig. 57 Effect of Aluminium, Chromium, Copper and Mercury on SOD activity in leaf of *Coleus amboinicus*



CATALASE ACTIVITY

Catalase activity of *Coleus amboinicus* showed an increasing trend in control plants (Table 22; Figs. 58-60) during growth up to 20 days and thetrend of activity was more or less uniform. Maximal CAT activity was recorded in the leaf samples than stem and root.

Plant treated with aluminium showed significant reduction of CAT activity in all tissues. In case of aluminium treated plant root tissues, the mode of CAT activity was more or less similar to the control. But CAT activity was very feeble in stem tissues throughout the growth period. The leaf of aluminium treated plants exhibited low catalase activity in comparison with the control, and the activity was slightly increasing duringgrowth.

Root tissues of *C. amboinicus* treated with chromium exhibited CAT activity more or less similar to that of control. Stem tissues showed only very low catalase activity and maximum activity was in 20th days of growth. Catalase activity of leaf tissues showed more or less similar in all stages of growth.

Due to copper treatment, catalase activity of root, stem and leaves was lower than the respective control tissues. Only negligible changes wereobserved in the CAT activity of root and stem during the entire period of growth whereas CAT activity of leaf tissue was comparatively higher thanroot and stem which remained unchanged during growth. Compared to therespective control the activity was very low.

Plants treated with mercury registered very feeble activity in root and stem whereas leaf tissues exhibited more activity compared to root andstem. CAT activity was very low than control plants during all stages of growth.

Table 22 Effect of Aluminium, Chromium, Copper and Mercury on Catalase activity of root, stem and leaf in *Coleus amboinicus*

Unit activity/mg protein (Specific activity)

	Tissues	**Interval Days**		
		4	**12**	**20**
Control	Root	1.09 ±0.02	1.62 ±0.07	2.32 ±0.032
	Stem	1.52 ±0.012	2.06 ± 0.02	3.96 ±0.043
	Leaf	2.53 ±0.03	3.54 ± 0.021	5.2 ±0.007
Aluminium (500µM)	Root	0.98 ±0.001	1.21 ±0.043	1.56 ±0.034
	Stem	0.94 ± 0.013	1.18 ±0.04	1.34 ±0.08
	Leaf	1.45 ±0.01	1.74 ±0.023	2.84 ±0.03
Chromium (150µM)	Root	0.89 ±0.023	0.98 ±0.08	2.19 ±0.023
	Stem	0.76 ±0.05	0.91 ±0.08	2.06 ±0.009
	Leaf	1.76 ±0.02	1.82 ±0.023	1.98 ±0.004
Copper (80µM)	Root	0.72 ±0.031	0.87 ±0.071	0.98 ±0.008
	Stem	0.86 ±0.012	0.96 ±0.03	1.03 ±0.034
	Leaf	1.69 ±0.014	1.89 ±0.08	1.97 ±0.021
Mercury (10µM)	Root	0.29 ±0.012	0.38 ± 0.02	0.76 ±0.018
	Stem	0.54 ±0.014	0.62 ±0.023	0.67 ±0.014
	Leaf	1.62 ±0.015	1.7 ±0.045	1.92 ±0.06

Values given are mean of 5 replicates ± S.E

Fig. 58 Effect of Aluminium, Chromium, Copper and Mercury on catalase activity in root of *Coleus amboinicus*

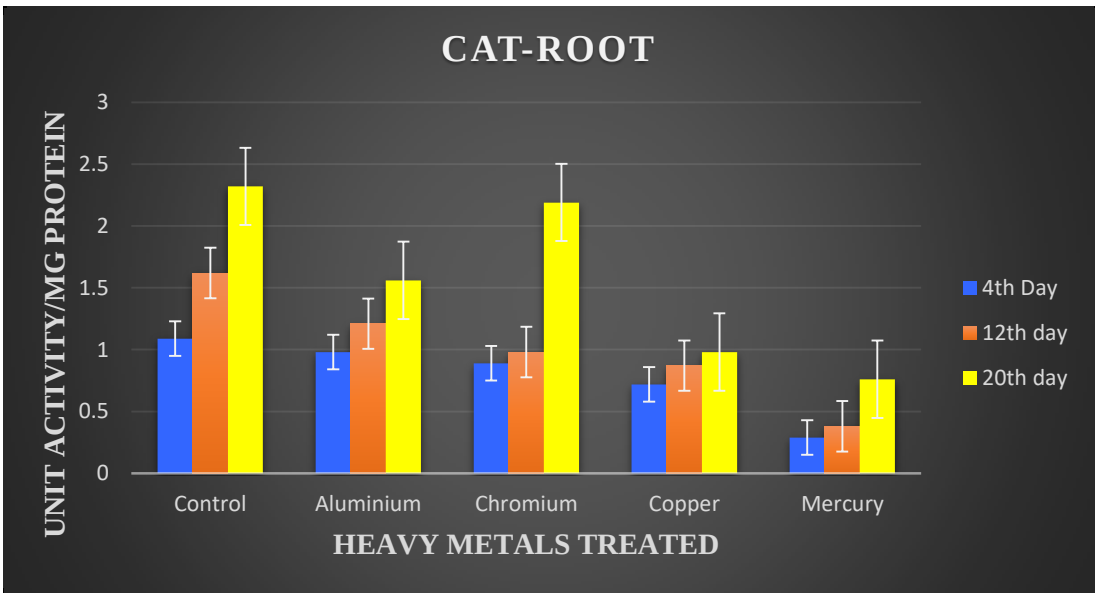


Fig. 59 Effect of Aluminium, Chromium, Copper and Mercury on catalase activity in stem of *Coleus amboinicus*

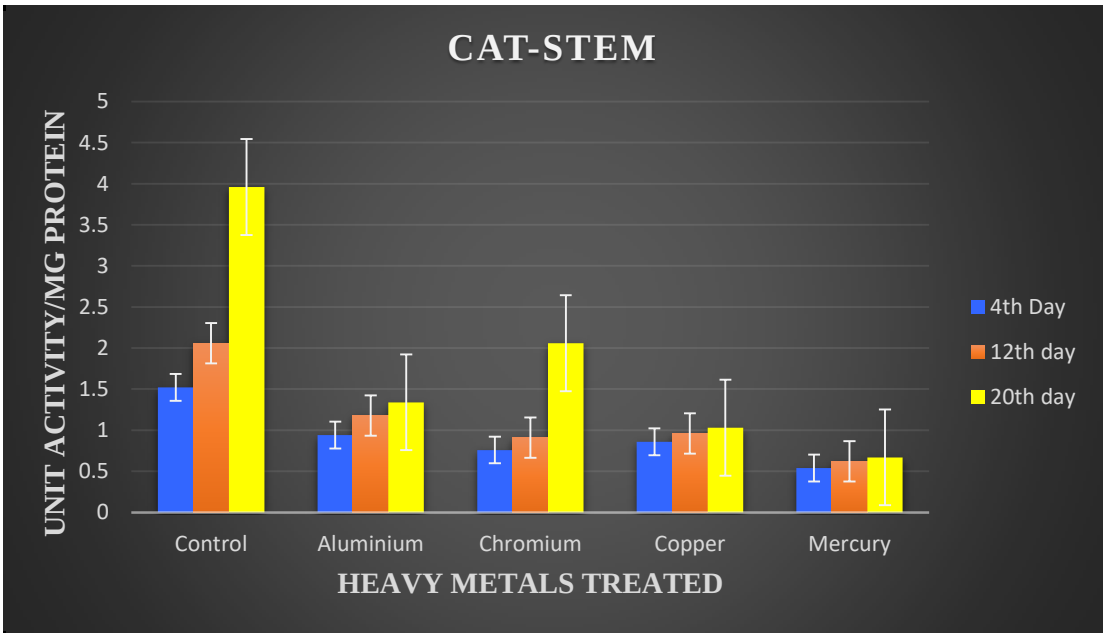
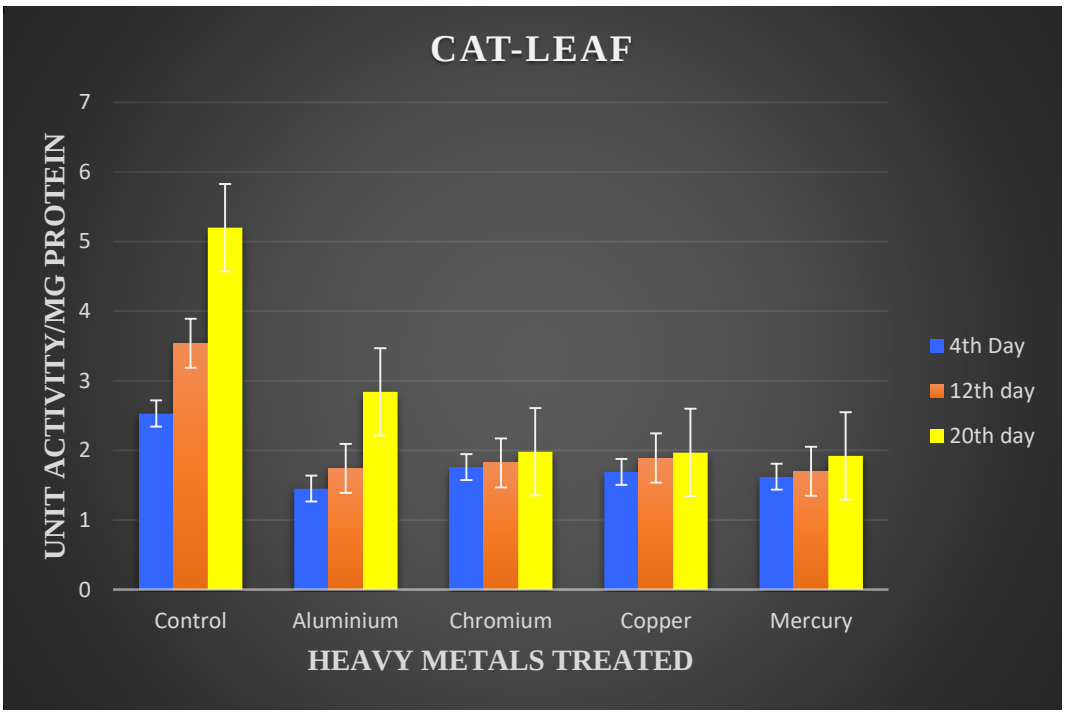


Fig. 60 Effect of Aluminium, Chromium, Copper and Mercury on catalase activity in leaf of *Coleus amboinicus*



BIOACCUMULATION

Bioaccumulation pattern of aluminium, chromium, copper and mercury in different parts-root, stem and leaf of *C. amboinicus* were ascertained at different intervals of growth (Table 23). Accumulation of aluminium in the roots on fourth day of treatment was comparatively lower and content was continuously increased throughout the growth period and the rate of accumulation was significant during 12-20 days. Aluminium content accumulated the stem showed significant increase during 12th day when compared to the control. More aluminium was present in the roots compared to stem and leaves during all phases of growth.

Plants treated with chromium exhibited considerable amount of chromium accumulated in the root and gradually and significantly increased during all phases of growth. Stem showed low content of chromium than roots and leaves in the initial stages followed by significant increase on 20th day($P<0.01$).

Treatment with copper on *C. amboinicus* resulted in comparatively more accumulation of copper in the roots than stem and leaf. Increase of copper accumulation in roots was increased significantly on 12^{th} day followed by a hike (two fold) on 20^{th} day($P<0.05$). Presence of copper in leaf was below detectable level on 4^{th} day and showed gradual increase thereafter.

Comparatively mercury bioaccumulation was meagre in all tissues than the other metals. The accumulation was more in root than other tissues. Accumulation of mercury only showed insignificant changes in the stem tissues whereas in the leaf mercury content was below detectable level on 4^{th} day followed by significant increase during further growth.

Analysis of aluminium, chromium, copper and mercury present in the residual solution (metals present in the culture solution after 20^{th} day of growth) showed remarkable changes dependent on metals. A general trend of reduction was observed in all case of metals during growth. An interesting observation in the distribution of mercury in the residual solution was comparatively very low amount was detected on 4^{th} day followed by almost absence since the metal present was only below detectable level.

Table 23 Bioaccumulation patterns of Aluminium, Chromium, Copper and Mercury on *Coleus amboinicus* grown in Hoagland solution containing standardized concentrations of heavy metals

Bioaccumulation µg/g dry weight

Treatments	Tissues	Interval days		
		4	12	20
Aluminium	Root	17.41±0.11	19.82±0.16	26.24±0.15
	Stem	5.67±0.13	6.63±0.13	9.78±0.19
	Leaf	3.34±0.09	4.59±0.17	7.5±0.13
	Residue	9.27± 0.11	4.67± 0.12	3.09± 0.14
Chromium	Root	13.46±0.22	19.31±0.21	22.4±0.02
	Stem	2.1±0.02	4.32±0.06	8.61±0.17
	Leaf	2.32±0.12	4.69±0.02	5.12±0.05
	Residue	8.02±0.07	5.22±0.14	3.74±0.08
Copper	Root	13.52±0.17	19.65±0.04	36.72±0.12
	Stem	3.62±0.06	8.65±0.09	14.37±0.13
	Leaf	2.15±0.03	4.16±0.02	5.91±0.02
	Residue	11.23± 0.12	7.44± 0.12	5.09± 0.08
Mercury	Root	8.58±0.04	6.13±0.02	6.11±0.11
	Stem	4.13±0.21	2.02±0.13	1.21±0.02
	Leaf	BDL	BDL	0.16±0.01
	Residue	2.312 ± 0.06	BDL	BDL

Values are mean of 5 replicates ±S.E

BDL : Below Detectable level

BIOCONCENTRATION FACTOR AND TRANSLOCATION FACTOR

Bioaccumulation was also analysed by two factors such as BCF andTF. When a comparison is made between the Bioconcentration factor- BCF (ratio of metal content of the roots to the medium) and Translocation factor- TF (ratio of metal concentration in shoot to the root), the response of *C. amboinicus* plants towards the different metals showed significant variations in both values.

Bioaccumulation was also analysed by two factors such as BCF and TF. When a comparison is made between the Bioconcentration factor- BCF (ratio of metal content of the roots to the medium) and Translocation factor- TF (ratio of

metal concentration in shoot to the root), the response of *C. amboinicus* plants towards the different metals showed significant variations in both values.

The BCF values of aluminium-treated plants gradually increased during growth. TF values was less than one in all stages of growth. Significant variations were observed in TF values in *C. amboinicus* treated with aluminium during growth.TF values showed an increase in first phase (4-12thday) followed by a reduction in last phase (12-20th day).

BCF values of chromium treated plants increased meagrely on first growth phase ie,4-12 days and a significant increase in the second phase 12-20.TF values were less than one in all intervals.

BCF values of plants treated with copper increased gradually during all stages of growth. On 12th day only, TF is nearly to one and in all other stages TF is below one. Compared to aluminium, chromium and copper, plants treated with mercury showed low values of BCF during growth. The TF value was lower than 1 during all stages of growth.

Table 24 Bio concentration factor and translocation factor

Treatments	INTERVAL – DAYS					
	4		12		20	
	BCF	TF	BCF	TF	BCF	TF
Aluminium	0.005	0.51	0.005	0.56	0.007	0.65
Chromium	0.006	0.32	0.008	0.46	0.01	0.61
Copper	0.02	0.42	0.03	0.65	0.05	0.55
Mercury	0.05	0.48	0.05	O.32	0.04	0.22

BCF: metal concentration ratio of plant roots to medium
TF: metal concentration ratio of plant shoots to roots

GCMS ANALYSIS OF *COLEUS AMBOINICUS* LEAVES

GCMS analysis of methanolic extract of *Coleus amboinicus* enabled the identification of eighteen secondary metabolites and the area percentage of compounds represent the quantity of the compounds. The compounds identified were 5-isopropyl-2-methylphenol, phytol, squalene, gamma-Sitosterol, neophytadiene, methylpalmitate, 1,6-Nonadien-3- ol,3,7-dimethyl, gamma-Tocopherol, vitamin E, alpha-linolenic acid methyl ester, Alpha-bergamotene, caryophyllene, 9,12-octadecadienoic acid, methyl ester, Methyl commate a, Beta sitosterol and Alpha - amyrin. Quantitativeexpression of these secondary metabolites was done by considering the area percentage of each compound. Abundantly occurring component was 5-isopropyl-2-methylphenol and is commonly known as carvacrol and least occurring secondary metabolite in control plants was vitamin E.

GCMS ANALYSIS SPECTRUM OF *COLEUS AMBOINICUS*

Figure: 61

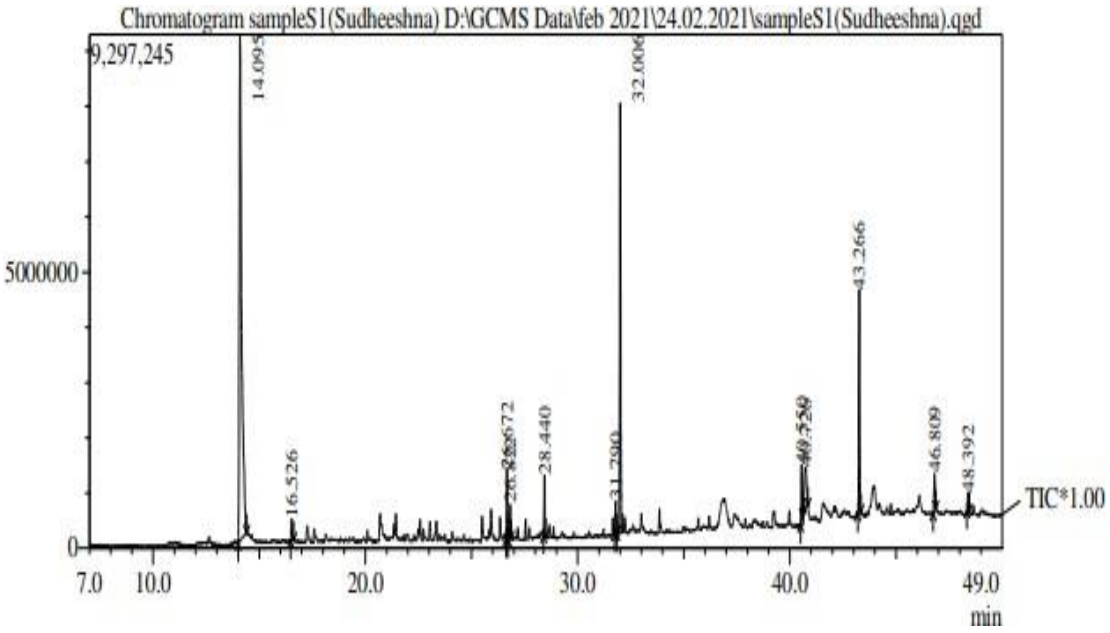


Table 25 GCMS Analysis *Coleus amboinicus*

Compounds	**Control**
5-isopropyl-2-methylphenol	55.88
Tetradecane	1.47
Neophytadiene	2.55
Methylpalmitate	12.24
alpha-linolenic acid methyl ester	3.52
Phytol	11.79
1,6-Nonadien-3-ol, 3,7-dimethyl	2.02
gamma.-Sitosterol	4.07
gamma.-Tocopherol	2.01
Vitamin E	1.02
Methyl stearate	3.45
Squalene	6.09
Alpha-bergamotene	14.67
Caryophyllene	4.78
9,12-octadecadienoic acid,methyl ester	16.43
Methyl commate a	6.34
Beta sitosterol	3.67
Alpha – amyrin	4.23

DISTRIBUTION OF SECONDARY METABOLITES IN *COLEUS AMBOINICUS* TREATED WITH ALUMINIUM

GCMS analysis of plants treated with Aluminium resulted in the occurrence of sixteen secondary metabolites such as 5-isopropyl-2- methylphenol, 9,12-Octadecadienoic acid, Phytol, Methylpalmitate, methyl commate a, gamma-Sitosterol, Methyl stearate, tetradecanoic acid, 12-methyl-, methyl ester. In plants treated with Al three secondary metabolites were absent along with the formation of one new component (Table 26). The components which were absent due to Al treatment include vitamin E, tetradecane and 1,6-nonadien-3-ol,3,7-dimethyl. The newly formed compound was Tetradecanoic acid,12-methyl-methyl ester. In the plants treated with Al, 5-isopropyl-2-methylphenol was the abundantly occurring compound andthe least occurring component was alpha-linolenic acid methyl ester.

GCMS ANALYSIS SPECTRUM OF *COLEUS AMBOINICUS* TREATED WITH Al

Figure 62

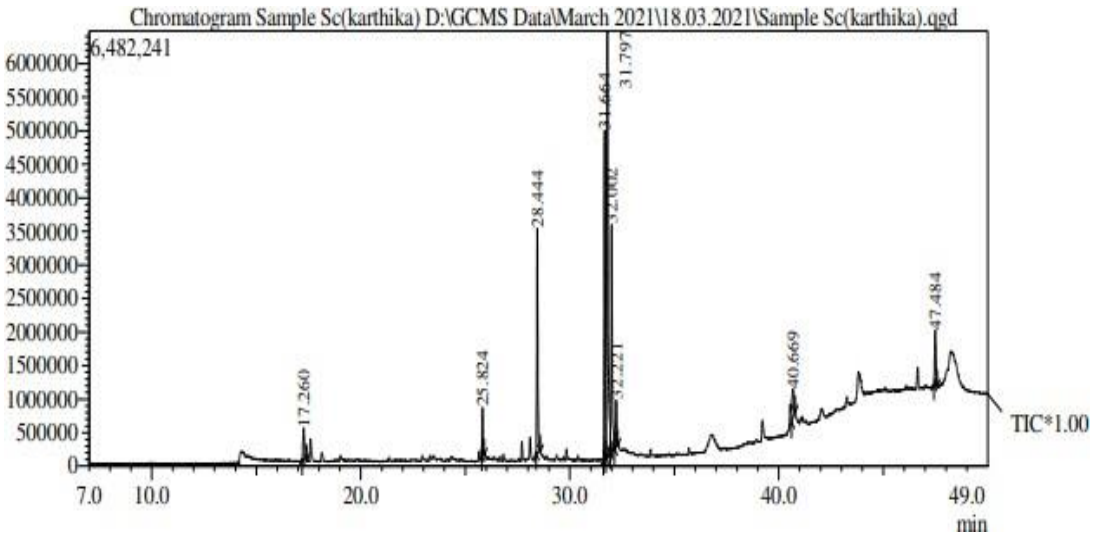


Table 26 GCMS analysis *Coleus amboinicus* treated with Aluminium

Compounds	**Aluminium**
5-isopropyl-2-methylphenol	49.49
Neophytadiene	3.12
Methylpalmitate	9.81
alpha-linolenic acid methyl ester	1.32
Phytol	13.79
gamma.-Sitosterol	3.84
gamma.-Tocopherol	2.89
Methyl stearate	3.18
Tetradecanoic acid,12-methyl-methyl ester	2.05
Squalene	5.21
Alpha-bergamotene	12.89
Caryophyllene	3.14
9,12-octadecadienoic acid,methyl ester	13.82
Methyl commate a	4.3
Beta sitosterol	2.18
Alpha – amyrin	5.67

DISTRIBUTION OF SECONDARY METABOLITES IN *COLEUS AMBOINICUS* TREATED WITH CHROMIUM

5-isopropyl-2-methyl phenol, phytol, Methyl commate a, Beta sitosterol, Methyl palmitate, Alpha-amyrin, Gamma-tocopherol and neophytadiene were some of the secondary metabolites present in *Coleus amboinicus* treated with chromium. The most abundant compound was 5-isopropyl-2-methylphenol and the least abundant was Tetradecanoic acid,12-methyl-methyl ester. The least compound was the newly formed secondary metabolite in plants subjectedto Cr treatment. Vitamin E, tetradecane, alpha-linolenic acid methyl ester, 1,6- Nonadien-3-ol, 3,7-dimethyl, caryophyllene and squalene were absent in plants treated with chromium in comparison with control.

GCMS ANALYSIS SPECTRUM OF *COLEUS AMBOINICUS* TREATED WITH Cr

Figure: 63

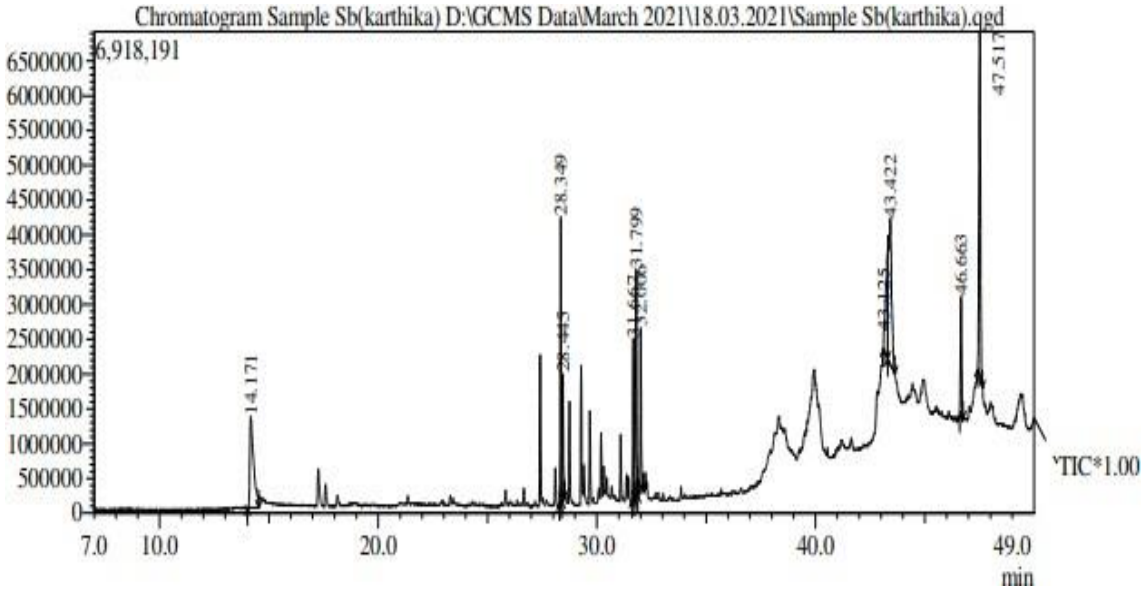


Table 27 GCMS analysis *Coleus amboinicus* treated withChromium

Compounds	**Chromium**
5-isopropyl-2-methylphenol	41.53
Neophytadiene	4.67
Methylpalmitate	8.62
Phytol	18.58
gamma.-Sitosterol	1.54
gamma.-Tocopherol	8.36
Methyl stearate	2.15
Tetradecanoic acid,12-methyl-methyl ester	1.12
Squalene	3.32
Alpha-bergamotene	10.56
9,12-octadecadienoic acid,methyl ester	11.87
Methyl commate a	3.72
Alpha – amyrin	6.7

DISTRIBUTION OF SECONDARY METABOLITES IN *COLEUS AMBOINICUS* TREATED WITH COPPER

GC-MS analysis of methanolic extract of copper treated *Coleus amboinicus* showed the occurrence of seventeen secondary metabolites namely 5-isopropyl-2-methyl phenol, Methyl stearate, Phytol, methyl palmitate, Isolongifolol methyl ether, tetradecanoic acid 12-methyl-methyl ester, phenol-2,4-bis(1,1-dimethylethyl), alpha-Bergamotene and Caryophyllene. The most abundant compound was 5-isopropyl-2-methyl phenol and the least one was Methyl stearate. The newly synthesized compound were Phenol,2,4-bis(1,1-dimethylethyl), Tetradecanoic acid,12-methyl-methyl ester and Isolongifolol, methyl ether. Tetradecane, alpha-linolenic acid methyl ester ,vitamin E and 1,6-nonadien-3-ol,3,7-dimethyl were absent in plants treated with Cu in comparison with control.

GCMS ANALYSIS SPECTRUM OF *COLEUS AMBOINICUS* TREATED WITH Cu

Figure: 64

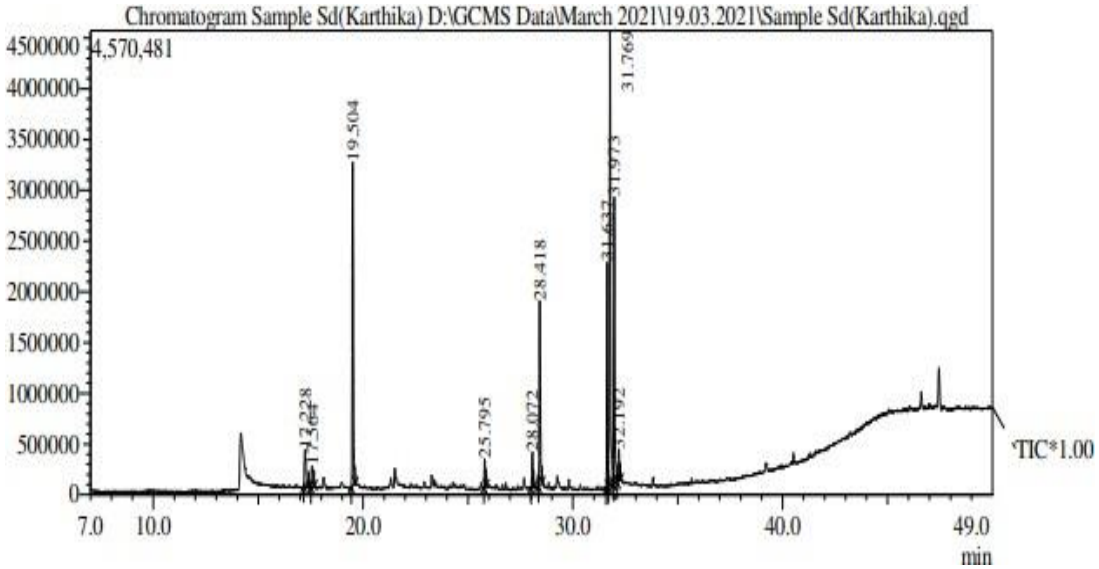


Table 28. GCMS analysis *Coleus amboinicus* treated withCopper

COMPOUNDS	**COPPER**
5-isopropyl-2-methylphenol	46.42
Neophytadiene	7.96
Methylpalmitate	11.02
Phytol	16.87
gamma.-Sitosterol	2.23
gamma.-Tocopherol	5.81
Methyl stearate	1.09
Isolongifolol, methyl ester	3.98
Tetradecanoic acid,12-methyl-methyl ester	1.45
Squalene	3.76
Phenol,2,4-bis(1,1-dimethylethyl)	1.09
Alpha-bergamotene	1.77
Caryophyllene	2.74
9,12-octadecadienoic acid,methyl ester	12.95
Methyl commate a	5.78
Beta sitosterol	1.66
Alpha – amyrin	4.23

DISTRIBUTION OF SECONDARY METABOLITES IN *COLEUS AMBOINICUS* TREATED WITH MERCURY

The compounds identified in *C. amboinicus* treated with mercury were 5-isopropyl-2-methylphenol, Neophytadiene, Methylpalmitate, Phytol, gamma.-Sitosterol, gamma.-Tocopherol, Methyl stearate, Tetradecanoic acid,12-methyl-methyl ester, Squalene, Alpha-bergamotene, 9,12-octadecadienoic acid,methyl ester, Methyl commate a and Alpha – amyrin.The abundant compound was 5-isopropyl-2-methyl phenol and the least one was gamma.-Sitosterol. Tetradecanoic acid,12-methyl-methyl ester was the newly synthesized compound after Hg treatment. The compounds removed after the treatment were vitamin E, tetradecane, alpha-linolenic acid methyl ester, 1,6-nonadien-3-ol,3,7-dimethyl, caryophyllene and beta sitosterol.

GCMS ANALYSIS SPECTRUM OF *COLEUS AMBOINICUS* TREATED WITH Hg

Figure 65

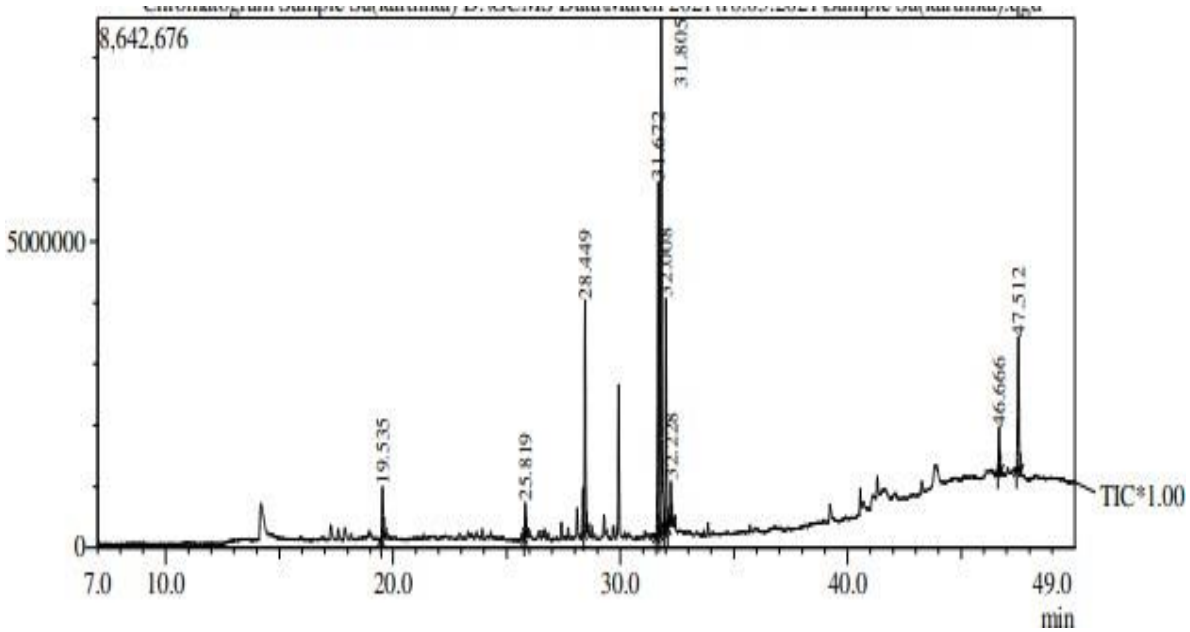


Table 29. **GCMS Analysis *Coleus amboinicus* treated with Mercury**

Compounds	**Mercury**
5-isopropyl-2-methylphenol	37.45
Neophytadiene	4.81
Methylpalmitate	9.45
Phytol	19.35
gamma.-Sitosterol	1.42
gamma.-Tocopherol	9.23
Methyl stearate	1.98
Tetradecanoic acid,12-methyl-methyl ester	1.51
Squalene	2.12
Alpha-bergamotene	9.12
9,12-octadecadienoic acid,methyl ester	12.12
Methyl commate a	2.56
Alpha – amyrin	5.74

DISCUSSION

To investigate the effect of heavy metals such as Al, Cr, Hg and Cu on *Coleus amboinicus*, screening experiments were conducted with different concentrations of the salts of aluminium ($AlCl_3$), chromium ($K_2Cr_2O_7$), copper ($CuSO_4$) and mercury ($HgCl_2$) and it was confirmed that 500 µM Al, 150 µM Cr, 10 µM Hg and 80 µM Cu exhibited toxicity symptoms which showed 50% growth retardation. According to an exhaustive evaluation by Foy *et al*. (1978), plant sensitivity and/or tolerance to heavy metals varies depending on the species and metal. These authors stated that, in order to determine the ideal concentrations for studying the toxicity of heavy metals, trial experiments with a range of values that demonstrate around 50% growth retardation while maintaining plant growth and survival should be used as a reference.

Prominent effect of heavy metal toxicity on plants is the visible symptoms of growth retardation. Growth pattern and rate assessed by morphological changes such as root and shoot length, leaf area and total biomass productivity of *C. amboinicus* cultivated in Hoagland nutrient medium containing known quantities of aluminium (500 µM), chromium (150 µM), copper (80 µM) and mercury (10 µM) showed significant variations as compared to the control. The remarkable differences in the concentrations that impose almost similar growth retardation and survival maintenance indirectly reveal the toxicity levels pertaining to each metal. The concentration of metals selected for the culture studies to impart more or less similar growth retardation retaining the survival of plant vary significantly reveals the difference in the magnitude of toxicity of individual metals on *C. amboinicus* (Tables 3, 4 and 5; Figs.3, 4 and 5).

Specific and nonspecific alterations are caused by different heavy metals, and these changes differ among plant species and metals. Since the primary toxicity mechanisms of different metal ions may be as different as their chemical properties vary. Membrane damage, inhibition of root growth, altered enzyme activity that results in secondary effects like altered water relations, insufficient vital nutrients,

and inhibition of physiological aspects like photosynthesis, respiration, and photoassimilate translocation are the primary characteristics of heavy metal stress (Huang and Cunningham, 1996; Singh, 2005; Solanki and Dhankhar, 2011). Ions, irrespective of their essentiality for plant growth, are known to interfere with passive and active transport of nutrients with structural changes in the plasma membrane, particularly ion channels which play important role in osmoregulation, growth, signalling, movement and long-distance transport of nutrients (Pantoja, 2021).

The selection of heavy metals of present investigation for treatments on *C. amboinicus* includes root length, shoot length, and leaf area as morphological characteristics to evaluate the growth pattern.

Chronological increase of root growth of *C. amboinicus* in terms of root length is gradual and cumulative in the control as well as all the treatments. But among the metals considerable reduction of root length is observed whereas in the shoot length distribution only negligible reduction occurs (Table 4; Fig. 3). However, only insignificant changes take place in the leaf area among the metals. Generally, growth retardation rate of root and shoot is negligible in all the metals except Hg in which growth retardation is significant compared to the control plants. But leaf area values remain almost unchanged in all the treatments.

Effect of each metal on root growth of *C. amboinicus* differ considerably (Table 3). Owning to the direct contact of growing root to heavy metal ions present in the nutrient medium, immediate effect is root growth retardation in the case of Al, Cr, Cu and Hg. Root length of *C. amboinicus* treated with Al is significantly reduced during 4-20 days. Primary toxicity symptoms of Al are root and root hair growth inhibition which are entitled to involve in interrupted uptake of mineral ions such as Ca, P and K (Linderberg and Greger, 2002). Earlier, Kochian (1995) stated that in addition to root growth inhibition, Al interferes with nutrient absorption, growth retardation and low productivity. Stunted root growth and Al toxicity are correlated in such a way that root apical region accumulates more Al than the growth region of the root (Delhaize *et al*., 1993; Matsmoto, 2000). According to those authors, Al accumulation in the growing root tissue is related to Al sensitivity and inhibition of

root growth is proportional to the Al content in the root tissues. Al has been reported to interfere with cell division quickly, damage the cell structure, decrease nutrient and water intake, and block root elongation and growth (He *et al*., 2019).

Moreover, the toxic or beneficial effect of Al on plant growth depends largely on the growing conditions, Al concentration and duration of exposure, plant species and physiological age (Huang *et al*., 1992; Bojó rquez-Quintal *et al*., 2017; Ofoe *et al*., 2022).

Nonetheless, the Al treatment barely affected the growth of the roots and shoots (Table 3&4). However, Al phytotoxicity is associated primarily with disruption of roots structure, physiology and functions of plants in general and roots in particular (Kochian and Shaff, 1991; Taylor, 1998). Since roots are the first organ to come in contact with the toxic heavy metals, root growth rate is affected largely. Several studies have shown the adverse effect of Al on plant growth in general and root growth in particular (Kochian, 1995; Matsmoto, 2000).

According to Chang *et al*. (1999), Al forms cross links between cell wall protein- extensin and pectins within the cell wall and also can bind to membrane protein and lipids (Linderberg and Griffiths, 1993). Most of the Al ions are bound into the cell wall and only very few enter plasma membrane. According to Matsmoto (2002), toxic effect of Al includes stunted root growth, poor root hair development, swollen root apex, stubby and brittle roots which reduces poor absorption of water. However, root growth elongation, by Al toxicity differ among plant species and cultivars. The inhibition of root elongation has been extensively used as a trait for screening Al-tolerant cultivars (Dipierro *et al*., 2005; Lu *et al*., 2020).

Aluminum leads to biochemical/physiological/anatomical alterations in roots. It is widely accepted that the most pronounced impact of the Al-induced phytotoxicity is blockage of root growth (Wang *et al*., 2016; Jaskowiak *et al*., 2019). A heavy impact on the cellular membrane of root cells emerges just in the early minutes of the exposure (Rout *et al*., 2001; Singh *et al*., 2017).

Over the past decades, several studies have demonstrated the effect of Al on growth and productivity in several plant species (Kochian *et al*., 2005; Sade *et al*., 2016; Bojó rquez-Quintal *et al*., 2017; Singh *et al*., 2017). Generally, most of these studies reported the toxic effects of Al and the tolerance mechanisms of plants (Awasthi *et al*., 2019; Borges *et al*., 2020; Fang *et al*., 2020), while a few reported beneficial effects on plant growth (Muhammad *et al*., 2019; Sun *et al*., 2020). On the other hand, stimulation of root and whole plant growth has been recognized as a beneficial effect of Al in several plant species (Arasimowicz-Jelonek *et al*., 2014; Muhammad *et al*., 2019; Liu *et al*., 2020).

Aluminium treatment resulted in only negligible differences in leaf area compared to control (Table 5). In fact, early in the growth phase, leaf growth is found to increase slightly, suggesting a slight stimulating effect of Al. This observation is consistent with the findings of Kochian (1995); Taiz *et al*. (2015), who suggested that Al has a slight beneficial effect on plant growth at lower concentrations.

Inhibition of root growth in *C. amboinicus* subjected to Cr is more or less similar to Al (Table 3). However, the involvement of each metal in the root growth is not similar. Decrease in the root growth is a well-documented effect of Cr on plants (Tang *et al*., 2001). Iqbal *et al*. (2001) reported root growth inhibition and DW decrease in *Caesalpinia pulcherrima* by Cr stress. General root growth inhibition by Cr is reported to be due to impaired cell division (Suseela *et al*., 2002).

As a tolerance mechanism in plants, stress-induced root length reduction reduces the region of exposure to metal stress (Miras-Moreno *et al*., 2014). The reduction in root length of plants on Cr treatment was also reported in *Cicer arietinum* (Medda and Mondal, 2017), *Citrus aurantium* (Shiyab, 2019), *Camellia sinensis* (Tang *et al*., 2012), *Oryza sativa* (Ma *et al*., 2016; Chen *et al*., 2017), *Pisum sativum* (Rodriguez *et al*., 2011) and *Vigna radiata* (Singh *et al*., 2021). Cr (VI)-mediated root growth inhibition may result from a reduction in cell size and an inhibition of cell division in the elongated region (Peralta *et al*., 2001) or the chromosome distortion in root apex cells, or the stress ethylene production

(Shinwari *et al*., 2015; Shahid *et al*., 2017). Plants take up Cr through roots and it gets transported to shoot, which affects the growth of stem and leaves. The Cr stress induced decrease in shoot length may be due to ultrastructural damage in leaf mesophyll cells, which impairs photosynthesis and ultimately results in decreased shoot development (Singh *et al*., 2021). The reduction in shoot length and leaf area was supported by the studies on *Helianthus annuus* (Fozia *et al*., 2008), *Allium cepa* (Nematshahi *et al*., 2012), *Citrus aurantium* (Shiyab, 2019), *Camellia sinensis* (Tang *et al*., 2012), *Myriophyllum spicatum* (Chandra and Kulshreshtha, 2004), *Vigna radiata* (Singh *et al*., 2021), and *Oryza sativa* (Ma *et al*., 2016; Chen *et al*., 2017).

Root length of *C. amboinicus* treated with 80 µM Cu is almost similar to that of Cr (Table 3). At all intervals of growth, Cu induces slight reduction of root growth compared to the control. Excess Cu is reported to cause root growth retardation in plants (Navari-izzo *et al*., 2006). According to those authors, root growth retardation before shoot growth inhibition is due to the preferential Cu accumulation in the roots. Reduced root growth is presumably due to the quick transport of Cu to the shoot as reported by Sheldon and Menzees (2005).

Shoot length of *C. amboinicus* subjected to excess Cu resulted in only negligible differences in all samples compared to the respective controls. According to Nair and Chung (2015), concentration of Cu in plants must always be in an optimal level as a prerequisite for proper functions since higher concentration of Cu persuade amendments in almost all metabolites.

Another effect of Cu toxicity in plants is decline in leaf area. In *C. amboinicus*, leaf area is reduced significantly as a result of Cu treatment. Garcia *et al*. (1999) and Reckova *et al.* (2019), opined that decline in leaf area under Cu environment is attributed to lignin accretion in xylem and ultimately leads to thickening and hardening of cell walls and hence pose adverse effect on cell wall development and leaf enlargement by decreasing its elasticity.

Mercury treatment resulted in maximum root growth retardation as compared to the control as well as other treatments (Table 3). Root length is the

earlier prime effect of heavy metal toxicity and as an important parameter indicative of the magnitude of toxic effects and the concentration of Hg (10 µM) given to impart growth retardation symptoms are not directly proportional. The time taken to induce root growth decrease is also very short and it starts right from the beginning of the exposure to Hg treatment (Table 3). At higher concentrations, mercuric chloride can interfere with the physiological and biochemical functions of *Centella asiatica*. It can lead to a decrease in morphological parameters, such as plant size, leaf size, and root development (Snehalatha and Jayaram, 2016). Toxicity of Hg has been reported in many plants like exposure to Hg in barley reduces shoot and root growth, stomatal conductance, and alters gene expression related to water stress (Lopes *et al.*, 2013). Mercury treated chickpea seedlings showed reduced growth and leaf pigment content in a concentration-dependent manner (Ahmad *et al*., 2018). Mercury toxicity induces oxidative stress in cucumber seedlings, resulting in plant injury and reduced root and shoot length (Cargnelutti *et al*., 2006). According to Malar *et al*. (2015), *Eichhornia crassipes* plants showed inhibited growth, decreased photosynthetic pigment synthesis, and accumulation of Hg ions in root, leaf, and petiole tissues after Hg treatment. Long-term Hg pollution leads to growth inhibition and quality degradation of ginger, reducing its yield by nearly 25% (Xu *et al*., 2020) and even at very low concentration of Hg it causes hazards to growth and development (Sandmann and Boger, 1983; De *et al.*, 1985; Orcutt and Nilsen, 2000). The *Vigna radiata* plants showed a significant reduction in seed germination, seedling and root length, and seedling DW after Hg treatment (Muhammad *et al*., 2015). The higher the concentration of Hg, the greater the negative effect on the plants.

An interesting observation in the stem morphology of plants treated with Hg is the development of many outgrowths appearing as rudimentary branches, and in the transverse sections, they appear as lenticels consisting of loosely arranged cells (Fig.2). These structures are exactly similar to normal lenticels, originating from the periderm (Fahn, 1982) in the transverse section of *C. amboinicus* (Fig.11). The function of the lenticel is connected with gas exchange similar to that of stomata of leaves (Fahn, 1982, 2000). In *Chromalaena odorata*, the lenticels contain densely

stained material without any specific cellular structure and these stained masses could be accumulated Hg complexed with the stain toluidine blue (Swapna *et al*., 2015). Through the opening of these lenticels, volatile form of Hg may be getting expelled by volatilization in accordance with the view of Moreno *et al*. (2005, 2008). In addition to the trichomes developed on the leaf epidermis, large numbers of lenticels present on the stem also are involved in the phytovolatilization of Hg which is a mode of detoxification.

Tolerance indices based on root length are regarded as a good metric for determining how resilient plants are to heavy metal stress (Majeed *et al*., 2019). Tolerance index calculated on the basis of root length of the treatment plants in comparison with that of the control (Turner, 1994). The low TI value found in *C. amboinicus* after heavy metal treatments suggested that the high concentration of heavy metal severely inhibits root growth and interferes with cell division. Decrease in TI was gradual in each interval and the difference in TI was insignificant when compared with each treatment (Table 6; Fig. 6).

Primary toxic effect of heavy metals is found to be root growth inhibition and this parameter is an ideal scale or index to measure the degree of tolerance (Wilkins, 1978; Wong and Bradshaw, 1982). Rooted propagules of *C. amboinicus* exhibit growth retardation soon after the exposure of Al, Cr, Cu and Hg. Tolerance index of *C. amboinicus* towards excess metal concentration also be correlated to the bioaccumulation potential of the plant which will be explained in detail under the section on bioaccumulation in the forthcoming pages.

The tolerance index of plants after heavy metal treatment varied depending on the specific plant species and the type of heavy metal. In the study, it was found that *Thespesia populneoides*, *Leucaena leucocephala*, and *Delonex regia* showed different levels of tolerance to lead and cadmium toxicity (Gladkov and Gladkova, 2021). *Leucaena leucocephala* exhibited the best germination response and was least affected by lead and cadmium toxicity, while *T. populneoides* and *D. regia* showed moderate tolerance (Ismail *et al*., 2013). In another study, it was mentioned

that plants have the ability to tolerate and alleviate metal pollutants, acting as filters or traps.

Maximum DW % was observed in the order Hg > Cu > Al > Cr compared to the control (Table 12). DW distribution of the roots of *C. amboinicus* is found to be more compared to the stem and leaf. Irrespective of these significant differences in the optimal concentration of Al, Cr, Cu and Hg on *C. amboinicus* to impart toxicity maintaining survival, DW distribution in the root showed gradual increase and varied between the metals. Maximum DW was observed in Hg treatment compared to other elements and control. DW content of stems exhibited insignificant reductions in plants treated with heavy metals. The possibility of reduction of DW in stem tissues might be the disruption of vascular tissues leads to the impaired photosynthesis and damaged cellular functions.

Dry matter content of leaves of plants treated with Hg exhibited enhancements as compared to control and other treatments, in which the increase of DW% was highest on 20^{th} day. Water absorption and translocation get impaired in the root itself, *ie*., it adversely affects relative water content in leaves. Since the DW is reciprocal to MC, maximum DW% was resulted in Hg treatment. The presence of Hg-binding peptides in the leaves suggests the ability of plants to accumulate and sequester Hg ions may have led to changes in the leaf composition, potentially affecting the DW. Mercury treatment resulted in cell degeneration and thickening of cell walls in rice plants. This result can be directly correlated to increased DW%. Increased DW% of roots and leaves of plants treated with all heavy metals is found to be related to the water potential of the plants because water potential is known to be affected by heavy metal absorption (Costa and Morel, 1994) and resultant stunted growth (Lepp, 1981; Shaw and Rout, 1998; Orcutt and Nilsen, 2000; Fodor, 2002).

Aluminium exposure of plants may result in decreased productivity and show sign of toxicity symptoms (Kochian, 1995; Silva, 2012). Toxicity and tolerance levels vary from plant to plant and according to whom plant responses both tolerance and toxicity phases causing significant growth retardation (Reichman, 2002). Total DW% of *C. amboinicus* plants cultivated in nutrient solution containing

Al is more or less similar to the control plants which is an indicative of the negligible stress induced by Al and it is well established that mild concentration of Al resulting in no change at lower concentration rather than metal is known to stimulate growth, though it is not an essential metal for plants (Kochian, 1995).

Treatment with Cr and Cu results in reduction of moisture content since DW is reciprocal to moisture content, while plants subjected to Hg treatment exhibit significant decrease in moisture content as compared to the control and other treatments. The pattern of moisture content in *C. amboinicus* is control > Al >Cu > Cr > Hg. Moisture content is an important controlling factor of metabolism and resultant growth which undergoes drastic changes due to these metals. To what extend can *C. amboinicus* undergo moisture stress variation is solely depend on the metal species in one hand and the concentration employed in the present study, on the other. Total dry matter production in *Nymphea alba* under heavy metal toxicity in general and Cr in particular get severely affected by Cr concentration above 2.5 µM in the nutrient medium (Vajpayee *et al*., 2001).

Decreased DW due to Cr treatment is in correlation with the reports of Del Bubba *et al*. (2013) in *Nicotiana langsdorffi*, Basit *et al*. (2022) in *Glycine max*, Qing *et al*. (2015) and Wakeel *et al*. (2018) in *Medicago sativa*, *Festuca arundinaceae*, and *Trifolium repens*, Chen *et al*. (2020) in *Pennisetum sinese*, Singh *et al*. (2021), and Jabeen *et al*. (2016) in *Vigna radiata*. Phyto-toxic effects of Cr on growth and biomass production have also been studied in *Brassica oleracea* (Ahmad *et al*., 2020), *Solanum lycoperscium* (Javed *et al*., 2021), *Cicer arietinum* (Singh *et al*., 2020), *Brassica juncea* (Handa *et al*., 2018) and *Brassica parachinensis* (Kamran *et al*., 2021).

Copper stress resulted in the reduction in the tissue RWC and MC% but enhanced DW% in the root, cotyledonary and primary leaves of *R. communis* seedlings, and the variations were severe in the cotyledonary leaves (Sameena and Puthur, 2023). The reduction of DW% due to Cu treatment was associated with reduced water use efficiency and photosynthesis under abiotic stress conditions, including heavy metal stress (Sharma *et al*., 2020). According to reports, increased

trace metal concentrations inhibited plant growth by causing nutritional imbalances, water shortages, and ultimately reduced photosynthesis (Bankaji *et al*., 2014).

The plant DW% decreased after Hg treatment in all the studies. In the study by Muhammad *et al*. (2015), Hg treatment at concentrations of 1, 3, 5, and 7 mM resulted in significant reductions in seedling DW of mungbean compared to the control. In the study by Malar *et al*. (2015), exposure of *Eichhornia crassipes* seedlings to varying concentrations of Hg led to a significant inhibition of plant growth rate. In the study by Muddarisna *et al*. (2013), the addition of ammonium thiosulphate increased the accumulation of Hg in plant shoots and roots, and the average increase in DW of maize grown on previously remediated media was lower compared to the control treatment. In the study by Mukarlina *et al*. (2016), the Hg treatment did not have a significant effect on the DW of *Wedelia trilobata* plants. In the study by Ansari *et al*. (2009), the growth characteristics, including plant dry mass, were inhibited by increasing levels of Hg treatment

Morphological modifications and/or impacts of these selected metals Al, Cr, Cu and Hg are also be correlated with the anatomical changes observed under light microscopic and scanning electron microscopic studies. Metal accumulation in plants resulted in alterations of the root, stem and leaf anatomy (Adejumo *et al*., 2021).

Aluminium treatment resulted in significant reduction of root growth which is super imposed with disappearance of roots hair and damaged piliferous cells (Fig.8A). These observations have already been done in plants subjected to Al (Linderberg and Greger, 2002). According to whom, Al toxicity leading to low root growth inhibition causes nutrient deficiency resulting in crop productivity. Another effect of Al on root anatomy is the occurrence of distinct endodermis with specific cell wall thickening and conspicuous cortical region. According to Chang *et al.* (2002), Al forms cross links between pectin and protein within the cell wall and also can bind to membrane proteins (Linderberg and Griffiths, 1993) and these changes all together result in cell wall thickening of endodermis of roots. Root growth inhibition and changes in root morphology such as swelling of root tips and atrophy

of root hair also have reported due to Al toxicity of plants (Huang *et al*., 2014b). According to Wang *et al*. (2016), Al in micromolar concentration can cause severe toxic effect on plants particularly in roots. According to Riyaz *et al*. (2018), root tip plays a decisive role in sensing Al toxicity and tolerance to Al mainly depends on the structure of roots. In order to reduce Al accumulation associated damage, the plants develop a series of physiological mechanisms (Kochian *et al*., 2015; Riaz *et al*., 2018). Anatomy of *C. amboinicus* stem treated with Al results in the increased number of xylem vessels (Fig. D). Anatomy of *C. amboinicus* leaves treated with Al also shows slight variations in the reduced size and vasculature in the midrib region of lower epidermis (Fig. E). Scorched appearance of leaves is one of the toxicity symptoms of Al in plants (Yan *et al*., 2019).

Aluminium treatment caused various anatomical changes in the plants. In rice, Al toxicity resulted in a decrease in root and shoot diameter, reduced number of metaxylem vessels in the root, and closure of stomata in the leaves (Samad *et al*., 2019). The midrib region of the lower epidermis was found to be reduced in size so also the vasculature due to the Al treatment in leaves. According to Silva *et al*. (2016), the Al stress caused an increase in leaf blade thickness and parenchyma layers, as well as lignification of root tissues in the Al-sensitive sunflower cultivar. These changes in plant anatomy can be used as markers of Al sensitivity in sunflower. According to Čiamporová, (2002), after Al treatment, there are alterations in root morphology, including root thickening, disturbances of root peripheral tissues, and initiation of lateral roots closer to the root tip.

Chickpea plants exposed to Al showed a reduction in the length of the primary root, number of lateral roots, and size and number of vessels in the root. The stem had a larger area of sclerenchyma cells, and the leaf had a reduced number of palisade parenchyma cells (Samad *et al*., 2021). It resulted in closure of stomata, and increased number of trichomes on the leaves. Corn plants grown in Al solutions exhibited inhibited root growth, fewer lateral roots, and morpho-anatomical alterations in the leaf, including a thinner cuticle layer and smaller metaxylem and protoxylem diameter in the vascular bundle (Batista *et al*., 2013). *Stenocalyx*

dysentericus seedlings showed tolerance to Al, with increased root relative elongation and internal detoxification mechanisms observed in the root tissue (Rodrigues *et al*., 2016). Water mimosa plants exposed to Al sulfate exhibited yellowish leaves, softer roots, shrinkage of the stele area, and color change in the leaf tissue.

Chromium treatment induced significant structural alterations in the plant organs (root, stem and leaves) of *C. amboinicus*. Conspicuous stelar region consisting of large xylem vessels and pith are observed in *coleus amboinicous* treated with Cr. Disappearance of root hair and damaged piliferous layer of roots are found to occur due to Cr stress in *C. amboinicus* (Fig. 9A). The depositions were the most noticeable alteration, according to SEM photographs. The unique characteristics of the epidermis, cortex, vascular region, and pith were visible in the scanning micrograph of the control root and shoot; however, these tissues became distorted when exposed to Cr. This outcome is consistent with reports from plants such as *Cicer arietinum* and *Vigna radiata* (Chandra *et al*., 2010; Medda and Mondal, 2017). Compared to the leaf, the root and stem had more damage, with the cell structures severely deformed (Fig. 14F) and some depositions observed in the tissues. This is consistent with the findings of Wakeel *et al*. (2020). The reduction of Cr (VI) to Cr (V) during the symplast-mediated endodermis passage may account for the root's deformities. Cr (V) is then retained in the root cortex cells.

Chromium may translocate from the root to the stem as a result of ongoing exposure to Cr. Stem anatomical deformities may result from increased ROS production at greater Cr concentrations, which leads to oxidative damage at the cellular level (Shanker *et al*., 2005). According to Shanker *et al*. (2004), cell dysfunction brought on by Cr build up in roots and the removal of Ca^{2+} ions from binding sites in cell walls and plasma membranes can also cause harm to root and stem tissues. The sequestration of Cr in the root vacuoles and poor translocation from root to shoots thus alters the structure of the root (Shanker *et al*., 2004) and in *C. amboinicus*, it can be correlated with the reduced root growth. Scanning electron micrographs showed the presence of some aggregates on the mesophyll cells of the

Cr treated leaves (Fig. 14D). Rucinska-Sobkowiak (2016) reported that apoplast resistance to water flow is increased by cell wall thickening caused by metal deposits and/or incrusting substances in the cell walls. This will in turn eventually reduces the transportation of water and soluble mineral nutrients from the root to the shoot and this observation can be corroborated with the decreased macro and micro-elements content detected in shoot than roots in the SEM-EDX data.

The increase in xylem wall thickness was observed in the root, stem, and leaf tissues of the *Parkinsonia aculeata* plants treated with Cr (Zhao *et al*., 2011). Distinct structural modifications, such as an increase in the thickness and diameter of the xylem walls, were observed in *Cicer arietinum* in response to Cr stress (Medda and Mondal, 2017). Additionally, the presence of cell structural distortions and Cr deposit inclusions in the xylem wall and inner parenchyma cells were observed in *Zea mays* seedlings (Singh *et al*., 2015). According to Purohit and Varghese (2003), in tomato and brinjal the increase in xylem wall thickness and structural modifications in the xylem were indicative of the plant's response to Cr stress. These findings are in accordance with our result.

Coleus amboinicus subjected to Cu treatment showed complete disappearance of root hairs, damaged piliferous layer, indistinct vasculature and thick-walled angular xylem vessels. Since Cu is an essential metal taking part in many metabolic processes, Cu toxicity damage roots with the symptoms ranging from disruption of roots piliferous layer and reduced root hair proliferation to severer deformation of root structure (Sheldon and Menzies, 2005). Marques *et al.* (2018) and Hossain *et al.* (2020) stated that, at higher concentration (sub-lethal) of Cu affect root growth and morphology. According to Cai *et al.* (2014) and Bochientio *et al.* (2015), Cu treatment results in reduced root growth and declined in the root biomass and root volume which is directly allied with cell division. Batool *et al.* (2015) highlighted that apparent decline in root growth linked with decreased cell division leading to enhancement in cell wall thickness of roots after Cu stress. Noticeable anatomical feature of the roots of *C. amboinicus* subjected to Cu is the presence of densely stained spots / particles distributed appear as embedded over the

section (Fig. 15D). These spots indicate the Cu content stained and localized in the root.

The plant anatomy was affected by Cu treatment in different ways. In *Alternanthera tenella*, high Cu concentrations led to a decline in cell sizes of stem and leaf tissues, increased formation of druse crystals, and a lower number of active reaction centers (Martins *et al*., 2020). According to Gong *et al*. (2019), spinach seedlings showed changes in cell ultrastructure, such as thinner chloroplast membranes and disruption of cell walls, when exposed to high Cu concentrations. In spring barley, excess Cu caused degradation of the epidermis in roots, a decrease in the diameter of the stele, and disruption of cell walls and cytoplasmic membranes (Minkina *et al*., 2020). *Billbergia zebrina* exhibited anatomical changes in roots, such as increased stomatal index and thicker exodermal cell walls, as a response to high Cu concentrations (Martins *et al*., 2016). According to de Souza *et al*. (2017), *Erythrina fusca* showed cell plasmolysis in the root cortical area and changes in thylakoid membranes when exposed to high Cu concentrations.

Essential oils (EOs) are oily aromatic liquids extracted from aromatic plants. Essential oils are naturally extracted plant constituents that capture the scent and flavor of the plant, and are mainly found in leaves and flowers. *Coleus amboinicus* are rich in essential oils. According to Sharifi-Rad *et al*. (2016), EOs are complex mixtures of volatile phytoconstituents, primarily terpenes, and can be extracted from various parts of the plant using techniques such as hydrodistillation and steam distillation. EOs have been used in traditional medicinal and healing systems for their diverse array of bioactive phytoconstituents, which have shown antioxidant, antimicrobial, anticancer, and anti-inflammatory properties, among others (Moccia *et al*., 2020). In addition to their therapeutic uses, EOs are widely utilized in various industries such as aromatherapy, cosmetics, food preservation, and insecticides. The extraction of EOs can be done using conventional methods or advanced techniques, with the latter being more environmentally friendly, efficient, and time-saving. Overall, EOs play a significant role in plants by providing their characteristic scent

and flavor, as well as offering potential health benefits and applications in various fields.

Oil globules in *C. amboinicus* play a significant role in its medicinal properties. The essential oil of *C. amboinicus* contains active compounds such as carvacrol and β-caryophyllene, which have antimicrobial properties (Changbunjong *et al*., 2022). The oil globules in *C. amboinicus* also contain other compounds such as rosmarinic acid, apigenin, and caffeic acid, which contribute to its antifungal activity against *Microsporum canis* (Mangathayaru *et al*., 2005). Additionally, the oil globules in *C. amboinicus* have been found to inhibit the biofilm formation of *M. canis*, making it a potential source of natural antifungal compounds. Overall, the oil globules in *C. amboinicus* play a crucial role in its antimicrobial and antifungal properties, making it a promising candidate for the treatment of various infections. The genres in which they could be found are sorted in a small number of families: Lamiaceae, Lauraceae, Asteraceae, Rutaceae, Myrtaceae, Poaceae, Cupressaceae and Piperaceae (Bruneton, 1999). EOs are biosynthesized, accumulated and stored in specialized histological structures, the secretory glandules (Bouwmeester *et al*., 1995; Bruneton, 1987). Svoboda and Greenaway (2003) confirmed that, there are two types of secretory glandules: those located on the plant surfaces with exogenous secretion and those located inside the plant in internal organs with endogenous secretion. They are also localized in the cytoplasm of some secretory cells in one or more plant organs.

External secretory tissue is located outside of the plant which includes epidermal papillae, glandular trichomes (secretory glandules or bristles) and non-glandular trichomes (Ascensão and Pais, 1998; Turner *et al*., 2000; Baran *et al*., 2010; Rezakhanlo and Talebi, 2010; Kremer *et al*., 2014). Internal secretory tissue is located inside the plant, and they include secretory canals, schizogenous pockets (or secretory pockets) and cells with intracellular secretion (Asbahani *et al*., 2015).

Coleus amboinicus shows both external and internal secretory tissues. The schizogenous pockets (or secretory pockets) are an intercellular space, often spherical, which is filled by EOs droplets synthesized by the cells which border it. It

is evident in the SEM images of *C. amboinicus* (Fig. 17A). Due to their hydrophobic nature of EOs and their density often lower than that of water, they are generally lipophilic, soluble in organic solvents, immiscible with water hence they attain a spherical or globular structure.

An interesting observation in the Cu treatment of *C. amboinicus* is the removal of oil globules from stem and leaf. Scanning electron microscopic images gives the clear evidence for the removal of oil globules (Figs. 17B & 18B). The possible reason for its removal may due to the reduction of a secondary metabolite named alpha-bergamotene after Cu treatment. It is a major component of essential oils in various plants and it contributes to the aromatic profile and therapeutic properties of essential oils. The characters and properties of this component are well explained in the forthcoming pages.

Induction of defense mechanisms may involve the production of secondary metabolites or in resource allocation. Such responses could indirectly affect the presence of oil globules. Zheljazkov and Nielsen (1996) indicate that elevated concentration of metals in growth medium affect essential oil content in some aromatic species. According to Zheljazkov *et al*. (2006) Cu treatment (150 mg/L) reduced oil content in *Anethum graveolens*. According to Elzaawely *et al*. (2007), the yield of oil extracted from Cu-treated *Alpinia zerumbet* plants (0.07%) was lower than that extracted from non-treated leaves (0.08%). Removal of oil globules is in agreement with previous studies which reported that when plants exposed to metal stress such as Cu, a decrease in volatile oil production occurred (Hashem and Sahab, 1999; Rajeswara Rao *et al*., 2000: Abou Zeid, 2002).

Mercury toxicity imposes many adverse effects on root growth of *C. amboinicus*. Similar to Cr, root hairs were totally absent in plants treated with Hg (Fig. 11A). Multilayered and larger rhizodermal cells showing broken and uneven margins were another impact of Hg toxicity. Occurrence of many trichomes and lenticels are characteristic features of *C. amboinicus* stem treated with Hg. Stem consisted of thick-walled endodermal cells and reduced number of vasculatures. Toxicity of Hg imparted substantial modifications in the leaves. Presence of large

number of epidermal hairs on both upper and lower epidermis of leaves is another feature of Hg toxicity. Many epidermal hairs are transformed into developed trichomes. Effect of Hg on root growth of *C. amboinicus* is reduction of root length and damaged root morphology and anatomy, and thickening of cell wall showed breakage also. Inhibition of root growth is a typical characteristic of Hg toxicity in plants as reported in tomato (Cho and Park, 2000) and cucumber (Cargnelutti *et al*., 2006), and the effect is found to be concentration dependent. Increased DW, if roots subjected to Hg, is found to be related with the abnormal proliferation of cells. The absorption is in consistent with the hypothesis part done by Arduinni *et al*. (2004) who obtained that Hg at lower concentrations induces cell proliferations.

Scanning electron microscopic studies confirmed most of the observations of light microscopy and cellular damages such as breakage of epidermal cells, damaged cells of medullary ray and broken cell walls of xylem cells. Epidermal layer of stem *C. amboinicus* treated with Hg exhibit several trichomes and numerous well-developed lenticels (Figs. 16E & 16F). These lenticels appear as blisters on the stem and can be seen with naked eyes. The well-developed trichomes and more distributed lenticels are entitled to function in crucial role as one of the detoxification mechanisms of Hg toxicity in *Chromalaena odorata* (Swapna *et al*., 2015).

A characteristic observation of leaf anatomy in *C. amboinicus* subjected to Hg stem is the development of large number of epidermal hairs on both upper and lower epidermis (Fig. 11E) and most of them got developed into trichomes. Similar to the structural modifications of stem under Hg stress, leaf structural changes also are directly related to detoxifications. Formations of structural details of lenticels on the stem of *C. amboinicus* treated with Hg and the epidermal cells appear as broken due to the occurrence of lenticels. Structure of leaf epidermal patterns are clearly seen in SEM studies (Fig. 16E). Breakage of thin-walled mesophyll cells results in the formation of some lacuna in the mesophyll tissue. Functional aspects of the structural modifications in the stem as well as leaf dealt with and correlated to the

mechanism of detoxification of Hg toxicity are discussed in the forthcoming sections.

The leaf micromorphological characters of *C. amboinicus* were significantly modified upon exposure to heavy metals. Stomatal distribution showed significant differences between the stomata of lower and upper epidermis and more values were showed by stomata of lower epidermis (Table 7). The increased number of stomata may be attributed to the enhanced ability of plants to regulate gas exchange and water balance under stressful conditions (Swapna *et al*., 2015). Heavy metal stress frequently favours stomatal closure, which is one of the major limitations to photosynthesis under heavy metal stress in plants (Sagardoy *et al*., 2010). The disturbances in the stomatal opening and closing trigger enhanced production of ROS and inhibit the electron transport chain, and thereby reducing the plant metabolism (Hoque *et al*., 2021). According to Gálusová *et al*. (2020), the reduction in the size of the stomata during heavy metal stress is to avoid further loss of water from the leaves. Moreover, heavy metals stimulate the accumulation of stress hormone ABA in the cells, and ABA mediates a series of signal cascades to regulate the stomatal aperture by guard cells and thereby reducing the transpirational loss of water (Bharath *et al*., 2021). Some plants excrete heavy metal complexes to the outside of the plant body via salt glands, trichomes, or subsidiary and guard cells of stomata, all of which protect plant cells from heavy metal toxicity (Choi *et al*., 2001; Broadhurst *et al*., 2004; Cosio *et al*., 2005; Rascio and Navari-Izzo, 2011).

Effect of Al, Cr, Cu and Hg on leaf anatomy is directly linked with morphological aspect such as stomatal index and structural modifications of guard cells, size and shape of stomatal pore. Stomatal index of control on both upper and lower epidermis remained unchanged during the growth of 20 days. In *C. amboinicus* treated with Al only negligible changes occur in the upper epidermis whereas, in the lower epidermis stomatal index values are more. Stomatal index values for *C. amboinicus* treated with Cu are more than the control. Maximum values of stomatal index occur in plants subjected to Hg and the values increased significantly during growth. Mercury and Cr effects resulted in an increase of

stomatal index compared to control and other treatments. Increased stomatal index in both upper epidermis and lower epidermis due to the exposure of Al, Cr, Cu and Hg may cause enhanced transpiration rate and resulted water stress. A significant role of increased stomatal index in the detoxification of Hg is apparent as it is related to the bioaccumulation pattern and phytovolatilization of Hg from the leaf through the stomata as reported in *Brassica juncea* (Moreno *et al*., 2004a, 2007).

Stomatal variations due to heavy metal treatments have been observed in several plants. In faba bean (*Vicia faba*), different doses of cadmium did not lead to a change in the number of stomata, but the stomatal index was decreased at higher doses (Piršelová *et al*., 2021). In soybean (*Glycine max*), exposure to arsenic and cadmium resulted in diverse adjustments of stomata and pavement cells, with one cultivar showing a decrease in stomatal size and the other using larger stomata (Gálusová *et al*., 2020). According to Sujatha and Priyadarshini (2011), in pigeon pea (*Cajanus cajan*), lead and cadmium caused significant reductions in photosynthetic activity and affected stomata, with cadmium having a greater effect. In faba bean, heavy metals such as Hg^{2+}, Pb^{2+}, Zn^{2+}, and La^{3+} inhibited stomatal movements, possibly by affecting water channels, while K^{+}, Na^{+}, and Mg^{2+} had no visible effects (Yang *et al*., 2004). Overall, heavy metal treatments can lead to changes in stomatal characteristics and functions, including stomatal size, density, conductance, and net photosynthesis rate.

Scanning electron micrographs of *C. amboinicus* stomatal distribution showed significant differences in number of stomata per unit area (cm^2). Stomatal distribution of Al and Cu treated plants with the control appeared more or less similar (Table 7), whereas Cr and Hg treatment resulted in little increase. Stomatal opening also found to be affected due to heavy metal treatment. In the control, almost all stomata appeared opened while during metal treatments stomata changed its structure (Fig. 21A). Stomatal opening was more found in the lower epidermis of Hg treated plants and in the leaves of Cr treated plants, stomata are widely opened.

Stomatal index values are indirectly related to water relations, particularly transpiration (Meidner and Mansfield, 1968). Increased stomatal indices in *C.*

amboinicus due to Cr and Hg treatment indirectly related to the water stress tolerance. The increased stomatal index values in *C. amboinicus* due to Cr treatment is in consonance with the view of Barcelo *et al*. (1985); who suggested that, leaf water potential in Cr treated *Phasolus vulgaris* remain unchanged.

Aluminium treatment has been found to cause significant changes in stomatal density, stomatal index, and stomatal shape coefficient in common buckwheat (*Fagopyrum esculentum*) (Smirnov *et al*., 2014). Özyiğit and Akinci (2009), reported that stomatal closure and no difference in total stomatal potential conductance index as characteristics of resistance in *Urtica pilulifera* plants treated with Al, cadmium and drought. *Urtica pilulifera* seedlings exposed to Al treatment showed significant effects on stomatal perimeters, diameters, and areas in all treated plants compared to control plants.

The structural variation of stomata after Cr treatment was observed in several studies. In *Vigna radiata* seedlings, Cr treatment resulted in altered stomatal structure, with deformed and less differentiated stomata frequently present (Gautam *et al*., 2021). Similarly, in tomato and brinjal plants, Cr caused a decrease in the number of stomata and epidermal cells, as well as a decrease in stomatal size (D Subrahmanyam, 2008). Örçen *et al*. (2013) reported that in Asian tobacco varieties, an increase in Cr concentration led to a significant increase in stomatal density, while stomatal length decreased. Additionally, in sweet potato plants, higher levels of Cr treatment resulted in damage to stomatal traits and a reduction in stomatal size (Purohit and Varghese, 2003). Overall, these studies demonstrated that, Cr stress can lead to structural variations in stomata, including changes in stomatal density, size, and morphology, which is in accordance with the present study.

The studies conducted by Fu *et al*. (2015) in Cu accumulator and excluder plants revealed that, heavy metal accumulators show substantially higher leaf stomatal density, numerous and longer epidermal hairs, and thick cuticle covered on the guard cells than metal excluder plants. It was reported that *Ricinus communis* plants have higher stomatal resistance during water-scarce situations, which is one of the drought tolerance mechanisms along with the early physiological reactions

(Papazoglou *et al*., 2020). This would ensure the increased resistance of the plant towards reduced tissue water status and help the plant to acclimatize the stress situations. Furthermore, the closing of the stomata during heavy metal stress resulted in reduced transpiration and hence reduced the translocation of toxic metal ions from roots to shoots (Shi *et al*., 2019). According to Sameena (2023), the closure of stomata during Cu stress increases the tolerance of the *Ricinus communis* plant towards reduced tissue water status and helps the plant to acclimatize the stress situations. Furthermore, stomatal closure resulted in reduced transpiration and hence reduced the translocation of Cu^{2+} from roots to shoots.

Scanning Electron Microscopy and Energy Dispersive X-ray analysis (SEM-EDX) was conducted on *C. amboinicus* to understand the structural details of anatomy of root, stem and leaf and also analysis the quantity of mineral ion presents in different region of the plant body. Scanning electron microscopic studies conducted on the root, stem and leaf of *C. amboinicus* treated with Al, Cr, Cu and Hg provided more details of cellular structure and localization of the metals in the section (Figs.22-26). Compared to the light microscopic observation of histologically stained sections, a confirmation of SEM and EDX enabled the quantitative and qualitative distribution of all nutrient elements as well as heavy metals given to the plant present in the section exposed to SEM followed by EDX. A combination of SEM-EDX shows the distribution pattern of different elements in specific tissues (root, stem and leaves) of plants. SEM images provide a magnified view permitting the observation of ultrastructural details. In addition to SEM, EDX enables to visualize the metal ions distribution in the tissues also.

The distribution of nutrient elements in plants was disrupted as a result of the conflict between the poisonous and nutrient elements for binding sites (Shackira and Puthur, 2019). In addition to being necessary for plant growth and development, mineral nutrients also aid in the reduction of various stresses, including heavy metal stress (Jalloh *et al*., 2008). According to Courbet *et al*. (2019), from a chemical perspective, chemical interactions and nutrient dynamics may arise from chemical

similarities or analogies between elements that share a common transporter for element uptake.

Aluminum is not found in its pure form; rather, it is combined with fluoride, silicon, and other elements (Silva, 2012). In the cytoplasm, Al creates irreversible macromolecular complexes (Poschanrider *et al*., 2008). Due to the release of Al chelating ligands and the interaction of Al with the cell wall, Al inhibits the elongation of the root tip (Delhaize and Ryan, 1995; Matsmoto, 2000).

Heavy metal treatment of *C. amboinicus* causes a decrease in the amount of carbon in the roots. This reduction in carbon export to the roots in the form of photoassimilates may be the result of the restricted ability of leaves to fix CO_2. The reduced oxygen concentration in the roots likewise showed this restricted CO_2 fixation. There has been a decrease in carbon absorption due to the insufficient ability of leaves to absorb CO_2 (Ruehr *et al*., 2009).

The enhanced Ca distribution in the roots of Cu-treated plants indicates the activation of the Ca-mediated signaling processes and confirms its role in stomatal closure and thereby preventing excess water loss during stress. It was reported that there was a cross-talk between the Cu-induced intracellular signals like calcium and H_2O_2 levels, leading to the activation of Ca-dependent gene expression of antioxidant proteins via CaMs and CDPKs in *Ulva compressa* exposed to Cu stress (González *et al*., 2012). The intake of essential minerals like calcium (Ca), magnesium (Mg), phosphorus (P), and iron (Fe) has been shown to be reduced by excessive Cr, which masks the sorption sites and forms insoluble complexes (Basile *et al*., 2012). In addition to being necessary for plant growth and development, mineral nutrients also aid in the reduction of various stresses, including heavy metal stress (Jalloh *et al*., 2009).

The accumulation of P content in the root tissues of *C. amboinicus* seedlings was significantly enhanced during all metal stress. Phosphorous is involved in various metabolic and regulatory processes in plants and constitutes the structural skeleton of biomolecules such as ATP, NADPH, nucleic acids *etc*. (Razaq *et al*., 2017; Bechtaoui *et al*., 2021). Therefore, the enhanced accumulation of P content

helps the plant to counteract the heavy metal induced growth and photosynthetic damages *via* enhanced production of ATP and NADPH. This would also ensure the enhanced mitochondrial activity and activation of antioxidant enzymes. According to the report of Hayes *et al*. (2019), enhanced Ca levels in the plant also modulate the leaf cell-specific allocation of P to the palisade mesophyll cells during high P conditions that also contribute to the efficient photosynthesis in these leaves even under Cu toxicity.

The increased activity of antioxidant enzymes is attributed to the higher level of K in the Cr-treated roots, and K also serves as a coenzyme or activator of a number of crucial enzymes (Mengal, 2007). K^+ ions activate a vast variety of enzymes, which encourage cell elongation and maintain osmotic balance.

Exposure of *C. amboinicus* to heavy metal stress enhanced the distribution of S in the root. Sulphur has several functions in plant growth and development, ranging from being a structural element of macro-biomolecules to influencing multiple physiological processes including abiotic stress tolerance (Zenda *et al*., 2021). Being an essential component of amino acid cysteine, and thereby GSH and phytochelatins, S offers the plants protection against oxidative stress induced by heavy metals. Therefore, the enhanced S in roots of *C. amboinicus* seedlings can be correlated to enhanced synthesis of S containing amino acids and thereby GSH and phytochelatins. The toxicity imparted by Cu^{2+} in *C. amboinicus* might be partially alleviated by the enhanced accumulation of these metal chelators.

Silicon improves the vigor and resistance of plants when exposed to environmental stresses as well as gives mechanical strength to the stem (Luyckx *et al*., 2017). The significant enhancement in the distribution of Si was observed in the root tissues. It has been reported that Si forms complexes with heavy metals, and their deposition and redistribution are responsible for better resistance of the plants against toxic metal ions (Lux *et al*., 2020; Janeeshma *et al*., 2021).

Copper may compete with other micro and macroelements like Zn, Ca, Mg, Fe, etc. for uptake by transceptors or sensors across membranes because it is a divalent cation (Fan *et al.*, 2021). The distribution of Fe was found to be

significantly enhanced in the root tissues of *C. amboinicus*. Iron is essential in many of the vital processes of plants, including DNA synthesis, photosynthesis, respiration and nitrogen reduction (Rai *et al*., 2021). In photosynthesis and respiration, Fe serves as a critical cofactor for electron transport components in the chloroplast and mitochondrion (Rout and Sahoo, 2015). The crosstalk between Cu and Fe has been documented in many previous studies (Rai *et al*., 2021). In order to prevent heavy metal mediated inhibition of photosynthesis and respiration, the entry and accumulation of Fe were promoted, which helps to alleviate metal toxicity to some extent.

Chlorine distribution was also significantly enhanced in the roots of *C. amboinicus* exposed to heavy metal stress. Due to the osmotic property of Cl^- ions, Cl accumulation resulted in the lowering of osmotic potential and increase in water contents of plants under stress (Koch *et al*., 2021). As B is an essential micronutrient and boric acid is a major component of the Hoagland nutrient medium, a basal level of B was observed in the root tissues of the plant. Though B is required for the normal growth and development of plants, its specific role in plant metabolism is still being debated (Lewis, 2019; Brdar-Jokanović, 2020).

Coleus amboinicus plants underwent substantial damage to their root cortex and epidermal cells as a result of Cu stress. The inhibition of metal entry into cells during heavy metal stress is linked to this cellular breakdown of shoots, one of the plant tolerance strategies used when subjected to severe stress (Ren *et al*., 2016). Similar to our findings, Al faifi and El-Shabasy (2021) noted the deterioration of the cell wall materials of cortex and epidermis in the roots of *Cenchrus ciliaris* plants cultivated near to a cement dust factory contaminated with several heavy metals, including Cu. Copper mediated interferences in plant water relations may be the cause of changes in the number and size of xylem vessels in the roots mediated by Cu stress, which lowers the moisture content and tissue water status in the shoots.

Cu^{2+} sequestration in the xylem walls of roots inhibits the harmful ions from moving to the shoots, as evidenced by the substantial increase in xylem wall thickness and the presence of clotted depositions in the xylem. According to reports,

cell walls are the primary location of metal immobilization, and the polysaccharides found in cell walls have the highest capacity to bind metal (Chmielowska-Bąk and Deckert, 2021).

Plants respond to metal stress by modulating the composition of their cell walls by increasing the quantities of polysaccharides, callose, and pectins. This thickens the cell wall, increases its potential to bind metal, and immobilizes it (Sarath *et al*., 2022). Comparable to the current investigation, Rucinska-Sobkowiak *et al*. (2013) noted thickening of the cell walls and deposits of calcium in the vascular cylinder of lupin roots subjected to lead stress.

Similarly, Ca^{2+} modifies the physical properties of the cell wall by ionically interacting with pectin, most specifically in the unmethylated homogalacturonans (most abundant polymer of pectin, having negative charge when unmethylated), resulting in the enhanced rigidity of the cell wall (Thor, 2019). Thus, the improvement in Ca distribution following $CuSO_4$ treatment provides clear evidence that stress-mediated improvements in cell wall composition facilitate metal sequestration processes (Olmedo *et al*., 2021; Wang *et al*., 2021). These findings suggest that the inhibition of water transport from the apoplast that envelops the xylem tissues may also be caused by metal-induced wall thickening and depositions. Moreover, the decreased water content could also be explained by the Cu deposition in the xylem walls.

The highest enhancement in the xylem wall thickening was observed in the root, stem and leaves of *C. amboinicus* subjected to Cu stress, along with higher distribution of Ca in roots, as evidenced from the EDX results. The regulation of metal transport from root to shoot was reviewed in detail by Angulo-Bejarano *et al*. (2021), and they observed that, as the roots are the first contact zone of heavy metals, significant anatomical changes can occur in the roots along with the efficient cell wall sequestration of the metal. Likewise, majority of the transported metal was sequestered in the cell walls of stem and a comparatively to a lesser extent in the leaves also. The enhanced synthesis of cell wall materials and associated thickening and metal sequestration to the cell wall was reviewed by Chmielowska-Bąk and

Deckert (2021), and the authors observed that about 50-70% of the uptaken metal could sequester into the cell wall.

The leaves are shielded from metal-induced photosynthetic damages by the binding of the metal ions in the cell walls of stem, which stops the ions from travelling further to the leaf. According to Hasan *et al*. (2017), metal-induced autophagy, a biological self-destruction mechanism that maintains cellular homeostasis by engulfing damaged organelles into the vacuole during stressful situations, is responsible for the minimal breakdown of xylem tissues shown in the SEM pictures of stem.

The distribution pattern of chlorophyll a, chlorophyll b and total chlorophyll content of leaves is found to be altered due to the exposure with the treated metals (Table 13). Since changes in pigments are associated with visual symptoms of growth disorders and photosynthetic production, chlorophyll is frequently measured to evaluate the effects of environmental stress (Parekh *et al*., 1990). Photosynthesis is a key phenomenon that contributes significantly to plant growth and development (Kalaji *et al*., 2017). Heavy metal stress resulted in the degradation of the thylakoid membrane protein complex, which resulted in the blockage of electron transfer and thereby diminished the photosystem activities (Shackira and Puthur, 2019). The degradation of photosynthetic pigments that cause deficiency in light-harvesting capacity is caused by exposure to high levels of heavy metals (Srivastava *et al*., 2021). Inhibition of biosynthesis of photosynthetic pigments is one of the primary events in plants during heavy metal stress and as a consequence, it shows lower photosynthetic efficiency, slower plant growth and development.

During the period of growth, in the control plants an exponential increase of chl a, chl b and total chlorophyll content was observed. As a result of Al stress, chl a content was decreased insignificantly, whereas chl b registered considerable increase during 4-8 days thereafter remained almost similar to control. Total chl content of *C. amboinicus* treated with Al was lower than the control plants. Inhibition of total chlorophyll has been reported due to Al stress in Buck wheat and soybean leaves (Milivejevic *et al.*, 2000). Since Al treatment resulted maintenance or slight

stimulation in the chlorophyll contents is an indicative of the lack of toxicity in *C. amboinicus* due to Al as observed in the other parameters of the present study. But the continuous exposure leads to its limited toxicity.

Negreanu-Pirjol *et al*. (2019) carried out bioaccumulation studies and effects of Al on plant growth in three culture plants species. According to them, sunflower showed enhanced synthesis of photosynthetic pigments, specifically chl a and carotenoids, at the highest Al concentration (250 mg/kg) whereas, wheat showed a decrease in chlorophyll and carotenoid synthesis with increasing Al concentration. In the case of mustard, variations in pigment levels could not be linked to Al concentration.

The ratio of chl a/b in the leaves of *C. amboinicus* subjected to Al stress is declined drastically than the control (Table 13). In rice plants reduction of chlorophyll content and low values of chl a/b ratio was reported which was accompanied by marked decrease in gross photosynthesis and photosynthetic rate. Al stress resulted in a reduction of chl a, chl b and carotenoid contents in the leaves of rice plants (Samad *et al*., 2020). According to Tohidi *et al*. (2015) *Brassica napus* plants, that treated with Al, the significant decrease in shoot length, root length and chl a, b content was observed but malondialdehyde (MDA) and total sugars content significantly increased.

Coleus amboinicus plants treated with Cr showed an increase in chl a during early period of growth followed by a decline and almost similar trend was shown in the case of chl b content also due to Cr toxicity (Table 13).

Inhibitory effect of Cr (VI) on chlorophyll molecules have been documented in a number of plants (Singh *et al.*, 2013) and distribution pattern and quantity are concentration and exposure period dependent. According to Vajpayee *et al.* (2001), chl a is more sensitive to Cr (VI) in *Nymphaea alba*. In *Phyllanthus amarus*, chl b is more sensitive to Cr (VI) (Rai and Mehrothra, 2008). The decreased content of chlorophyll pigments in response to Cr (VI) response has been attributed to impaired activities of various enzymes such as delta aminolevulinic acid dehydratase (ALAD)

and protochlorophyllide reductase involved in chlorophyll biosynthesis (Vajpayee *et al.*, 2001; Shanker *et al.*, 2005; Ganesh *et al.*, 2009).

The reduction in chlorophyll content of the plantlets treated with Cr makes it easy to assess the enhanced toxicity. Chinmayee *et al*. (2014) had also reported similar findings in *Jatropha curcas*. The degradation of photosynthetic pigments that cause deficiency in light-harvesting capacity is caused by exposure to high levels of heavy metals (Srivastava *et al*., 2021). Chromium stress induced reduction in chlorophyll content was reported in plants like *Citrus aurantium* (Shiyab, 2019), *Triticum aestivum* (Subrahmanyam, 2008). *Najas indica*, *Vallisneria spiralis* and *Alternanthera sessilis* (Chandra and Kulshreshtha, 2004). The adverse effects of Cr on the vital photosynthetic pigments including chlorophylls were reported by Boonyapookana *et al*. (2002) and Henriques (2010). Gill *et al*. (2015) hypothesized that Cr at higher doses inhibits the activity of d-aminolaevulinic acid dehydratase, an essential enzyme in pigment synthesis that results in inhibiting chlorophyll biosynthesis. Studies on Cr stress in *Vigna radiata* (Singh *et al*., 2021), *Nicotiana tabacum* (Bukhari *et al*., 2016), *Sorghum bicolour* (Kumar *et al*., 2019), *Brassica juncea* (Mahmud *et al*., 2017; Singh *et al*., 2017), *Cicer arietinum* (Singh *et al*., 2020), *Zea mays* (Habiba *et al*., 2019), *Brassica napus* (Gill *et al*., 2015), and *Brassica oleracea* (Ahmad *et al*., 2020) have reported similar results.

According to Bera *et al.* (1999), chl pigments-chl a, chl b and total chl in mung bean seedlings are found to be decreased irrespective of the concentrations of Cr. In the present study, chl a/b ratio declined significantly compared to the control during early stages of growth and thereafter remained unaltered (Table 13; Figs. 30-33). Reduction of total chl, chl a and chl b in wheat (Sharma *et al*.,1992) and chl a/b ratio in *Salvinia minima* (Nichols *et al.*, 2000) revealed the adverse effect of Cr mainly due to inactivation of the protein and enzymes involved in chlorophyll synthesis.

Since photosynthetic pigments like chlorophyll a and b are inhibited by Cr, the net photosynthetic rate is impacted, which lowers dry biomass (Liu *et al*., 2008). Shanker *et al.* (2004) stated that a reduction in the chl a/b ratio by Cr toxicity is an

indication of chloroplast damage like reduced size of the peripheral part of the chloroplast antenna complex. Those authors further opined that the decrease in chl b due to Cr toxicity could be due to the destabilization and degeneration of the proteins of the peripheral part of the chloroplast. Another possibility suggested by Shankar *et al.*, (2005) is the inactivation of photosynthetic enzymes involved in the chlorophyll biosynthetic pathway as reported in most plants under Cr stress.

In *C. amboinicus* plants treated Cu stress, chl a showed an increase initially and then retained the status quo throughout the growth period. It showed an enhanced chl a and chl b content compared to the control and the a/b ratio remained unaltered (Table 13). Cu stress induces various toxicity symptoms in the metabolism of plants, of which photosynthesis is the most sensitive process (Giannakoula *et al*., 2021). Total chl content was very high in Cu treated *C. amboinicus* (Fig. 32). Even though the changes in the distribution of chl pigments in *C. amboinicus* are significant, a regular or uniform pattern cannot be seen in the experimental plants as a result of Cu stress. It can be speculated that the fluctuations in the chl pigment content is not directly related to the chl synthesis and Cu content because excess Cu seems to affect biosynthesis of photosynthetic system by altering protein and pigment composition resulting in disturbing chloroplast and thylakoid membrane and triggers oxidative stress on the content of pigments electron carriers and subsequently photosynthetic electron transport (Vassilev *et al*., 2003; Gongaley-Mandoza *et al*., 2013; Rehman *et al.*, 2019). Kupper *et al.* (2009) suggested that Cu induced inhibition of photosynthesis is due to Cu toxicity on photosystem II reaction centre in *Crassula helmsii* plants. According to Hossain *et al*. (2020), photosynthetic pigments such as chl a and b in lentil plants get reduced at higher concentration of Cu (3 mM $CuSO_4$). In *C. amboinicus* enhanced contents of chlorophyll pigments indicates lack of high toxicity of Cu the standardized concentration of Cu is only 80 µM (Table 13) and at this level, toxicity symptoms are imparted but the plants survive at this concentration. Hence a speculation can be made that the toxicity of Cu to interfere in the chlorophyll synthesis is concentration dependent in accordance with the view of Hossain *et al*. (2020). According to whom only at high concentration of Cu, synthesis of chlorophyll pigments gets affected adversely and

since Cu is an essential nutrient element for general metabolism and chlorophyll synthesis and functions in particular, the homeostasis of Cu toxicity is concentration dependent. Cu toxicity induces chlorosis and the primary target of Cu toxicity in plants is photosystem II in the thylakoid membrane (Yruela,2009; Bernel *et al*., 2004).

According to Vijayarengan and Jose (2014), toxic levels of Cu substitute for Mg in the chlorophyll and also induce deficiencies in iron and magnesium ions in the plant, leading to the inhibition of protochlorophyllide and phytoene synthesis, which are the intermediary products of chlorophyll and carotenoid biosynthesis respectively, and thereby causing reduction in the chlorophylls and carotenoid levels. Binding of Cu to the -SH group of ALA (aminolevulinic acid) dehydratase (converts aminolevulinic acid to porphobilinogen) and protochlorophyllide reductase (participates in the porphyrin and chlorophyll metabolism) also destroys the structure and function of chloroplast (Myśliwa Kurdziel et al., 2004). Dall'Osto *et al*. (2012) reported that, carotenoids protect the chlorophylls from peroxidation, so that elevated levels of chlorophyll destruction were observed due to reduction in carotenoids during Cu stress. Xue *et al*. (2013) noticed that, the prime reason for leaf chlorosis during metal stress is the destruction of pigments in the older leaves and inhibition of its biosynthesis in younger leaves.

Chlorophyll content of *C. amboinicus* treated with Hg showed significant variations rather fluctuation compared to the control during all intervals (Table 13). Chlorophyll a exhibited an increasing trend during early intervals followed by decline than the control whereas chl b remained almost unchanged, so also, chl a/b ratio. Toxicity of Hg has been reported in many plants and even at very low concentration, it causes hazardous to plant growth (Sandman and Boger, 1983). Effect of Hg in chlorophyll synthesis and content is not yet elaborately studied due to several limitations and complexity of involvement of Hg ions in metabolism in general and chlorophyll synthesis in particular. Mercury is an inhibitor of protein synthesis and function as well as enzyme activity (Baker and Walker, 1989; Cseh,

2002; Sahu *et al.,* 2012). The photosynthetic carbon reduction cycle enzymes are inhibited by mercury (Van Assche and Clijsters, 1990; Shaw, 1995).

Coleus amboinicus plants are very sensitive to Hg stress at very low concentration of $HgCl_2$ (10 µM), at this concentration manifestations of toxic symptoms such as stunted root growth, inhibition of germination etc have been reported (Orcutt and Nilsen, 2000). Inhibition of photosynthetic enzymes activity in *Phaseolus* seedlings have been reported as a result of Hg toxicity (Prasad and Prasad, 1987; Parmer *et al.*, 2002). Results of these studies, show a decline in the chlorophyll content due to Hg toxicity and according to those authors, the decline chlorophyll content is linked to photosynthetic efficiency. According to Prasad *et al.* (1991), Hg imposes a direct effect on photosynthetic electron transport causing generation of reactive oxygen species (ROS).

As mentioned earlier, substantial fluctuations occur in the distribution of chlorophyll pigments in *C. amboinicus* as a result of Hg toxicity. So, it seems that the concentration (10 µM) selected for the investigation on *C. amboinicus* imparts very mild and irregular impact of Hg on the metabolism of chlorophyll pigments. But exact mechanism and role of Hg ions in the pathway is obscure. Nevertheless, inhibitory effect of enzymes on chlorophyll synthesis and photosynthetic carbon cycles cannot be ruled out because many enzymes such as RUBISCO and other functional proteins are vulnerable to Hg toxicity (Shaw, 1995). Impact of Hg ions on proteins/enzymes structure and function are based on the affinity of Hg ions to -SH group of amino acid component of the proteins. According to Jain and Puranik (1993), in *Zea mays* one of the mechanisms by which Hg exert the toxicity is by interaction with essential -SH group of enzymes and structural proteins. Another impact of Hg is the competition with other metals such as Cu or zinc within the cell (Marschner, 1983).

Almost all toxic effects of Hg on general metabolism particularly chlorophyll synthesis pathways in *C. amboinicus* are being shown by the distribution of chlorophyll components in such a way that very low toxicity is imposed by the $HgCl_2$ (10 µM) given to the plants. Translocation and accumulation pattern of Hg

(which will be dealt with the results in forthcoming pages) also play a significant role in the synthesis of chlorophyll molecules. During growth for a period of 20 days in nutrient solution, Hg content is reduced due to the loss of Hg by phytovolatilization and the proportional reduction of Hg results in low toxicity and considerable occurrence of chlorophyll content during later period of growth.

Inhibition of chlorophyll synthesis due to Hg toxicity was reported in many plants (Kupper *et al*., 1998; Mystiwa-Kurdziel and Strazalka, 2002). Kupper *et al*. (1998) interpreted the results of many trace elements- Cu, Cd, Hg, Pb, Zn etc. interfering with chlorophyll synthesis by substituting Mg^{2+} of chlorophyll molecules and resultant inhibition of photosynthesis. According to Ahmed and Tajmir-Riahi (1993), Hg interacts with light harvesting process of chlorophyll in *Lactica sativa* leaves. In wheat varieties treated with heavy metals, total chlorophyll content was decreased to 70% and the reduction may be the result of inhibition of enzymes involved in chlorophyll biosynthesis. Striking changes have been reported in the chloroplast fine structure, reduction in grana stacks and amount of stroma etc. by the toxicity of heavy metals (Kupper *et al*., 1998). Stefanov *et al*. (1993) suggested that, the reduced rate of photosynthesis is associated with chloroplast damage in maize plants treated with heavy metals like Pb.

In all photosynthetic plants, other than chlorophylls a second group of pigments are carotenoids. These molecules participate in light absorption and photoprotection and also play a minor role as an accessory pigment in the light energy harvesting molecule absorbing and transferring of light energy to chlorophyll pigments. In addition to the role of the assembly of light harvesting the complex molecules of carotenoids play another indispensable function in protecting the photosynthetic apparatus from photooxidative damage such as formation of singlet state of oxygen and damage caused by these molecules and its reactive products. Carotenoids are integral constituents of the thylakoid membrane and are usually associated intimately with many of the protein that makeup the photosynthetic apparatus.

In *C. amboinicus* carotenoid content show no significant increase in control plants whereas in plants treated with Al, there occurred a significant reduction during early period of growth and only negligible changes occurs in between the intervals. The increase of carotenoids in the control plants is found to be related to normal functions. Cr > Hg > Al > Cu is the order of carotenoids in *C. amboinicus* after the stress. Cr and Hg shows highest carotenoid content shows the level of imparted toxicity in *C. amboinicus* plants. Carotenoid content of plants treated with Cr is very high and gradually increase throughout the growth period (Fig. 33). This observation reveals the magnitude of Cr toxicity in *C. amboinicus* as shown by other growth parameters. In *C. amboinicus* plants treated with Cu, carotenoid synthesis shows only negligible changes and values of carotenoids is a sign of minute level of toxicity induced by Cu. Distribution patterns of carotenoids in the plants subjected to Hg, also reveals the enhanced level of toxicity induced by Hg. The distribution pattern of carotenoids in the control and experimental samples appears as a marker or scale of toxicity of Hg in *C. amboinicus*, because reduced values of chlorophyll molecules indicating the toxicity pattern and the defensive role played by carotenoids function hand in hand.

Carotenoid pigments play an essential role in photoprotection when the photosynthetic membrane gets damaged by large amount of energy which cannot be sorted by normal phytochemical reactions (Taiz *et al*., 2015). According to those authors carotenoids are one of the important ROS scavenging molecules. As mentioned earlier, Hg induces maximum toxicity in *C. amboinicus* in the terms of parameters like reduced SOD activity (Table 21), proline and MDA (Tables 18 and 20) content etc resultantly production of comparatively more ROS can be noticed and hence the ROS scavenging activity of carotenoid cannot be ruled out in *C. amboinicus*.

Numerous biochemical and physiological processes of plants get interfered and /or impaired by heavy metal toxicity. As mentioned earlier, growth retardation is the primary visual symptom of Al, Cr, Cu and Hg toxicity. Manifestations of toxicity can also be interpreted in terms of occurrence and distribution of primary

metabolites because the decreased or disturbed distribution and allocation of metabolites such as sugars, starch, amino acids and protein which are prone to get bind with ions of toxic metals.

Occurrence of sugars content in *C. amboinicus* plants exposed to Al remained almost unaltered in the roots, stem and leaves indirectly revealing negligible toxicity of this metal (Table 14; Figs. 34-36). Total sugar content of *C. amboinicus* plants treated with Cr and Cu found to increase in all parts of the plant compared to the control and between the metals Cr and Cu the difference is negligible whereas, Hg treatment results in a significant increase of sugar content compared to the control and other treatments. Significant variations in the distribution of sugars take place in leaves of control and all treatments and only slight fluctuations are observed in stem and root (Table 14). Maximum content of sugar is present in the leaves of *C. amboinicus* treated with Hg indicating more toxicity of this metal which has reported to interfere almost all enzyme activities of metabolism in actively growing plants (Orcutt and Nilsen, 2000; Moreno *et al*., 2004b).

Sugars are the primary fuel for energy metabolism, and are important signalling chemicals (Rosa *et al*., 2009). The characterizing of the changes in soluble sugars and starch accumulation in leaves is essential for determining the phytotoxicity of Cr (Sinha *et al*., 2018). The enhancement in the soluble sugars and starch concentration of plants when treated with Cr was reported by Prado *et al*. (2010) and Rodriguez *et al*. (2012) and their results can be corroborated with the findings of the present study. Distribution of total soluble sugar content of *C. amboinicus* plants treated with Al remain almost unaltered in the root, stem and leaf exhibiting more or less similar metabolism indirectly revealing the negligible Al toxicity (Table 14). Total sugar content of plants subjected to Cr and Cu is increased in all tissues compared to the control. In contrast to our results, Armendariz *et al*. (2016) observed the reduced accumulation of photoassimilates in soybean plants exposed to arsenic stress due to lower leaf area and and damage to the photosynthetic system.

In plants, occurrence of enhanced sugar content indicates impaired metabolism which is pertinent to metal toxicity (Cseh, 2002). Irrespective of the difference in the nutrient essentiality between Cr and Cu, toxicity related metabolism of sugars is more or less similar in *C. amboinicus*. Maximum sugar content in the roots, stem and leaves is an indicative of the lack of normal metabolic activity pertaining to the energy required activities. According to Hall (2002), toxicity of heavy metals is reported to be resulted from the decreased content of primary metabolites due to interaction of metabolism particularly enzymes. Significant increase (accumulation) of sugar in all parts of *C. amboinicus* indirectly indicates the disrupted metabolism of sugars. This observation is in accordance with the view of Orcutt and Nilsen (2000) and they opined that increase in accumulation of sugar is associated with the alteration of metabolite composition due to environmental constraints. The increase in sugar content subjected to heavy metal stress is an indication of metal induced up-regulation of sugar metabolism in order to meet the increased energy demands to cope up with the stress situations (Mishra *et al*., 2014). Increased sugar content can contribute to osmoregulation, helping plants maintain water balance and turgor pressure. The accumulation of soluble sugars as a result of exposure to stress can be positively correlated with the increase of relative leaf water content and plays a main role in adjusting the osmosis of plant. Elevated sugar levels can stimulate the production of stress-related enzymes-peroxidases and catalases, which detoxifies ROS and protecting plant cells from oxidative damage (Gangola *et al*., 2018).

In growing plants, distribution of soluble sugars indirectly linked to photosynthetic rate which is integrated with chlorophyll pigment content. In *C. amboinicus* subjected to Al stress, sugar content remains almost unaltered showing negligible toxicity induced by Al which is found to be slightly stimulatory to growth. In addition to the pivotal role of sugars as metabolites, monomeric sugars (glucose, fructose etc.) play vital roles in plants under stress as compatible solute molecule (Bray *et al*., 2002). These monomeric sugars are released from polymeric forms such as starch fructans etc. in plants under various stresses particularly water

and metal stress and these monomers get polymerized to facilitate rapid and reversible osmotic adjustment when stress is removed.

According to Reichman (2002), Cu toxicity plays a role on root growth inhibition. Metal impact in the form of growth inhibition, since normal growth is impaired due to heavy metal and distribution pattern of metabolites, particularly primary metabolites vary in plants under stress. Metabolites such as sugars, amino acids and proteins register significant increase, different organs, growth inhibition, is imposed by difference in the allocation of metabolites due to binding of metallic ions to the metabolites.

Concentration of starch content in the root, stem and leaves of *C. amboinicus* showed marginal increase in the initial stages and then the content remained unchanged after the heavy metal treatment (Table 15). The plant may prioritize the maintenance of starch reserves for essential metabolic processes and growth even under stress conditions. But during last interval of growth, there occurred a significant depletion of starch in the leaves of plants treated with Cr, Cu and Hg and all these metals are found to impose more toxicity in the order Hg < Cr < Cu. This observation reveals the toxicity level of these metals on one hand and reduced tolerance potential because as mentioned earlier inter-conversion of sugars and starch is an important tolerance mechanism of plants under metal stress (Bray *et al*., 2002). In the presence of heavy metals, starch is immobilized and nutrient sources become limited. Moreover, a reduction in proteolytic enzyme activity and an increase in protein and amino acid content can be observed under heavy metal stress (Seneviratne *et al*., 2019).

The effect of Al treatment on starch content in plants varied depending on the plant species and the specific experimental conditions. According to Mishra and Dubey (2008), the starch content in rice plants decreased after Al treatment. Results suggest that Al^{3+} toxicity in rice seedlings impairs the metabolism of starch and sugars and favours the accumulation of hexoses by enhancing the activities of sucrose hydrolyzing enzymes. In another study done by Muthukumaran and Rao (2013), the starch content in rice seedlings declined during leaf senescence in

response to Al exposure. According to those authors, the activity of α-amylase, an enzyme involved in starch degradation, decreases with increasing periods of exposure to Al. Study conducted by Lidon *et al*. (1997) found that, increasing Al concentrations had no significant effect on starch concentrations in *Zea mays* plants. The starch concentration did not change significantly with increasing Al concentrations. This result can be corroborated with our result.

The starch content in plants was found to be affected by Cr treatment. In the study by Ezhilvannan and Sharavanan (2023), it was observed that higher concentrations of Cr in the soil resulted in a reduction of starch in *Gossypium hirsutum* due to the impact on the photosynthetic pigments. Similarly, Sihag and Joshi (2016) found that increasing levels of Cr (VI) in the soil led to a decrease in non-structural carbohydrates, including total soluble sugar and reducing and non-reducing sugar, in sorghum plants. Additionally, del Real et al. (2014), reported that *Silene vulgaris*, when grown with Cr (VI), exhibited stunted growth and accumulation of starch in the leaves, indicating an impairment in carbon utilization and assimilation. In our experiment, an exorbitant increase of starch content in leaves during initial days of Cr exposure followed by a decline was seen. The reason for the decline in final stage may be due to limiting factors like nutrient deficiency because no excess supply was provided. Eleftheriou *et al*. (2015) also observed alterations in starch accumulation in the root tip cells of *Arabidopsis thaliana* exposed to Cr (VI), with starch grains and electron dense deposits occurring in the plastids. Overall, Cr treatment had a negative impact on starch content in plants.

Sfaxi-Bousbih *et al*. (2010) stated that, Cu excess caused a limitation in the hydrolysis of sucrose and starch, leading to their subsequent hyperaccumulation in the cotyledons of bean seeds. This restricted the starch and sucrose breakdown in reserve tissue, as evidenced by the inhibition in the activities of α-amylase and invertase isoenzymes. Therefore, the starch content increased after Cu treatment. Starch content in *Trigonella foenum-graecum* was significantly decreased at both 30 and 60 days of growth when *Nostoc muscorum* was applied to the soil with different concentrations of Cu. Additionally, Cu stress resulted in a reduction in the activity

of antioxidant enzymes, such as peroxidase and superoxide dismutase. The study highlights the potential toxicity of Cu when present in excess amounts and the need for strategies to mitigate its negative effects on plant growth and metabolism.

Ruixin *et al*. (2022) found that, following Hg treatment in wheat plants, starch content increased, suggesting an acceleration of the aging process in the tissues. Following the Hg treatment, the chloroplasts lost their oval shape and contacts and gradually increased the amount of starch deposited, which suggested that the aging process of tissues had accelerated. The membrane cell was thereby deformed by mercury chloride. This was consistent with our findings that the addition of Hg to the treatment led to higher starch content when compared to the control and other treatments.

Amino acids are the precursors and components of proteins, which play a vital role in the metabolism and development of plants. During exposure to abiotic stresses, including heavy metal stress, plants accumulate particular amino acids, which may be involved in stress tolerance, including the role played as osmolytes, signalling molecules, modulation of ion transport and involvement in metal ion detoxification, regulation of pH and stomatal opening (Rai, 2002; Xu *et al*., 2012; Zemanová *et al*., 2015). Reduced amino acids content indicates more protein synthesis as reported in rice grown under heavy metal stress (Verma and Dubey, 2003).

As discussed earlier, growth retardation is the primary visual symptoms of toxicity due to Al, Cr, Cu and Hg. Manifestation of toxicity can also be interpreted in terms of the occurrence of primary metabolites because the decreased and/or disturbed distribution and allocation of metabolites such as sugars and proteins which are prone to get bind with metal ions of the toxic elements. In addition to the obvious role of amino acids in protein synthesis, amino acids perform essential function in both primary and secondary plant metabolism. Amino acids serve to assimilate nitrogen and transport nitrogen source to sinks and some others serve as precursor to secondary metabolites. Thus, the synthesis of amino acids directly or indirectly controls various aspects of plant growth and development. Recent

investigations of genes involved in amino acid biosynthesis revealed that this is a dynamic process controlled by metabolic, environmental and developmental functions.

Total free amino acid content of plant parts such as root, stem and leaves of *C. amboinicus* cultivated under different environment set up by cultivating rooted propagules in nutrient solution artificially contaminated with known quantities of Al, Cr, Cu and Hg reveals significant reductions in the contents of the amino acid during a specific period of growth. Decreasing trend of total free amino acid content was observed, which can be correlated with the accumulation of secondary metabolites. Amino acids form precursors of alkaloids, phenol and lignin, they are often channeled to secondary metabolism for their synthesis. It may occur by shifting of amino acid biosynthesis pathway to that of secondary metabolites (Rai, 2002).

Variations are observed in the distribution of total free amino acids among root, stem and leaves and these changes during comparable intervals of growth period. Treatment with Al resulted in negligible reduction of amino acids since toxicity of Al is very mild and total metabolism of *C. amboinicus* is not deviated from the control plants. Total free amino acid content in roots of *C. amboinicus* registered only insignificant fluctuations compared to the control and between the metals the changes were meagre (Table 16). But in the stem, there occurred considerable reduction of free amino acids. Total free amino acid content of leaves also reduced in the plants subjected to Cr and Cu while Hg treatment resulted in drastic reduction than the control as well as other treatments (Table 16). Reduced content of free amino acid of leaves indicates the impaired metabolism of amino acids synthesis or enhanced protein synthesis which gets stored transiently in the leaves as a result of metal stress.

It was reported that Cu forms stable complexes with amino acids in the xylem sap, facilitating the transport of metals from root to shoot in hyperaccumulator plants (Shabbir *et al*., 2020). The increase in amino acids creates a nitrogen reserve, resulting in the dynamic adjustment of nitrogen metabolism,

thereby enhancing antioxidation mechanisms and osmotic adjustment under stress conditions (Zhong *et al*., 2017).

Total protein content of stem and leaves of *C. amboinicus* exhibited a significant enhancement in plants treated with Al, Cr, Cu and Hg. Comparatively leaves are protein rich compared to root and stem in the control and treatments (Table 17). Protein distribution pattern in roots of *C. amboinicus* showed significant increase in plants treated with Cr and Hg while the increase in protein content in the root was only marginal. One of the reasons for increased protein content in *C. amboinicus* treated with Cr and Hg is found to be due to the synthesis of stress proteins particularly phytochelatins which have already been reported in plants treated with heavy metals such as Cr and Hg (Grill *et al*., 1985; Salt *et al*., 1998; Chowdary and Panda, 2005).

In order to reduce or prevent damage caused by heavy metals, plants synthesize small cysteine-rich oligomers, called phytochelatins (PCs) at the very beginning of metal stress (Ashraf *et al*., 2010; Pochodylo and Aristilde, 2017). Notably, PC synthesis plays the most crucial role in mediating plant tolerance to heavy metals (Clemens, 2006; Emamverdian *et al*., 2015). The mechanism of heavy metals detoxification is not only limited to the chelation, but also involves accumulation and stabilization of heavy metal in the vacuole through formation of high molecular weight (HMW) complexes with PCs (Jabeen *et al*., 2009; Furini, 2012). Alike PCs, metallothioneins (MTs) are also naturally-occurring intracellular cysteine-rich major metal-binding proteins, which are used by cells to immobilize, sequester, and detoxify metal ions (Capdevila and Atrian, 2011). The proposed roles of MTs include (a) participation in maintaining the homeostasis of essential transition metal ions, (b) sequestration of toxic heavy metals, and (c) protection against intracellular oxidative damage induced by stress (Hossain *et al*., 2012).

Transition metals such as Cu, Fe, Mn and Zn are essential for all organisms because they play critical roles in a variety of physiological processes. For example, Cu is required for photosynthesis, respiration, ethylene perception, ROS metabolism and cell walls in plants (Burkhead *et al*., 2009; Peñarrubia *et al*., 2010). A number of

studies suggested the involvement of plant MTs in the participation of metal ion homeostasis, especially for Cu, during both vegetative growth and senescence (Taiz *et al*., 2015).

A number of studies have shown that heavy metals and metalloids inhibit refolding of chemically denatured proteins in vitro, obstruct protein folding in vivo and stimulate aggregation of nascent proteins in living cells (Sharma *et al*., 2011; Jacobson *et al*., 2012). For example, Cr has been shown to trigger oxidative protein damage and protein aggregation in yeast by enhancing mRNA mistranslation (Holland *et al*., 2007). Exposure to heavy metals leads to over expression of proteins, stimulating stress-related genes and activating signaling mechanisms, causing oxidative stress and negative impacts on plant growth and cell death. The biosynthesis of metal binding cysteine rich peptides that function to immobilize, sequester and detoxify the metal ions is thought to be the central for detoxification of heavy metals (Clemens, 2001; Viehweger, 2014).

Irrespective of the difference in the concentration of metals given to the treatment, the variations of the protein content are found to be related to the impact of the toxicity level on the distribution and/or it is a detoxifying mechanism of metal toxicity as opined by Cheng (2003), according to whom, lack of solubility of protein is a detoxifying mechanism in plants and role of PCS also cannot be ruled out in this context. Plants exposed to heavy metals like Cd, Cr, Cu, Hg etc. synthesize cysteine rich polypeptides called PCs as demonstrated in many plants (Cobbett, 2000; Clemens, 2001).

Plants experience high osmotic stress as a result of the accumulation/deposition of metals inside the xylem vessels and other spaces, which reduces the amount of water that is available to the plant due to decreased water transport from the external environment to the shoot system (Haider *et al*., 2021). Osmotic adjustment is the term used to describe the lowering of the solute potential that takes place during osmotic stress. Plants exhibit strategies to deal with the cell osmoticum by the accumulation of suitable metabolites and the synthesis of stress proteins (Yadav *et al*., 2021). According to Slama *et al*. (2015), metabolites also

scavenge reactive oxygen species and shield/protect subcellular structures in halophytes.

Heavy metal stress is typically examined in the context of the primary processes affected, such as growth, photosynthesis, respiration, adaptations to stress conditions, and so forth, in research studies as well as reviews. However, little attention is usually given to the fact that proteins, primarily enzymes, are the molecules that are most frequently both the targets of heavy metal damage and the mechanism by which plants compact heavy metal stress.

Plants under heavy metal stress have been found to have impaired protein synthesis (Reddy and Prasad, 1992; Prasad, 1997). The scientists reported that heavy metals had an inhibiting influence on the production of proteins. The lack of necessary amino acids may be one of the causes of reduced protein synthesis according to Prasad (1997). Bishnoi *et al*. (1993) stated that, the inhibition of the mobilization of amino acids at the site of protein synthesis occurred in *Pisum sativum* upon exposure to Cr.

In some cases, metal stress affects the proteins linked with nutrient metabolism with an increase in proteins associated with transcription and translational regulation, antioxidant pathways, molecular chaperones and biosynthetic metabolism (Mustafa and Komatsu, 2016). In order to grow healthy in metal toxic environments, the plant initially synthesizes some proteins and molecules/metabolites which then induce the expression of particular genes (Maksymiec, 2007). It has been reported that abundant proteins involved in energy, disease and defence-related functions are differentially regulated in response to Cd stress in sunflower (Júnior *et al*., 2015).

The protein content in plants was found to be little increased by Al treatment. More or similar protein content in control and aluminium treated plant reported by Kochian, (1995). In tomato plants, long-term exposure to Al induced systemic alterations in the proteomes of roots and leaves, with changes in proteins involved in various cellular processes (Zhou *et al*., 2016). According to Wu *et al*. (2019), in citrus plants, Al treatment did not change the protein content of the root. In both

tomato and citrus plants, proteins related to detoxification, cellular transport, post-transcriptional modification, and oxidation-reduction homeostasis were found to interact with Al-responsive proteins, suggesting their involvement in Al tolerance. Additionally, in tomato plants, proteins related to the light reaction centres of photosynthetic machinery were identified as primary targets of Al-induced stress (Okekeogbu *et al*., 2014). But in *C. amboinicus* Al showed a beneficiary effect on protein synthesis.

The increase in protein content in plants after Cr treatment is believed to be a response to the stress caused by Cr toxicity. As a defense mechanism, Cr is known to induce production of PCs and other peptides like metallothioneins rich in cysteine residues (Klobus *et al.*, 2002). Increased protein content shows reduced level of stress as a result of stress protein synthesis which contribute to the occurrence of phytochelatins (Verma and Dubey, 2003; Mishra *et al.*, 2006). This increase in protein content is thought to be a protective response to the oxidative injury caused by Cr, as proteins play a crucial role in various cellular processes, including antioxidant defense and detoxification (Wang *et al*., 2013).

Plants have cellular mechanisms to manage the accumulation of metal ions inside the cell, and one of the ways they do this is by increasing the expression of genes related to protein synthesis and folding (Yadav *et al*., 2022). The activation of signal transduction pathways and the expression of stress response proteins, such as heat shock proteins and metallothioneins, have also been observed in plants under Cr stress, which may contribute to the increase in protein content (Wang *et al*., 2013). Overall, the increase in protein content in plants after Cr treatment is a complex response involving multiple cellular processes and gene expression changes. In contradictory to our results, Cr treatments significantly reduced the protein content in *Cichorium intybus* plants because Cr toxicity induces the degradation of proteins.

The enhanced synthesis of proteins during metal stress also contributes towards the specific protective functions and synthesis of enzymes involved in antioxidative defence mechanisms as well as in maintaining cellular membrane

integrity (Omidifar et al., 2021). Corroborating to the behavior of *C. amboinicus*, Olkhovych *et al*. (2016) reported the enhanced synthesis of stress proteins (reduced ascorbic acid) in *Pistia stratiotes* exposed to Cu nanoparticles. The observed reduction in amino acid contents may negatively affect plants adaptive reactions associated with *de novo* synthesis of stress proteins. Hence, the accumulation of proteins in *C. amboinicus* during Cu exposure implies enhanced metal tolerance mechanisms in the plant. Significant increase of total protein content of *C. amboinicus* treated with Cu metal, is found to be a typical characteristic of Cu as suggested by Chai *et al*. (2014), who opined that Cu stress induces expression of genes encoding for proline rich proteins, glycine rich proteins and hydroxy proline rich proteins, which are the three classes of structural proteins of plant cell walls and these proteins play important roles in the lifting of the plant's resistance to Cu stress.

Leaves of Al treated plants showed high metabolic activity since toxicity of Al was very feeble as already mentioned earlier. Increased protein content of leaves of Hg treated plants can be correlated also with the tolerance mechanism of *C. amboinicus* plant. Very low Hg content in leaves because toxicity of Hg is meagre to inhibit protein synthesis resulting in more protein content.

An important role of proline is the function of the amino acid as a compatible solute which is an osmotically active organic compound but does not interfere with metabolic enzymes activator even at higher concentration unlike organic and inorganic ion (Taiz *et al*., 2015). Therefore, when *C. amboinicus* is exposed to toxic metals like Al, Cr, Cu, and Hg, which are primarily metal ions, the metal ions accumulate in the vacuole to reduce stress and preserve the water potential equilibrium between the vacuoles and cytosole compartments, which causes the plants' compatible proline to increase.

According to Rai *et al*. (2004), proline is thought to play a number of functions during metal stress, including maintaining the osmoticum, scavenging free radicals, and stabilizing membranes. Proline serves as a suitable solute that not only regulates osmotic pressure but also shields enzymes from denaturation and ROS scavenging (Hussain *et al*., 2011). Proline is the only amino acid that accumulates in

plant leaves under stressful circumstances. According to Ganesh *et al*. (2009), proline build up in tissues causes osmotic adjustment and acts as a dependent marker for genotypes linked to stress tolerance. Another strategy for helping plants deal with metal stress is increased phenolics. By scavenging reactive oxygen species, phenol-coupled ascorbate peroxidase activity, and metal chelation, phenols shield plant cells from heavy metal stress (Lavid *et al*., 2001).

Proline has multiple functions and the important one is regulation of osmoticum change imposed by drought (Saradhi and Saradhi, 1991; Rout *et al*., 1997). Because of the presence of metal ions, the increased proline level in the roots when compared to the control indirectly indicates that the growth medium is lacking in water, and proline thus plays an important role in osmoregulation in the roots. However, proline content in roots treated with Cr and Cu is comparatively lower than that of roots treated with Hg. This is likely because Hg is more toxic than others, as evidenced by studies on *Rubia tindorium* treated with Hg (Iqbal *et al*., 2001), and *Caesalpinia pulcherrimia* (Moral *et al*., 1995). Proline accumulation in plants mitigates the water potential reduction caused by metal ions, according to Costa and Morel (1994).

One tactic plant uses to offset the harmful effects of heavy metal stress is the accumulation of proline (Clemens, 2006). *Sorghum bicolor* (Kumar *et al*., 2019), *Helianthus annuus* (Qadir *et al*., 2020), *Cicer arietinum* (Singh *et al*., 2020), *Zea mays* (Adhikari *et al*., 2020), and *Ocimum tenuiiflorum* (Rai *et al*., 2004) have all been shown to have significantly elevated proline levels as a result of Cr stress. Proline content of root exhibited only slight increase in *C. amboinicus* subjected to Al treatment, whereas stem and leaf contained significantly high amount of proline (Table 18). Distribution of proline in the root, stem and leaves was significantly increased in plants treated with Hg. Cu treatment resulted in significant increase in the roots, stem and leaves.

The highest enhancement in the xylem wall thickening was observed in the root, stem and leaves of *C. amboinicus* subjected to Cu stress, as evidenced from the EDX results (Table 8). Since Cu is an essential metal for plant growth, behaviour of

C. amboinicus in terms of proline accumulation can be corroborated with the anatomy as well as the cell wall thickness. Proline is crucial for cell wall proteins like extensins and arabinogalactans, influencing cell wall thickness and plant development (Kishor *et al*., 2015). According to Roberts *et al*. (1972), Hydroxyproline, found in plant cell walls, is associated with wall thickening, especially in stationary phase cells, suggesting a link between proline and cell wall thickness. Similar results shown by Muñoz *et al*. (1998), Proline-rich protein (PRP) CanPRP is linked to cell wall strengthening during growth and stress, indicating a connection between proline and cell wall thickness in *Cicer arietinum*.

Phenolics come under an important group of secondary metabolites and constitute a number of compounds/molecules. According to Harborne (1980), plants experiencing stress from the environment produce more phenolic synthesis. According to reports, root development inhibition is a result of Al (Goldbold and Kettner, 1991), Hg (Godbold and Huttermann, 1986), and Cr (Kahle, 1993). According to Cseh (2002), the toxic effects of heavy metals typically cause anatomical and morphological alterations such woody characteristics, thin growth, and thickening of the cell walls. Increased lignification of the cell wall is indicated by the woody structure of roots (Fahn, 1982).

Phenolics are a broad class of compounds that play a major part in defense mechanisms in plants like antioxidants. Plants produce phenolics as a stress response, which have the ability to chelate metals and act as antioxidants. These features can aid in the adaptation of the plant to the metal stress environment (Kısa *et al*., 2016). In the present study, treatment of *C. amboinicus* with all treatments resulted in the enhanced accumulation of phenolics contents in the root, stem and leaves, which helped the plants to scavenge the excess ROS molecules. Total phenolics content estimated during the stress treatments in *C. amboinicus* was found to increase, which can be correlated with the accumulation of secondary metabolites like flavonoids, anthocyanin and alkaloids. The antioxidant capacity of phenolics is related to their aromatic ring structure with –OH or – OCH3 substituents, which is suitable for trapping free radicals (Dumanović *et al*., 2021). Through Fenton

reactions, phenolic compounds containing o-dihydroxy groups can form a complex with hazardous metal ions, thereby preventing the generation of ROS. Therefore, during heavy metal stress, the phenolic compounds accumulated in *C. amboinicus* aid in the ability of plants to sequester hazardous quantities of toxic metals, thereby reducing ROS production. Plants may be subjected to several radical physiological changes as a result of the imbalances in the production and scavenging of ROS which is termed as an oxidative burst (Morina *et al*., 2010; Anjum *et al*., 2015). When certain heavy metals like copper (Cu), nickel (Ni), cadmium (Cd), chromium (Cr) and arsenic (As) are present in excessive quantity, they show a tendency to generate ROS (Körpe and Aras, 2011).

The anatomical modification within the root of *C. amboinicus* treated with Hg is positively correlated with increased phenolics. Increased phenolics of roots particularly plants subjected to Hg stresses are found to be indirectly correlated with woody structure of roots and also the toxicity exerted in roots. The shoot has a lower phenolic quantity than the roots. Lenticels and stomatal alteration may be the possible reason, since Hg is phytovolatilized and there is very minimal accumulation of shoots. As a result, the exerted toxicity is relatively minimal.

Increased phenolics of roots particularly plants subjected to Cu stresses is found to be indirectly correlated with woody structure of roots with thickened and lignified cell wall (Table 19). In cell wall formation process, cross linking of phenolics group of compounds such as tyrosine residue get attached to cell wall matrix polysaccharides and this process coincided with cell wall maturation which is believed to be mediated through peroxidase activity resulting in cell wall rigidification (Taiz *et al*., 2015). According to Buchanan *et al*. (2000), phenolics are the precursor of lignin and *de novo* synthesis of soluble phenolics occur under heavy metal stress and these phenolics act as intermediate in lignin biosynthesis (Michalak, 2006).

Phenolics protect plant cells against heavy metal stress by metal chelation, phenol- coupled ascorbate peroxidase activity and scavenging of reactive oxygen species (Lavid *et al*., 2001). Induction of biosynthesis of phenolics compounds has

been reported in maize in response to Al (Winkel-Shirley, 2002), in *Phaseolus vulgaris* exposed to Cd (Dietz *et al*., 1999) and in wheat in response to nickel toxicity (Diáz *et al*., 2001). These phenolic compounds also modify the lipid packing order in order to cause alteration in peroxidation kinetics and stabilize membrane by decreasing membrane fluidity (Arora *et al*., 2000). Thus hinder the flow of free radicals and restrict lipid peroxidation (Blokina *et al*., 2003).

Malonydialdehyde (MDA) content represents the magnitude of lipid peroxidation as an important impact of heavy metal toxicity in plants. The increased MDA levels in the root and shoot tissues at different concentrations of heavy metals are considered as a sign of oxidative damage due to external toxicity and thus can be considered as a sensitive index of oxidative stress (Yam and Tam, 2013; Hashem *et al*., 2016). As a result of lipid peroxidation, several products are produced from polyunsaturated precursors which comprises of small hydrocarbon fragments such as ketones and malondialdehyde (Garg and Manchanda, 2009). Once polysaturated fatty acids undergo peroxidation by ROS attack, it may lead to chain breakage and thereby cause increase in membrane fluidity and permeability (Sharma *et al*., 2012).

According to Djebali *et al*. (2005), when plants are exposed to stressful environmental conditions, the rate of lipid peroxidation gets triggered which ultimately results in loss of membrane integrity, this in turn results in leakage of essential elements. Enhanced metal uptake and accumulation in roots of *C. amboinicus* induce higher levels of free radical generation, which is indicated by high MDA content of roots. Cu-induced oxidative stress leads to a variety of stress responses, including increased ROS levels, electrolyte leakage, and decreased membrane and chlorophyll stability indices (Rehman *et al*., 2019). Similar case was reported in *A. ilicifolius* when subjected to Zn stress (Shackira *et al*., 2017). Exposure to hexavalent Cr compounds like potassium chromate can lead to the formation of protein carbonyls and MDA through protein oxidation and lipid peroxidation, contributing to DNA-protein crosslinks formation (Mattagajasingh *et al*., 2008).

When plants are under a range of environmental stressors, hydrogen peroxide is a key signaling molecule for a number of physiological processes that can lead to stress tolerance (Hieno *et al*., 2019). According to Apel and Hirt (2004), the primary sites where ROS are produced in plants are the chloroplasts (through photochemistry and electron transport), peroxisomes (through photorespiration), mitochondria (through respiratory electron transport), cell membranes, and cytosol (by peroxidase enzyme activity). Accordingly, an excess of ROS molecules causes irreversible oxidative damage to cellular constituents such as proteins and DNA, which in turn causes cell death (de Souza *et al*., 2017).

Overproduced ROS within the cells converts the membrane fatty acids into toxic lipid peroxides, leading to the destruction of the biological membranes, ion leakage by the plasma membrane, and ultimately causing cell death (Rangani *et al*., 2018; Kamran *et al*., 2020). The over-accumulation of MDA content is also an indication of the disturbances in the chlorophyll stability and associated photodamage in the leaves, which is in agreement with high MDA content in metal treated plant leaves.

An increased amount of MDA suggested that ROS produced as a result of environmental stresses has damaged the membrane to a greater extent (Meng *et al*., 2007). Numerous studies have reported that as Cu stress increases in different plants, there is a corresponding increase in lipid peroxidation and accumulation of MDA (Jiang *et al*., 2013). Increased lipid peroxidation due to Cr stress was reported in *Trticum aestivum* (Zhang *et al*., 2010; Ali *et al*., 2015), *Zea mays* (Maiti *et al*., 2012), *Chamomilla recutita* (Kovácik *et al*., 2014), *Brassica campestris* (Chandra *et al*., 2009) and *Cicer arietinum* (Singh *et al*., 2020). Exactly similar behaviour is shown by *Strobilanthes alternata* treated with Zn (Devarajan, 2022). Enhanced rate of MDA synthesis is shown in *Triticum aestivum* and *Brassica campertis* due to toxicity of Cr and Cd (Chandra *et al*., 2009).

The scavenging of toxic ROS from plant cells is mediated by a number of protective enzymes including SOD, APX, GPX, and CAT, which make up the enzymatic antioxidant defense system. Increased activity of antioxidant enzymes

leads to rapid scavenging of ROS, assisting the plant in escaping oxidative stress (Hashem *et al*., 2016). According to Gratão *et al*. (2005), SOD detoxifies superoxide radicals, preventing cellular damage caused by stress. The balance between SOD and APX or CAT activities in cells is crucial for determining the steady state level of superoxide radicals and hydrogen peroxide (Bowler *et al*., 1992). Catalase is less efficient than other peroxidases in scavenging H_2O_2 because of its low substrate affinity (Zhang *et al*., 2007). Enzymes like CAT, POD, and APX activities were induced, helping to remove H_2O_2 radicals from the cells, while the rate of H_2O_2 radicals increases due to SOD activity (Zhang *et al*., 2020).

SOD is the major antioxidant enzyme involved in primary resistance against ROS, catalyzing the dismutation of •O2 - and consequently producing 182 H2O2 and O2 (Gill et al., 2015). The enhanced level of •O2 - accumulations stimulates SOD enzyme activity; thereby •O2 - produced in cells are quickly transformed to H2O2 by the activity of SOD (Dixit et al., 2001). Beyond its ability to scavenge ROS, SOD has been recently found to play important functions in a variety of metabolic processes. It functions as an RNA-binding protein, a nuclear transcription factor, and a signal modulator of glucose metabolisms (Chung, 2017). Most of them function independently of their typical antioxidant characteristics, which obliquely aid in controlling oxidative stress (Wang *et al*., 2018).

Catalases are mostly peroxisomal enzymes that are active at relatively higher H_2O_2 concentrations and are capable of degrading H_2O_2 without the need for reducing power (Anjum *et al*., 2016). According to Sharma and Ahamed (2014) and Su *et al*. (2018), catalysis is involved in the direct conversion of H_2O_2 into H_2O and $^{\bullet}O_2^-$, as well as the indirect conversion that occurs when H_2O_2 oxidizes substrates such formaldehyde, formate, methanol, ethanol, and nitrite, releasing H_2O and $^{\bullet}O_2^-$.

When *C. amboinicus* is treated with Al, the SOD activity of the root, stem, and leaf does not change in comparison to the control (Table 21). The reduced SOD activity in Al treated *C. amboinicus* plants as compared to control plants suggests that Al has a less toxic effect. This behaviour is consistent with the toxicity symptoms displayed by parameters such as the absence of growth retardation

(Fig.1), impaired metabolism and anatomical variation (Fig.8) in plants treated with Al.

SOD enzyme activity in plants treated with Al varied depending on the species and concentration of Al. In rice and chickpea seedlings, Al stress caused a decrease in SOD activity in the roots and shoots of rice seedlings, while in chickpea seedlings, it caused an increase in SOD activity in the roots and leaves (Samad *et al*., 2020). Pea plants treated with Al showed increased SOD activity in the nodules and roots (Sujkowska-Rybkowska, 2012). In *Vicia faba*, SOD activity was mostly higher in leaves and roots exposed to Al compared to the control (Zhang *et al*., 2009). Overall, the effect of Al on SOD activity in plants is species-specific and can vary depending on the concentration and duration of Al stress. In *Lemna minor* plants, both the Zn and Al treatments dramatically enhanced SOD activity; however, the induction of the enzyme was most noticeable when the Zn content was raised relative to the control (Radic *et al*., 2009).

When Cr treated, plants are compared to those treated with other metals and controls, there is a significant increase in SOD activity in initial period of growth but it significantly decreased at final stages of growth. According to Giannopolitis and Ries (1977), SOD is the first enzyme that detoxifies highly reactive oxygen species in plants by turning O_2 radicals into H_2O_2. As a hazardous byproduct of SOD activity, H_2O_2 needs to be removed by being converted to water in a later process. Stress-related enzymes in plants, such as peroxidase and catalase, are thought to be crucial for scavenging H_2O_2 (Noctor and Foyer, 1998; Zhang *et al*., 2007). The upregulation of SOD activity may help in scavenging the superoxide radicals produced by Cr and reducing the oxidative damage to the plants. Rahman *et al*. (2010) reported that, when Cr was applied to *Kandelia Candel* plants, the amount of SOD in the roots increased and was effective in scavenging the superoxide that Cr created. An increase in metal concentration and exposure times was shown to cause an increase in malondialdehyde (MDA) content in both roots and leaves. According to their findings, a high concentration of an excessive supply of Cr may disrupt multiple metabolic pathways in seedlings, leading to plant toxicity manifested as

chlorosis, necrosis, impaired photosynthetic capacity, and ultimately, plant death. *Gossypium hirsutum* (Daud *et al*., 2014), *Zea mays* (Jinhua *et al*., 2009), *Brassica compestris* (Qing *et al*., 2015), and *Oryza sativa* (Panda, 2007) have all been shown to have increased SOD activity in response to Cr toxicity.

Numerous physiological investigations have demonstrated the function of phytochelatins in maintaining homeostasis and eliminating harmful metals, such as Cr. One of the most significant plant chemicals in the detoxification of Cr in plants is phytochelatin (Shanker *et al*., 2005; Singh *et al*., 2013). Phytochelatins bind Cr and other heavy metals in the cytosol before they are sequestered into vacuoles. Diwan *et al*. (2010) discovered that phytochelatins are induced by Cr toxicity in both the shoot and root of plants. This phenomenon is known as Cr-mediated induction and is significant for the Cr speciation effect in the species *Vigna radiata* and *Brassica juncea*.

SOD activity of root, stem and leaf of *C. amboinicus* plants treated with Cu treatment resulted in the significant increase. The findings of the present study can be corroborated with the reports from other plants such as in mung bean seedlings (Gaur *et al*., 2021). According to Yap *et al*. (2021), the antioxidant enzymes such as APX, CAT, POD and SOD activities were enhanced considerably to reduce the oxidative stress caused by ROS molecules during Cu stress in *Centella asiatica*. Copper affects the SOD activity of plants by both promoting and inhibiting its activity. At lower concentrations of Cu, SOD activity and chlorophyll content increase, indicating a protective function of plants against copper stress (Mazaheri-Tirani *et al*., 2021).

However, contrast to our results, as the concentration of Cu increases, SOD activity decreases drastically, indicating a negative effect on the plants. The addition of Cu^{2+} at various concentrations in water irrigation also increases SOD activity linearly in paddy plants (Mir *et al*., 2021). The effect of Cu on SOD activity is dose-dependent, with the lowest level of Cu inducing SOD activity and higher levels reducing it (Aryani *et al*., 2018). Overall, Cu can both enhance and inhibit SOD activity in plants depending on its concentration.

Effect of metal stresses due to Hg in *C. amboinicus* exhibited as structural changes, modifications, increased synthesis of MDA and proline clearly indicate the occurrence of drastic stress impact. So, it seems that since the stress is strong, plant modified for defense purposes. SOD activity is efficient to cope with metal activity. Study corroborated with our result includes all $HgCl_2$ treatments decreased the level of SOD in wheat seedlings, indicating that Hg affects the SOD activity of plants (İşkil *et al*., 2022).

CAT activity of root, stem and leaves of *C. amboinicus* plants treated with Al resulted in decreased activity of enzymes compared to the control presumably indicating, may be the reduced substrate affinity and this view is conceivable because increased activity of SOD in all treatments particularly in Hg treated plants results in the exorbitant production of H_2O_2 which is not properly functioned as substrate for catalase activity.

Shanker *et al*. (2004) suggested that, combined activity of SOD and CAT is critical in mitigating the effect of oxidative stress imposed by Cr in *Vigna radiata*. Numerous plant species, including *Zea mays* (Jinhua *et al*., 2009), *Gossypium hirsutum* (Daud *et al.,* 2014), and *Triticum aestivum* (Adress *et al*., 2015), have been shown to have a Cr-induced increase in CAT activity. But it is contradictory to our results. In *C. amboinicus* Cr treatment resulted in slight reduction of CAT activity.

Copper treatment also results in the reduction of CAT activity in *C. amboinicus* (Table 22). Contradictory to this observation, many plants including *Abutilon indicum*, *Withania somnifera*, bamboo, maize, and others, have been shown to exhibit Cu-stress mediated elevation of CAT activity (Emamverdian and Ding, 2017; Rout *et al*., 2017; Yap *et al*., 2021). This helps the plant withstand Cu-stress mediated oxidative stress and effectively alleviate H_2O_2. Su *et al*. (2018) concluded that a deletion mutation of CAT genes resulted in impaired plant growth, a disrupted redox state, and increased susceptibility to external stressors suggest that the CAT enzyme is crucial for the growth and development of stressed plants. Least CAT activity was shown by the Hg treated *C. amboinicus* plants. So, it seems that since

the Hg stress is strong, plant modified for defense purposes. CAT activity is efficient to cope with metal activity and it made the plant tolerant towards Hg stress.

According to Habeeb *et al*. (2021), the activity of antioxidant enzymes was triggered in *C. amboinicus* under varying levels zinc stress and drought. Because CAT and SOD were highly active in Zn-stressed plants, there was less H_2O_2 remaining in the cells than in the control sample. This suggests that the amount of H_2O_2 present was shown to affect CAT enzyme activity. ROS levels have increased due to increased Zn accumulation in plant tissue, and antioxidant enzymes are crucial in eliminating these harmful oxygen species (Parida and Das, 2005).

Bioaccumulation pattern of Al, Cr, Cu and Hg vary significantly between the metals. However, because there are notable variations in the treatment concentrations, the total amount of each metal cannot be compared because it depends on the ideal concentration that is provided to the plants to create a modest degree of toxicity. Nonetheless, a little proportionality between the treatment concentration and the contents deposited in the roots, stems, and leaves of the plant can be seen. However, it has been discovered that the differences in the bioaccumulation patterns of Al, Cr, Cu and Hg are caused by the specificity of each metal throughout the processes of absorption, translocation, and accumulation.

The trend of bioaccumulation in *C. amboinicus* after heavy metal treatment was root > stem > leaf. The bioaccumulation of heavy metal increased gradually with increase in the treatment period, and the major part of the up taken heavy metal was retained in the root itself, and only a small portion was found to be transported to the aerial parts. This is in accordance with the studies of Fulekar (2016), established that the metal content concentrates more in the roots than the shoots because the roots receive the metal entering the plants as a result of nutrient uptake. According to Peng *et al*. (2015), the immobilization of higher amount of metal in the root system is a chief tolerance mechanism against heavy metal toxicity in plants. Since heavy metal concentration was higher in the roots, it can strongly bind to the root cell wall, resulting in the restricted mobility of the metal and reduced translocation to the aerial parts (Marques *et al*., 2018). In plants, the absorption of

metal depends on the metal availability in the soil and the physiological requirement of the metal to the plant (DalCorso *et al*., 2013).

The amount of each metal given to the plant in Hoagland nutrient media can also be used to interpret the accumulation of heavy metals in the roots, stem, and leaves. Bioconcentration factor (BCF) and translocation factors (TF) are two important parameters used to evaluate the potential of plant to remediate a particular metal (Sinha *et al*., 2018). According to Yoon *et al*. (2006), BCF (Bioconcentration factor) and TF (Translocation factor) can be used to assess the distribution of metals in the root and stem. According to those authors, hyperaccumulation of metals occurs when a contaminant taken up by a plant is not degraded rapidly resulting in high accumulation. While hyperaccumulator plants absorb and translocate metals into their above ground biomass, tolerant plants typically limit the transfer of metals from the soil to the roots and shoots of the roots, resulting in a biomass that is roughly similar in terms of metal concentration. According to Fitz and Wenzel (2002), plants with TF and BCF values less than one (<1) are not appropriate for phytoremediation. BCF is a measure of the bioavailability of a metal from the soil, or growth medium, and its value is associated with each element's capacity for absorption (Yoon *et al*., 2006).

The roots of *C. amboinicus* having a very high concentration of accumulated Al, throughout all growth intervals whereas the stem and leaves have shown a considerable amount of Al content. The percentage of Al that enters the plant and remains in the residual solution is observed. The morphology, anatomy, and distribution pattern of the metabolites of plants treated with Al demonstrate a favorable influence on growth and metabolism rather than any discernible toxicity or stress symptoms. Hence the tolerance potential of *C. amboinicus* towards Al is very high since the availability of considerable quantity of Al in the nutrient solution never results in any inhibitory effect of Al.

Aluminium addition in *Melastoma malabathricum* seedlings stimulates growth up to an optimum value, with some populations showing a physiological response that optimizes elemental stoichiometry and growth rate (Mahmud *et al.*,

2020). According to Schmitt *et al*. (2016), Al accumulation in *Symplocos paniculata* plants negatively impacts sapling performance, suggesting that nutrient solutions with Al may improve plant growth. Nevertheless, Al phytotoxicity is associated primarily with disruption of roots structure, physiology and functions (Kochian and Shaff, 1991; Taylor, 1998). The exclusion of Al from the root apex and internal tolerance once Al reaches the plant symplast are the two mechanisms underlying the Al tolerance mechanism, as per the findings of Delhaize and Ryan (1995) and Kochian (1995).

Aluminum does not hinder growth at lower concentrations. In fact, early growth periods discover a slight increase in leaf growth, suggesting a slight stimulating effect. This observation is consistent with the findings of Kochian (1995) and Taiz *et al.* (2014), who suggested that Al might exert a slight beneficial effect on plant growth. One of the proposed mechanisms of Al toxicity involves Al interaction with ion transport systems functioning at root cell plasma membrane (Taylor, 1988; Kochian, 1995).

Aluminum exclusion is the main mechanism underlying Al tolerance in plants, as Al-sensitive plants absorb more Al than Al-tolerant plants (Kochian, 1995; Matsumoto, 2000). The exclusion mechanism involves secretion of Al chelating ligands binding of Al with the cell wall and mucilage, a plant induced pH barrier in the rhizosphere or root apoplasm, selective permeability of the plasma membrane and Al^{3+}efflux.

In contradictory, Al toxicity and resistance in plants are mainly influenced by the apoplast, with resistance mainly achieved through lower Al accumulation in root apical meristems (Horst *et al.*, 1995). Aluminum stress significantly reduces root growth in sorghum seedlings due to accumulation of Al in the root extension zone, callose accumulation, and impairment of plasma membrane integrity (Too *et al*., 2014).

Coleus amboinicus showed highest BCF values in the treatment with Al at the final stages of growth. TF values are less than one in all stages of Al treated plants and hence it is not suitable for phytoextraction with the view of Yoon *et al*.

(2006). TF values showed considerable increase throughout the period. Increased TF values of Al treated leaf during the last stage of growth indicate the enhanced translocation of Al from root to shoot and this observation is in agreeable with the concept of Kochian, (1995) who suggested Al ions get translocated to various parts of the plant without interfering the metabolism.

The bioaccumulation of Cr treated *C. amboinicus* plants shows highest accumulation in roots than shoot and are significantly increased at all growth stages (Table 23). Concentration of Cr in the root tissues increased steadily and linearly. The root, stem, and leaves had a comparatively higher concentration of Cr on the 20^{th} day of treatment. Quantity of Cr was very low in leaf compared to root and stem and increase was insignificant between 12^{th} and 20^{th} day of treatment. The Cr level of residual solution is extremely low and is gradually decreased during the course of the interval.

Laetues sativa (Singh, 2001) and *Nelumba nucifora* (Vajpayee *et al*., 1999) have been shown to exhibit progressive accumulation of Cr, with roots containing more Cr (10–200 times) than shoots. According to literature, *Veronica becanga* and a number of hydrophytes remove a significant amount of Cr from soil (Zurayk *et al*., 2001). Kabata-pendias and Pendias (2001) reported that the roots and shoots of *Helianthus annuus*, *Zea mays*, and *Vicia faba* exhibited a progressive increase in Cr accumulation.

In the present study, a higher accumulation of Cr was observed in the roots than shoots. Similar observations were found in *Colocasia esculenta* (Men *et al*., 2018), *Diectomis fastigiata* (Mohanty and Patra, 2020), *Melia azedarach* (Yan *et al*., 2020), and *Pennisetum sinese* (Chen *et al*., 2020), *Vigna radiata* (Singh *et al*., 2021), *Brassica oleracea* (Ahmad *et al.*, 2020), *Cicer arietinum* (Singh *et al*., 2020) and *Arachis hypogaea* (Zong *et al*., 2020). The fact that Cr is less mobile in plant roots and that Cr is sequestered in the vacuoles of root cells, which serves as a defense mechanism and gives plants an inherent resistance to Cr toxicity (Mangabeira *et al*., 2011).

Similar to our results, maximum bioaccumulation is seen in roots were found in *Vigna radiata* (Jabeen *et al*., 2016; Singh *et al*., 2021), *Zea mays* (Anjum *et al*., 2017), *Brassica napus* (Gill *et al*., 2015), *Brassica campestris* (Zhao *et al*., 2019), *Brassica oleracea* (Ahmad *et al*., 2020), *Cicer arietinum* (Singh *et al*., 2020), *Arachis hypogaea* (Zong *et al*., 2020), and *Oryza sativa* (Nagarajan and Ganesh, 2015), and *Tradescantia pallida* (Sinha *et al*., 2014). Sometimes, the root concentration of Cr exceeds the shoot concentration by 100 times (Shanker *et al*., 2005). This could be due to the low mobility of Cr in the plant roots compared to other metals or the sequestration of Cr in the vacuoles of root cells which acts as a protective mechanism and provides natural tolerance to plants against Cr toxicity (Mangabeira *et al*., 2011). Stress ethylene is induced by Cr in plant roots that cause strong cellular damage and is transported to shoots from root.

The BCF values of *C. amboinicus* treated with Cr are very low and rise over the growth phase (Table 24). TF values of *C. amboinicus* treated with Cr increase, indicating that the absorption pattern is proportionate to translocation with Cr mobilization pattern. Research has demonstrated that the roots of *Lactice sativa* (Singh, 2001) and *Nilumbo nucifera* (Vajpayee *et al*., 1999) display a gradual accumulation of Cr content rather than the shoots. It was similar to the results of *C. amboinicus* treated with Cr also. The plants such as *Dyera costulata* (Ghafoori *et al*. 2013), *Pluchea indica* (Sampanpanish *et al*. 2006), *Amaranthus dubius* (Mellem *et al*. 2012), *Convolvulus arvensis* (Gardea-Torresdey *et al*. 2005), showed a significant translocation of Cr from roots to aerial parts and thus, suggested that these species have a high phytoremediation potential.

Plants require Cu, a micronutrient that builds up in various sections of the plant. Following $CuSO_4$ treatment, *C. amboinicus* showed the following bioaccumulation trend: root > stem > leaf. The bioaccumulation of Cu by *C. amboinicus* increased gradually as the concentration of Cu in the medium and the length of the treatment period increased. The majority of the Cu that was taken up by the plant was found to remain in the root itself, with only a small amount being transported to the aerial parts.

Since Cu concentration was higher in the roots, it can strongly bind to the root cell wall, resulting in the restricted mobility of the metal and reduced translocation to the aerial parts (Marques *et al*., 2018). This result is corroborated with the increased xylem wall thickness in roots of *C. amboinicus* after Cu treatment. As the absorbed metal is highly concentrated in the roots, the detoxification of the metal is also crucial to reduce the bioavailability and toxicity in plants. The major detoxification mechanisms operational in plants are the binding of the metal ions to the cell wall as well as the sequestration into the vacuole (Zhou *et al*., 2017).

Martins *et al*. (2020) reported that while *Alternanthera tenella* plants exhibit a significant potential for Cu bioaccumulation and tolerance, they also clearly display indications of stress when exposed to excess Cu. Because of their tolerance and great capacity for Cu bioaccumulation, they can be employed in phytoremediation or bioindication. Lu *et al*. (2018) examined eight distinct aquatic plant species to determine the absorption capability and bioaccumulation of Cu in water: *Juncus effusus*, *Acorus calamus*, *Eichhornia crassipes*, *Sagittaria sagittifolia*, *Arundina graminifolia*, *Echinodorus major*, *Nymphaea tetragona*, and *Pistia stratiotes*. The Cu enrichment amount in aquatic plants was related to the content of lignin in plants, and higher the content of lignin, the greater the amount of copper.

Bioaccumulation pattern of Cu in *C. amboinicus* shows significant increase in BCF values revealing enhanced absorption to the roots compared to the solution. TF is also increasing during growth. According to Kumar *et al*. (2021), BCF was found to be greater than one, indicating the suitability of *Vetiveria zizanioides* for phytoremdiation of Cu and Cd from soil. Metal accumulation in roots was much higher than shoots (TF $\ll$ 1) indicating that the mechanism involved was of phytoextraction.

Coleus amboinicus treated with Hg shows highest accumulation in roots during growth (Table 23). Similar results were found in *Houttuynia cordata* plant, which was found to be a Hg accumulator, with the highest content in the roots (Wang *et al*., 2021). According to Quiñones *et al*. (2021), white lupin plants were

also found to accumulate high levels of Hg in nodules, roots, and cluster roots. A study on soil contamination near a former thermometer factory found that Hg concentrations in soil were correlated with Hg accumulation in the roots of *Sterculia* (Sinduja *et al*., 2020). Leafy vegetables grown in Hg-contaminated soils were found to accumulate Hg primarily in the roots, reducing the risk of bioaccumulation in the edible portions (Yang *et al*., 2019). These studies highlight the potential of plant roots to accumulate Hg.

Accumulation pattern of Hg in *C. amboinicus* is different from that of other metals. Very low Hg content was accumulated in the plant parts of *C. amboinicus* after Hg treatment. In the leaves, Hg was below detectable level except 20^{th} day reveals the phytovolatilization capacity of that metal. Through lenticels in stem (Fig.2) and also through stomata (Fig.21E), Hg was escaped before accumulation. Hence Hg was meagrely accumulated in *C. amboinicus*. BCF factor registered only very low change. But the TF values show decrease in the final stage of growth is indicative of the reduced translocation of Hg to the shoot meanwhile in the root system comparatively the Hg content remained unchanged presumably due to the exhaustion of the metal in the medium.

A great majority of phytochemicals do not directly participate in growth, development and reproduction of plants, hence such group of phytochemicals are named as 'secondary metabolites'. Secondary metabolites have got potential role in human nutrition, cosmetics drugs and their indispensable role in plant defense. Medicinal plants are an excellent source of different bioactive secondary metabolites used in developing innovative therapeutic agents and the bioactive plant product shows fewer side effects than manufactured drugs.

The GCMS analysis of the bioactive compounds revealed that both qualitative and quantitative variations were recorded in the leaves of *C. amboinicus* exposed to heavy metals as compared to that of the control. Considering the area percentage of GC-MS chromatogram (Fig.61) of alcohol soluble compounds revealed the presence of 18 bioactive components in *C. amboinicus* were identified

and those compounds belonging to different classes and most of them are reported to exhibit important biological activities.

5-Isopropyl-2-methylphenol/ Phenol, 2-methyl-5-(1-methylethyl) is the most prominent phytochemical determined in *C. amboinicus* and it is a phenolic constituent of many essential oils. Commonly it is known as Carvacrol and it is a monoterpenic phenol. Carvacrol, a phenolic monoterpene in *Coleus amboinicus*, exhibits a broad spectrum of medicinal properties. Its antimicrobial activity has been demonstrated against various microorganisms, including E. coli, *Staphylococcus aureus*, and *Candida albicans*, through membrane disruption and cell lysis (Suresh and Vasudeva, 2018). As an antioxidant, carvacrol scavenges free radicals, reduces lipid peroxidation, and enhances antioxidant enzyme activity (Lee and Lee, 2019). Its anti-inflammatory effects are mediated by inhibiting pro-inflammatory enzymes and cytokines, as well as NF-κB activation (Khan *et al*., 2018). Carvacrol also exhibits analgesic and antispasmodic activities, similar to morphine (Santos and Rao, 2017). Furthermore, its anticancer properties include inducing apoptosis, inhibiting cancer cell proliferation and migration, and enhancing chemotherapy efficacy (Ahmad *et al*., 2019). Additional studies have highlighted carvacrol's gastroprotective, neuroprotective, and antidiabetic activities, making it a valuable compound contributing to the therapeutic potential of *Coleus amboinicus* (Bhattacharya and Sahu, 2020).

Carvacrol-based nano-emulsion shows promise in attenuating hyperglycemia and neurodegenerative diseases in experimental diabetes (Hussein *et al*., 2020). Oral administration of essential oils (Carvacrol, Thyme and Oregano) can control immune stress and maintain intestinal health in broilers challenged by *Salmonella enterica* Lipopolysaccharide (Liu *et al*., 2020). Since carvacrol is a lipophilic compound, it is insoluble in water and soluble in organic solvents like ethanol, methanol and acetone. Carvacrol has neuroprotective properties against Cd-induced neurotoxicity in rats by decreasing inflammation and oxidative stress (Yıldız *et al*., 2022). Carvacrol induces apoptosis in human choriocarcinoma cells by disrupting

calcium homeostasis and causing oxidative stress, suggesting it may be a potential new therapeutic agent for choriocarcinoma control (Lim *et al.*, 2019).

Medicinal property of *C. amboinicus* is known earlier and has been used as a medicine for cough and cold as a folk medicine. Owning to the presence of several biological compounds, *C. ambionicus* exhibits different pharmacological activities including anti-microbial, anti-inflammatory, anti-tumor, wound cure, anti-epileptic, anti-larvicidal, antioxidant and analgesic property (Kumar and Kumar, 2020). Indian Borage's essential oil act against *A. ochraceus* contains carvacrol hence, exhibits efficient antifungal aspect (Bhatt *et al*., 2013). Due to the presence of triterpenoids, Indian Borage functions as anti-inflammatory agent that minimize redness and swelling, as well as itchiness and inflammation, in any of the insect bites and stings, eczema and psoriasis (Arumugam *et al*., 2016).

The reduction of Carvacrol in both drought and Zn stressed plants can relate to the development of other metabolites of the same pathway in stressed plants (Habeeb *et al.*, 2021). According to Janeeshma *et al*. (2021), heavy metals elicit alterations in the composition of various bioactive compounds in plants. The enhanced biosynthesis and accumulation of various bioactive secondary metabolites in plants is a tolerance mechanism adopted by the plants during heavy metal stress (Anjitha *et al*., 2021). Most of these secondary metabolites are phenolic or flavonoid compounds, having antioxidant properties (Karimi *et al*., 2012; Anjitha *et al*., 2021).

On exposure to heavy metal, the composition of phytol was significantly increased in the leaves as compared to the control. Since phytol is an isoprenoid alcohol bound with chlorophyll molecule *via* ester linkage, the degradation of chlorophyll results in the accumulation of phytol and its derivatives in the cells (Gutbrod *et al*., 2021). The most prevalent plant terpenoid is phytol, which is the side chain of the photosynthetic pigment chlorophyll (Davis and Croteau, 2000). It has been observed that the predominant constituent in essential oils found in *Glycosmis pentaphylla* leaves is phytol (Prakasia and Nair, 2015). It gets increased in drought stress which indicates severe pigment degradation and enrichment of different essential oils (Habeeb *et al.*, 2021). Phytol is having antimicrobial,

antinociceptive, antioxidant and immunostimulant propertie (Ryu *et al*., 2011: Santos *et al*., 2013). Ryu *et al*. (2011) studied anti-inflammatory and antiallergic effects of essential oil and phytol isolated from *Artemisia princeps* in mice. Santos *et al*. (2013) reported antinociceptive and antioxidant activities of phytol *in vivo* and *in vitro* models. Antimicrobial activity of phytol is well studied and has been shown by various authors. Rajab *et al*. (1998) and Saikia *et al*. (2010) investigated antimicrobial activity against *Mycobacterium tuberculosis*. Similarly antibacterial effect of phytol is shown in *Staphylococcus aureus* reported by Inoue *et al*. (2005).

Phytohormones and beneficial microbes play crucial roles in improving plant stress tolerance and defence response, aiding sustainable crop production in hostile environments (Egamberdieva *et al*., 2017). Photosynthesis occurs in rice mutants without reducing the geranylgeraniol side chain to phytol, but phytol is essential for preventing leaf chlorosis under light-stressed conditions (Shibata *et al*., 2004). Understanding the source of phytol for tocopherols in plants can enable breeding and engineering for vitamin E biofortification and enhanced stress resilience (Albert *et al*., 2022).

According to Ichihara and Fukubayashi (2010), fatty acids are the major component of lipids and the physical, chemical and physiological properties of lipids primarily depend on its fatty acid composition. Fatty acids have multiple roles in plant heavy metal stress tolerance (He and Ding, 2020). Significant reductions in the distribution of fatty acid methyl esters such as methyl palmitate and methyl stearate were observed in the leaves of *C. amboinicus* upon exposure to heavy metal. According to the studies conducted by Elloumi *et al.* (2014), a significant alteration in the fatty acid composition was observed in *Prunus dulcis* exposed to Cd, and the authors concluded that these variations were related to the extent of membrane lipid peroxidation in the plant. Similar variations in the relative abundance of fatty acids and their derivatives were observed in the roots of *Zea mays* under Cu stress (Chaffai *et al*., 2009). Cadmium induced suppression of fatty acid metabolism in the roots of *Sedum plumbizincicola* was reported by Sun *et al*. (2020). Therefore, the

significant enhancement in the distribution of fatty acids most severely indicates the extent of lipid peroxidation due to heavy metal stress in the leaves.

Methyl palmitate component is reduced in all treatments. Methyl palmitate is a fatty acid methyl ester of palmitic acid. According to Wang *et al*. (2009), Methyl palmitate is a common botanical compound that occurs naturally in many plants such as bark of Tonka bean (Nakano and Suarez, 1970), fruit of fig (Lin *et al*., 2005) and unripe green walnut husks (Wang *et al.*, 2009). Methyl palmitate was also reported as antagonistic to muscarinic receptors. Rats' lung inflammation and fibrosis caused by bleomycin are prevented by methyl palmitate, an anti-inflammatory and antifibrotic drug. It is employed in the manufacturing of plasticizers, detergents, emulsifiers, wetting agents, stabilizers, resins, and animal feeds.

Methyl palmitate effectively protects against silica-induced lung fibrosis in rats by counteracting inflammation, regulating ROS generation, and reducing collagen deposition (Sharawy *et al*., 2013). Methyl palmitate shows significant anti-inflammatory and anti-arthritic effects in a rat model of adjuvant induced arthritis, inhibiting synovial CD68 macrophage expression (Jaleel *et al*., 2021). Methyl palmitate and ethyl palmitate effectively combat inflammation in various experimental models, reducing edema, inflammation, and neutrophil infiltration (Saeed *et al*., 2012). Methyl palmitate does not inhibit human pregnant myometrial contractility, and alternative hypotheses must be pursued to explain the higher incidence of dysfunctional labor in obese women (Crankshaw *et al*., 2014). Oral methyl palmitate significantly decreases the formation of epidural fibrosis in rats, potentially due to its anti-inflammatory and antioxidant effects (Kızılay *et al*., 2018).

Methyl stearate is a fatty acid methyl ester and an octadecanate ester obtained by formal condensation of the carboxy group of stearic acid with the hydroxyl group of methanol. Methyl stearate is reduced in all treatments especially in Hg and in Cu. Methyl stearate is a nonionic surfactant that breaks up aggregates and unfolds proteins to increase the solubility of various substances. The compounds isolated such as methyl stearate and 9-octadecenoic acid from *Melastomastrum*

capitatum play a vital role as anticancerous compound (Ukwubile *et al*., 2019). The leaf methanol extract of it contains bioactive compounds that show potential in treating various diseases, including ovarian cancer. Methyl esters of saturated and unsaturated fatty acids can be isolated from organic material in seawater, with identification based on gas chromatographic retention indices and mass spectrometric fragmentation (Ehrhardt *et al*., 1980).

Vitamin E is present in the control *C. amboinicus* plants. Gamma-tocopherol is a form of vitamin E that is found naturally in a variety of plant-based foods, including nuts, seeds, and vegetable oils, and constitute the major antioxidative systems in plants. After heavy metal exposure, gamma-tocopherols enormously increased in *C. amboinicus*. Highest amount is present in Hg and Cr treated *C. amboinicus* plants. It has several important functions in plants. According to the observations of Ali *et al.* (2020), tocopherols can improve the heavy metal stress tolerance in *Brassica napus*. Tocopherols in plants protect lipids against oxidative stress and play a role in primary carbohydrate metabolism, with a significant proportion synthesized from free phytol during stress or senescence (Dörmann, 2007).

Oral supplementation of gamma-tocopherol (gT) increased angiogenesis in the placental vascular network in late pregnant ewes, potentially providing nutrients for fetus development and growth (Kasimanickam *et al*., 2010). In animal models, a combination of tocopherols rich in gamma-tocopherol suppresses the growth of lung, breast, colon, and prostate cancers, indicating great potential for use in the prevention of human cancer (Ju *et al*., 2010). According to Hensley *et al*. (2004), gamma-tocopherol is a more effective negative risk factor for myocardial infarction and some forms of cancer than alpha-tocopherol. Gamma-tocopherol is more effective in scavenging free radicals and nitrogen oxygen species that cause inflammation, which are components of neurodegenerative disorders (Usoro and Mousa, 2010).

Terpenoid compounds identified in *C. amboinicus* include neophytadine, squalene, methyl commate a, alpha amyrin, caryophyllene and alpha bergamotene.

Terpenoids play vital role in anti-inflammatory effects as well as aid defence against environmental stresses (Prakash, 2017). According to the observations of Rogowska and Szakiel (2021), during stress situations, the biosynthetic pathways of triterpenoids and sterols get changed significantly.

In the present study, the enhanced accumulation of a diterpene, neophytadiene, was observed in all leaves upon exposure to heavy metal as compared to the control. According to the observations of Rani *et al.* (2021), the increased levels of terpenes, including neophytadiene are the products of heavy metal stress related metabolism in plants, which help the plant to tolerate the higher concentrations of the metal. It was reported that the terpene synthase enzyme requires a divalent cation (Mg^{2+} or Mn^{2+} under normal conditions) as a co-factor for their optimal activity (Ashaari *et al*., 2021). During low concentrations of Cu^{2+} ions, it becomes the co-factor for terpene synthase and enhances the biosynthesis of terpenes. In contrast, at higher concentrations of Cu^{2+}, the enzyme activity was interrupted (Hojati *et al*., 2017). This may be the reason for the enhanced neophytadiene accumulation in Cu stressed leaves. Neophytadiene has been found to have anti-inflammatory and anti-tumour properties, which could potentially be useful in the treatment of certain diseases. Additionally, neophytadiene has been studied for its potential as an ingredient in cosmetic and personal care products due to its emollient and skin conditioning properties.

Squalene is another compound present in *C. amboinicus* plants. After the heavy metal stress, the quantity of squalene was reduced. Squalene is synthesized in plants as a precursor to other important compounds such as phytosterols, triterpenes, and carotenoids. Squalene is an important component of plant cell membranes and helps maintain their structural integrity. Its antioxidant, membrane structure, plant defence, anti-inflammatory, and hormonal regulation properties all contribute to the overall health and fitness of plants. Skin is the target tissue subjected to various environmental stimuli that result in oxidative stress, such as pollution, photooxidation, and UV light. Squalene molecules are relatively abundant in skin (Micera *et al*., 2020). Aioin *et al*. (1995) showed the scavenging activity of squalene

on superoxides formation in the Kerathionocytes exposed to oxidative stressors and suggested a protective role of squalene that acts in combination with SOD.

After heavy metal stress, the quantity of Methyl commate A is reduced. Methyl commate A is a natural product that belongs to the group of diterpenoid alkaloids. This component is significantly decreasing in all treatments especially in Cr and Hg treatments. Commercially it is isolated from the leaves of the plant *Gelsemium elegans,* which is commonly known as "heartbreak grass" or "yellow jasmine". Methyl commate A has been shown to have several pharmacological activities, including analgesic, anti-inflammatory, and antitumor effects. It has also been studied for its potential use as a treatment for neurological disorders such as Alzheimer's disease and Parkinson's disease (Zhang *et al*., 2019).

Three closely similar naturally occurring chemicals in the triterpene class are called amyrins. This compound was increased in *C. amboinicus* during heavy metal stress. Alpha-amyrin has been found to possess several biological activities, including anti-inflammatory, antitumor, and antidiabetic effects. It is also used in traditional medicine for various purposes, such as wound healing, pain relief, and respiratory illnesses. Some plant sources of alpha-amyrin include licorice, rosemary, and basil (Melo, 2011; Karen *et al*., 2020).

Gamma sitosterol is a type of phytosterol, which is a group of naturally occurring plant compounds that are chemically similar to cholesterol. This component was reducing after the heavy metal treatment. Sitosterol, found abundantly in plants, shows potential as a herbal nutraceutical for diabetic management, with various biological actions including anxiolytic, sedative, and antioxidant effects (Babu *et al*., 2020). Gamma sitosterol has been investigated for its potential health benefits, particularly for its ability to lower cholesterol levels and improve cardiovascular health. Some studies have suggested that gamma sitosterol may be effective in reducing LDL ("bad") cholesterol levels and improving overall cholesterol profiles, although results have been mixed and further research is needed to fully understand its effects. Gamma sitosterol has been investigated for its

potential anti-inflammatory and anticancer properties (Sundarraj *et al*., 2012; Gylling *et al*., 2014).

As a result of exceptional therapeutic qualities of gamma-sitosterol, it has the potential to be employed as an antidiabetic and was initially discovered in *Girardinia heterophylla* (Tripathi *et al*., 2013). Drought induced production of gamma-sitosterol was observed in *C. amboinicus* (Habeeb *et al*., 2021). Gamma-sitosterol, a major phytochemical in Jambolan seeds, may help reduce blood glucose levels in diabetes treatment (Ramalingam *et al*., 2020).

Sitosterol from *Moringa oleifera* effectively suppresses inflammation in cells by dispersing as nanoparticles and inhibiting key inflammation signalling pathways (Liao *et al*., 2018). Sitosterol serves as a starting material for the biosynthesis of other steroids in plants, such as progesterone, digitoxigenin, gitoxigenin, and digoxigenin (Bennett *et al*., 1969). Sitosterol and sitosterolin are important nutrients in human and animal nutrition due to their synergistic stimulatory effect on the immune system and their potential prophylactic effect against various diseases (Pegel *et al*., 1997). Sitosterol and its derivatives show promising anti-tumour effects, with potential applications in treating various malignant tumours and developing novel anti-tumour drugs (Bao *et al*., 2022).

Beta sitosterol decreased in *C. amboinicus* after the treatment with Al and Cu. But in case of Hg and Cr treatment, the compound vanished in *C. amboinicus*. Beta-sitosterol inhibits colon cancer cell growth and induces apoptosis through caspase-3 and caspase-9 activation and apoptosis-associated proteins (Choi *et al*., 2003). Plant sterols, such as beta-sitosterol, may help protect against colon tumor formation, enhancing the protective effects of vegetarian diets (Raicht *et al*., 1980). Beta-sitosterol, a novel plant-derived angiogenic factor, shows potential pharmaceutical applications for managing chronic wounds (Moon *et al*., 1999). Beta-sitosterol effectively inhibits breast cancer cell growth and activates Fas signaling, potentially serving as a preventive measure for breast cancer (Awad *et al*., 2007).

Tetradecane compound is deteriorating after heavy metal treatment. All the treatments resulted in the disappearance of tetradecane compound, which is a hydrocarbon that belongs to the alkane family and has 14 carbon atoms. It is commonly found in various natural sources, including plants. In plants, tetradecane has been identified as one of the volatile compounds that contribute to their aroma and flavour. It is often found in the essential oils of many plants, including herbs, spices, and flowers. Tetradecane may also have a role in plant defence against herbivores and pathogens. Some studies have shown that plants can release volatile compounds such as tetradecane to repel or deter insects and other pests. Overall, tetradecane is just one of the many chemical compounds that are present in plants and play important roles in their physiology and interactions with the environment (Yaseen *et al.*, 2015).

1,6-Nonadien-3-ol, 3,7-dimethyl is a chemical compound that is also known as DMNT (dimethyl nonatriene). It is present in the control *C. amboinicus* plants. But all the metal treatments resulted in the disappearance of DMNT. It is a volatile organic compound that is found in certain plants, such as tomatoes, corn, and tobacco, and is produced by some insects, including aphids and spider mites (Annett *et al*., 2016). DMNT has been studied for its potential use as a natural insect repellent, as it has been shown to repel certain insect pests, including the whitefly and spider mites (Diaz, 2016). It has also been investigated for its potential use as a plant growth regulator and as a component of pheromone blends used to attract beneficial insects (Shiojiri *et al*., 2017).

Alpha-linolenic acid methyl ester is a chemical compound that is derived from alpha-linolenic acid, an essential omega-3 fatty acid that is found in certain plant-based foods, such as flaxseed, chia seeds, and walnuts. Dietary components chlorophyllin, beta-carotene, and alpha-linolenic acid methyl ester effectively inhibit chromosomal aberrations caused by mutagens, with alpha-linolenic acid methyl ester being the most effective (Renner e*t al*., 1990). Only the Al treatment is retaining this compound, and all other metals Cr, Cu and Hg resulted in the deletion of this compound. Alpha-linolenic acid is an important nutrient for human health,

and it has been associated with a range of potential health benefits, including reducing inflammation, improving heart health, and supporting brain function. Alpha-linolenic acid is an omega-3 fatty acid that may help reduce the risk of heart disease by improving cholesterol levels, reducing blood pressure, and decreasing the risk of blood clots.

Caryophyllene is a natural bicyclic sesquiterpene found in many plants, particularly in spices like black pepper, cinnamon, and cloves. It is also found in herbs such as oregano, basil, and hops, as well as in some strains of *Cannabis*. Caryophyllene has been found to have a variety of pharmacological properties, including anti-inflammatory, analgesic, and anxiolytic effects. It has also been shown to have potential therapeutic benefits in the treatment of conditions such as anxiety, depression, and addiction (Vallejo *et al*., 2006). Al and Cu treatment retained caryophyllene but the quantity is reduced. Complete disappearance happens in case of Cr and Hg.

Alpha-bergamotene is a sesquiterpene hydrocarbon that is found in the essential oils of various plants. Alpha-bergamotene has been shown to have a range of potential health benefits, particularly in the areas of anti-inflammatory and antioxidant activity. Some studies have also suggested that alpha-bergamotene may have anti-cancer properties. This component is drastically reduced after Cu treatment which leads to the removal of oil globules from stem and leaf of *C. amboinicus* which is already discussed earlier.

2,4-Bis(1,1-dimethylethyl) phenol is also known as butylated hydroxytoluene (BHT), which is a synthetic antioxidant commonly used as a food additive and in cosmetics, pharmaceuticals, and industrial applications. It is not produced by plants naturally. But after the Cu treatment, this compound is formed in *C. amboinicus*.

At higher concentration, it promotes photosynthesis in hop seedlings (Zhang and Xin, 2011). Oxidation in alcoholic solvents produces benzylic ethers, which serve as convenient intermediates for syntheses of related compounds. It shows strong free radical scavenging activity and stable phenoxy radical formation when

oxidized with lead and production in avocado roots may contribute to resistance against *Phytophthora cinnamomi* by regulating hydrogen peroxide production (Bhimanagoud *et al*., 2019).

Tetradecanoic acid, 12-methyl-methyl ester is a newly formed compound after the heavy metal treatment and is a type of fatty acid methyl ester that is commonly found in plants and other natural sources. Anti-inflammatory and antioxidant properties of this compound were studied (Bahar, 2014). It has a variety of uses in industry and can also have potential health benefits. It is not present in the control plants but after all treatments this compound was identified. Hence it is evident that all metal treatment causes deterioration of the medicinal potential *i.e*., antioxidant property of *C. amboinicus*. Even though the newly synthesized component, Tetradecanoic acid, 12-methyl-methyl ester plays direct role in antioxidation process, it is evident that the *de novo* synthesis maintains the medicinal potential of *C. amboinicus* plant.

Isolongifolol, methyl ester is a chemical compound that is found Cu treated *C. amboinicus* plants not in the control ones. This component is particularly present in the essential oils of some coniferous trees such as *Pinus radiata*. While there is some limited research on isolongifolol and its potential health benefits, there is currently not enough evidence to support its use in human health or as a therapeutic agent. Antimicrobial activity of *Warburgia ugandensis* resulted in the formation of isolongifolol methyl ester (Abuto, 2016). Isolongifolenone effectively repels mosquitoes and ticks, offering an inexpensive and safe alternative to synthetic chemical repellents for large human populations (Zhang *et al.*, 2009).

Treatment resulted in the reduction of 9,12-octadecadienoic acid methyl ester, also known as methyl linoleate, which is a fatty acid methyl ester commonly found in plant oils, such as sunflower oil and soybean oil. In terms of human uses, 9,12-octadecadienoic acid methyl ester is used as a flavoring agent and as an ingredient in various cosmetic and personal care products. It is also used in the production of biodiesel.

In conclusion, *Coleus amboinicus* exposed to aluminum, chromium, copper and mercury displays notable metabolic, structural, and metal-specific tolerance mechanisms in addition to toxicity responses additionally varies amongst metals. Heavy metal tolerance potential by *C. amboinicus* is thus undoubtedly proved under laboratory experimental conditions. Different adaptations developed by the plant in response to the increased level of toxic metal ions in the growth medium provide confirmatory evidences for suggesting *C. amboinicus* as a promising tolerant candidate for the heavy metal pollution. Since *Coleus amboinicus* is a wild plant that grows naturally in marshy and shaded places, it is seldom exposed to heavy metal contamination and retains its medicinal properties, such as its antioxidant capacity, in its natural habitat.

SUMMARY AND CONCLUSIONS

Coleus amboinicus (syn; *Plectranthus amboinicus*) is a potential medicinal herb belonging to the family Lamiaceae and is known by a bunch of vernacular names ranging from Indian borage to "Panikoorka" in Malayalam.

In the present investigation, an attempt is made to analyse the effect of selected heavy metals – Aluminium, Chromium, Mercury and Copper on the medicinal plant *Coleus amboinicus*. A comparative study on the parameters such as anatomical/histochemical changes in the root, stem and leaves of plants subjected to known quantities of aluminium, chromium, mercury and copper cultivated under Hoagland nutrient solution were followed to evaluate the impact of heavy metals on growth, physiology and metabolic changes in general and medicinal property in particular.

The plants growing in half strength Hoagland medium without any metal treatment served as the control. Analyses of various functional and structural aspects in the root, stem and leaves were carried out on 4, 8, 12, 16 and 20 days of heavy metal treatment. For the standardization of heavy metal concentrations that induce toxicity symptoms so as to impart about 50% growth retardation, rooted cuttings were grown in different concentrations of aluminium chloride ($AlCl_3$), potassium dichromate ($K_2Cr_2O_7$), copper sulphate ($CuSO_4$.5H2O) and mercuric chloride ($HgCl_2$). The concentrations of metal salts in which the rooted propagules of *C. amboinicus* survived but exhibited approximately 50% growth retardation were selected for the research study. The optimal concentrations were determined by trial-and-error method. Rooted propagules were grown in different concentrations such as Aluminium chloride – 500 µM, Potassium dichromate-150 µM, Mercuric chloride-10 µM and Copper sulphate - 80 µM. *C. amboinicus* plants treated with these standardized concentrations of heavy metal used for the further analysis.

A comparative study on the parameters such as anatomical/histochemical changes in the plant parts subjected to known quantities of Al, Cr, Cu and Hg

cultivated in Hoagland nutrient solution were followed to evaluate the impact of these heavy metals on growth, physiology and metabolic changes in general and medicinal property in particular. Tolerance index also was calculated and taken as a parameter to assess tolerance potential. In addition, qualitative and quantitative analyses of protein, phenolics, proline, chlorophyll and carotenoid pigments were done. Since the production of ROS is induced by heavy metal toxicity, the function of scavenging enzymes such as catalase, superoxide dismutase activity, and MDA production, was also examined in different organs such as the root, stem, and leaves. In order to confirm the anatomical modifications in the different plant parts, a scanning electron microscopy (SEM) analysis was also conducted. The SEM-EDX (SEM linked to Energy Dispersive X-ray analysis) technique was also used to determine the specific involvement and distribution pattern of heavy metal ions provided to the nutrient medium. ICP-OES was used to analysethe potential of *C. amboinicus* for bioaccumulation of heavy metals. Since this plant is medicinal, GC-MS analysis was also used to examine the distribution and occurrence of bioactive secondary metabolites.

The following conclusions are drawn after analysing, comparing, and discussing the observation data using appropriate recent literature.

- The growth retardation and morphology of *Coleus amboinicus* were observed to be somewhat similar to different concentrations of Al, Cr, Cu and Hg. However, there was a notable variation in the concentration required to cause visible toxicity symptoms, meaning that the tolerance potential of plant varied depending on the metal.

- The anatomical observations like impaired growth, removal of root hairs, disruption of roots, and impaired vessels in the treatment of Cu indicate that this element is more toxic than Al, even though it is an essential element and the morphological changes caused by heavy metals are minimal.

- SEM study of leaf surface reveals development of multicellular uniseriate long hairs and glandular bladder like trichomes. Number and size vary from

plant to plant. Maximum trichomes were shown by leaves treated with Hg. These structures are also destined to sequester metal ions and also function as a pathway for the exit of volatile forms of metal particularly Hg.

- In plants treated with Hg, there is an increased number of stomata and a wider stomatal opening; these traits are directly linked to the escape of volatile forms of Hg, which is an indicator of toxicity sequestration.

- The development of lenticels is found to be linked with the exit of metals from the plants treated with Hg, since Hg is already known to exit by the process of phytovolatilization from the parts of the plant.

- The vital role of the cell wall in Cu sequestration is indicated by the increased cell wall thickness, higher distribution of Ca in the xylem walls, and a greater amount of deposited Cu in the roots.

- The precision of the SEM-EDX study is confirmed by the remarkable increases in the weight percentage of Al, Cr, Cu and Hg in their respective treatments as compared to the distribution of other elements.

- Water absorption and translocation get impaired in the root itself, *i.e.*, it adversely affects RWC in leaf. The presence of Hg-binding peptides in the leaves suggests that the plant's ability to accumulate and sequester Hg ions may have led to changes in the leaf composition, potentially affecting the dry weight. Hg treatment resulted in increased DW.

- Increased protein reveals the possibility of phytochelatin synthesis which is considered as a major effect of heavy metals in general and Cr and Hg in particular, since phytochelatins are involved in heavy metal sequestration.

- Proline functions to quench ROS in *Coleus amboinicus* as well, since the activity of antioxidant enzymes registers only very feeble activity. Proline concentrations in the roots of plants treated with Hg continuously increase, indicating the compatible role of proline and osmoregulation in mitigating the toxicity of heavy metal ions in the roots.

- Enhanced proline content indirectly reveals the water deficient in the growth medium due to the presence of metal ions and hence proline plays the role of osmoregulation in the roots. Because of the reduced root growth, decreased uptake of essential elements, and root dieback due to metal stress, the metabolic activities in the roots become highly disturbed.

- The total free amino acid concentration showed a declining trend, which is consistent with the accumulation of secondary metabolites. Amino acids are the building blocks of lignin, phenol, and alkaloids; their production is frequently facilitated by secondary metabolism. It could happen if the pathway for the production of amino acids shifts to one for secondary metabolites.

- Bioaccumulation profile of all the four metals differed and found to be dependent on process of absorption, translocation and accumulation in *C. amboinicus*. Maximum accumulation of Cu and minimum accumulation of Hg happened.

- Less accumulation of Hg can be interpreted in terms of concentration of Hg given and the mode of loss through different passages of the plant body like lenticels and stomata. Despite the increased content of Hg in the root system and Hg content is below detectable level in the leaves. Loss occurs by phytovolatilization through modified trichomes on the midrib of leaves as well as through open wide stomata.

- Translocation potential of Al is very high and this observation can also be correlated with the maximum concentration given in the treatment.

- Effect of heavy metals resulting in the absence of many secondary metabolites which is vital for the antibacterial property of *Coleus amboinicus* whereas occurrence of some new bioactive components especially Tetradecanoic acid,12-methyl-methyl ester shows similar properties.

- On exposure to Al, Cr, Cu and Hg, the composition of phytol was significantly increased in the leaves as compared to the control. Since phytol is an isoprenoid alcohol bound with chlorophyll molecule via ester linkage, the degradation of chlorophyll results in the accumulation of phytol and its derivatives in the cells. Phytol is the side chain of the photosynthetic pigment chlorophyll, is the most abundant plant terpenoid.

- An interesting observation in the Cu treatment of *C. amboinicus* is the removal of oil globules from stem and leaf. SEM images give a clear evidence for the removal of oil globules. The possible reason for its removal may due to the reduction of a secondary metabolite named alpha-bergamotene after Cu treatment. It is a major component of essential oils in various plants and it contributes to the aromatic profile and therapeutic properties of essential oils.

- Defensive mechanisms/strategies in terms of different metabolites distribution, antioxidant potential of particular primary and secondary metabolites, antioxidant enzymes, and bioaccumulation potential comply with the tolerance/sensitivity of *C. amboinicus* cultivated under simulated experimental setup, even though there is significant variation in the molar concentrations of each metal.

RECOMMENDATIONS

1. Field Validation of Laboratory Findings

To fully understand the implications of the findings and their applicability in real-world scenarios, it is crucial to validate them under field or natural conditions. While laboratory experiments offer controlled environments that allow for precise manipulation and observation, they may not fully replicate the complex interactions and variability present in natural ecosystems. Field studies provide an opportunity to assess the generalizability of our findings in more realistic settings, where plants are subject to a range of environmental factors and stressors beyond just heavy metal exposure. By extending research from laboratory to field conditions, it can enhance the relevance and applicability of our findings, contributing to a more comprehensive understanding of the impacts of heavy metal contamination on medicinal plants in their natural habitats.

2. Assessment of Heavy Metal Biomagnification in *C. amboinicus* Consumption Chains

This research has revealed the impact of heavy metal contamination on *C. amboinicus*, highlighting potential risks associated with its consumption, particularly for vulnerable populations such as infants who use it for cold and cough remedies. Understanding the potential for biomagnification of heavy metals in the consumption chain is essential for assessing the health risks posed by contaminated medicinal plants. Infants, due to their developing physiology and higher sensitivity, are particularly vulnerable to the adverse effects of heavy metal exposure due to the continuous usage. Investigate the pathways through which the contaminated medicinal plant enters the consumption chain, including harvesting, processing, and preparation for oral administration to infants. Determine the extent to which heavy metals may be transferred and concentrated at each stage. Use appropriate risk

assessment models and guidelines to estimate the likelihood of adverse health outcomes. By assessing the potential for biomagnification of heavy metals in the consumption chain of *C. amboinicus*, particularly those used for infant healthcare, further studies can contribute to safeguarding public health and informing regulatory policies to ensure the safety and efficacy of traditional medicinal practices.

REFERENCES

Abdel-Basset, R., Ozuka, S., Demiral, T., Furuichi, T., Sawatani, Y., Baskin, T. I., Matsumoto, H. and Yamamoto, K. (2010). Aluminium reduces sugar uptake in tobacco cell cultures: A potential cause of inhibited elongation but not of toxicity. *Journal of Experimental Botany*, **61**: 1597–1610. https://doi.org/10.1093/jxb/erq053

Abid, S. and Touqueer, S. (2015). Antimicrobial and antioxidant activity of *Breynia disticha* and *Vernonia elaeagnifolia*. *Journal of Applied Pharmacy*, **7**: 178–182.

Abou Zeid, A. H. S. (2002). Stress metabolites from *Corchorus olitorius* L. leaves in response to certain stress agents. *Food Chemistry*, **76**: 187–195. https://doi.org/10.1016/S0308-8146(01)00324-6

Abuto, J. O. (2016). Antimicrobial activity and phytochemical profiles of *Warburgia ugandensis Sprague* (Canellaceae) extracts from different populations across the Kenyan Rift Valley (Doctoral dissertation, Kenyatta University, Nairobi, Kenya).

Adejumo, S. A., Oniosun, B., Akpoilih, O. A., Adeseko, A. and Arowo, D. O. (2021). Anatomical changes, osmolytes accumulation and distribution in the native plants growing on Pb-contaminated sites. *Environmental geochemistry and health*, **43**: 1537–1549. https://doi.org/10.1007/s10653-020-00649-5

Adhikari, A., Adhikari, S., Ghosh, S., Azahar, I., Shaw, A. K., Roy, D., Roy, S., Saha, S. and Hossain, Z. (2020). Imbalance of redox homeostasis and antioxidant defense status in maize under chromium (VI) stress. *Environmental and Experimental Botany*, **169**: 103873. https://doi.org/10.1016/j.envexpbot.2019.103873

Adrees, M., Ali, S., Iqbal, M., Bharwana, S. A., Siddiqi, Z., Farid, M., Ali, Q., Saeed, R. and Rizwan, M. (2015). Mannitol alleviates chromium toxicity in wheat plants in relation to growth, yield, stimulation of antioxidative enzymes, oxidative stress and Cr uptake in sand and soil media. *Ecotoxicology and Environmental Safety*, **122**: 1–8. https://doi.org/10.1016/j.ecoenv.2015.07.024

Aery, N. C. (2012). Plant defence against heavy metal stress. In J. Mérillon and K. Ramawat (Eds.), *Plant Defence: Biological Control*,**12**: 241-269.

Aguilera, J. G., Teodoro, P. E., Da Silva, J. P., Pereira, J. F., Zuffo, A. M. and Consoli, L. (2019). Selection of aluminum-resistant wheat genotypes using multi environment and multivariate indices. *Agronomy Journal*, **111**: 2804–2810. https://doi.org/10.1002/agj2.20144

Ahmad, A., Khan, M. A. and Mujeeb, M. (2019). Carvacrol induces apoptosis and inhibits proliferation in human breast cancer cells. *Journal of Cellular Biochemistry*, **120**: 7611-7621. doi: 10.1002/jcb.28353

Ahmad, P., Ahanger, M. A., Egamberdieva, D., Alam, P., Alyemeni, M. N. and Ashraf, M. (2018). Modification of osmolytes and antioxidant enzymes by 24-epibrassinolide in chickpea seedlings under mercury (Hg) toxicity. *Journal of Plant Growth Regulation*, **37**: 147–155. https://doi.org/10.1007/s00344-017-9730-6

Ahmad, R., Ali, S., Abid, M., Rizwan, M., Ali, B., Tanveer, A. and Qin, X. (2020). Glycinebetaine alleviates chromium toxicity in *Brassica oleracea* L. by suppressing oxidative stress and modulating plant morphology and photosynthetic attributes. *Environmental Science and Pollution Research*, **27**: 1101-1111. https://doi.org/10.1007/s11356-019-06431-5

Ahmed, A. and Tajmir-Riahi, H. A. (1993). Interaction of toxic metal ions Cd^{2+}, Hg^{2+} and Pb^{2+} with light-harvesting proteins of chloroplast thylakoid membranes: An FTIR spectroscopic study. *Journal of Inorganic Biochemistry*, **50**: 235–243. https://doi.org/10.1016/0162-0134(93)80014-G

Ahn, S. J., Sivaguru, M., Chung, G. C., Rengel, Z. and Matsumoto, H. (2002). Aluminium-induced growth inhibition is associated with impaired efflux and influx of H^+ across the plasma membrane in root apices of squash (*Cucurbita pepo*). *Journal of Experimental Botany*, **53**: 1959–1966. https://doi.org/10.1093/jxb/53.376.1959

Ahn, S. J., Sivaguru, M., Chung, G. C., Rengel, Z. and Matsumoto, H. (2002). Aluminium-induced growth inhibition is associated with impaired efflux and influx of H+ across the plasma membrane in root apices of squash (*Cucurbita pepo*). *Journal of Experimental Botany*, **53**: 1-9.

Aioin, A., Shimizu, T. and Kuriyama, K. (1995). Effect of squalene on superoxide anion generation induced by a skin irritant, lauroylsarcosine. *International Journal of Pharmaceutics*, **113**: 159–164. https://doi.org/10.1016/0378-5173(94)00334-6

Aiyegoro, O. A. and Okoh, A. I. (2010). Preliminary phytochemical screening and in vitro antioxidant activities of the aqueous extract of *Helichrysum longifolium* DC. BMC *Complementary and Alternative Medicine*, **10**: 1–8. https://doi.org/10.1186/1472-6882-10-1

Albert, E., Kim, S. S., Magallanes-Lundback, M., Bao, Y., Deason, N., Danilo, B., Wu, D., Li, X., Wood, J., Bornowski, N., Gore, M., Buell, C. and DellaPenna, D. (2022). Genome-wide association identifies a missing hydrolase for tocopherol synthesis in plants. *Proceedings of the National Academy of Sciences*, **119**: e2113488119. https://doi.org/10.1073/pnas.2113488119

Alfaifi, T. and El-Shabasy, A. (2021). Effect of heavy metals in cement dust pollution on morphological and anatomical characteristics of *Cenchrus ciliaris* L. *Saudi Journal of Biological Sciences*, **28**: 1069-1079. https://doi.org/10.1016/j.sjbs.2020.11.036

Ali, E., Hassan, Z., Irfan, M., Hussain, S., Rehman, H. U., Shah, J. M. and Ali, S. (2020). Indigenous tocopherol improves tolerance of oilseed rape to cadmium stress. *Frontiers in Plant Science*, **11**: 1536-1540. https://doi.org/10.3389/fpls.2020.01536

Ali, S., Chaudhary, A., Rizwan, M., Anwar, H. T., Adrees, M., Farid, M., Irshad, M. K., Hayat, T. and Anjum, S. A. (2015). Alleviation of chromium toxicity by glycinebetaine is related to elevated antioxidant enzymes and suppressed chromium uptake and oxidative stress in wheat (*Triticum aestivum* L.). *Environmental Science and Pollution Research*, **22**: 10669–10678. https://doi.org/10.1007/s11356-015-4193-4

Allan, J. E. (1969). The preparation of agricultural samples for analysis by atomic absorption spectrometry. *Varian Techtron Bulletin*, **2**: 12–69.

Almeida Rodrigues, A., Filho, S., Almeida Rodrigues, D., Lino Rodrigues, C., Sales, J. and Vital, R. (2016). Influence of aluminum on root growth and anatomy of *Stenocalyx dysentericus* (DC.) O. Berg. *African Journal of Biotechnology*, **15**: 1193–1200. https://doi.org/10.5897/AJB2016.15383

Alves, A., Ribeiro, R., Azenha, M., Cunha, M. and Teixeira, J. (2023). Effects of exogenously applied copper in tomato plants' oxidative and nitrogen metabolisms under organic farming conditions. *Horticulturae*, **9**: 323-329. https://doi.org/10.3390/horticulturae9030323

Amin, H., Ahmed Arain, B., Abbasi, M. S., Amin, F., Jahangir, T. M. and Soomro, N. U. A. (2019). Evaluation of chromium phyto-toxicity, phyto-tolerance and phyto-accumulation using biofuel plants for effective phytoremediation. *International Journal of Phytoremediation*, **21**: 352–363. https://doi.org/10.1080/15226514.2018.1524837

Amin, H., Arain, B. A., Amin, F. and Surhio, M. A. (2013). Phytotoxicity of chromium on germination, growth and biochemical attributes of *Hibiscus esculentus* L. *American Journal of Plant Sciences*, **4**: 9-16. https://doi.org/10.4236/ajps.2013.412A2002

Anderson, J. A. (1995). Lipid peroxidation and plant tissue disorders: Introduction to the workshop. *HortScience*, **30**: 196–197.

Andersson, M. (1988). Toxicity and tolerance of aluminium in vascular plants – a literature review. *Water, Air and Soil Pollution*, **39**: 439–462. https://doi.org/10.1007/BF00279487

Angulo-Bejarano, P. I., Puente-Rivera, J. and Cruz-Ortega, R. (2021). Metal and metalloid toxicity in plants: An overview on molecular aspects. *Plants*, **10**: 635-643. https://doi.org/10.3390/plants10040635

Anjitha, K. S., Sameena, P. P. and Puthur, J. T. (2021). Functional aspects of plant secondary metabolites in metal stress tolerance and their importance in pharmacology. *Plant Stress*, **2**: 100038. https://doi.org/10.1016/j.plants.2021.100038

Anjum, H. A., Ashraf, U., Khan, I., Tanveer, M., Shahid, M., Shakoor, A. and Wang, L. (2017). Phyto-toxicity of chromium in maize: Oxidative damage, osmolyte accumulation, anti-oxidative defense and chromium uptake. *Pedosphere*, **27**: 262–273. https://doi.org/10.1016/S1002-0160(17)60315-1

Anjum, N. A., Singh, H. P., Khan, M. I. R., Masood, A., Per, T. S., Negi, A., Batish, D. R., Khan, N. A. and Ahmad, I. (2015). Too much is bad - an appraisal of phytotoxicity of elevated plant-beneficial heavy metal ions. *Environmental Science and Pollution Research*, **22**: 3361–3382. https://doi.org/10.1007/s11356-014-3438-5

Anjum, S. A., Tanveer, M., Hussain, S., Shahzad, B., Ashraf, U., Fahad, S., Hassan, W., Jan, S., Khan, I., Saleem, M. F., Bajwa, A. A., Wang, L., Mahmood, A., Samad, R. A. and Tung, S. A. (2016). Osmoregulation and antioxidant production in maize under combined cadmium and arsenic stress. *Environmental Science and Pollution Research*, **23**: 11864-11875. https://doi.org/10.1007/s11356-016-6308-9

Annett, R., Claudia, S., Zhiwu, Z., Alexander, E. L., Feng Tian, Tobias G. K., Christiane S., Susanne P., Sandra I., Georg J., Willhelm, B., Jonathan G., Edward S. Buckler and Jörg D. (2016). Characterization of biosynthetic pathways for the production of the

volatile homoterpenes DMNT and TMTT in *Zea mays*. *The Plant Cell*, **28**: 2651–2665. https://doi.org/10.1105/tpc.16.00551

Ansari, M. K. A., Ahmad, A., Umar, S. and Iqbal, M. (2009). Mercury-induced changes in growth variables and antioxidative enzyme activities in Indian mustard. *Journal of Plant Interactions*, **4**: 131–136. https://doi.org/10.1080/17429140902782845

Apel, K. and Hirt, H. (2004). Reactive oxygen species: Metabolism, oxidative stress and signal transduction. *Annual Review of Plant Biology*, **55**: 373–399. https://doi.org/10.1146/annurev.arplant.55.031903.141701

Arasimowicz-Jelonek, M., Floryszak-Wieczorek, J., Drzewiecka, K., Chmielowska-Ba□k, J., Abramowski, D. and Izbianska, K. (2004). Aluminum induces cross-resistance of potato to *Phytophthora infestans*. *Planta*, **239**: 679–694. https://doi.org/10.1007/s00425-004-1335-4

Arduini, I., Masoni, A., Mariotti, M. and Ercoli, L. (2004). Low cadmium application increases *Miscanthus* growth and cadmium translocation. *Environmental and Experimental Botany*, **52**: 153–164. https://doi.org/10.1016/j.envexpbot.2004.01.006

Armendariz, A. L., Talano, M. A., Villasuso, A. L., Travaglia, C., Racagni, G. E. and Reinoso, H. (2016). Arsenic stress induces changes in lipid signalling and evokes stomata closure in soybean. *Plant Physiology and Biochemistry*, **103**: 45–52. https://doi.org/10.1016/j.plaphy.2016.02.029

Arnon, D. I. (1949). Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant Physiology*, **24**: 1–15. https://doi.org/10.1104/pp.24.1.1

Arora, A., Byrem, T. M., Nair, M. G. and Strasburg, G. M. (2000). Modulation of liposomal membrane fluidity by flavonoids and isoflavonoids. *Archives of Biochemistry and Biophysics*, **373**: 102–109. https://doi.org/10.1006/abbi.1999.1523

Arumugam, G., Swamy, M. K. and Sinniah, U. R. (2016). *Plectranthus amboinicus* (Lour.) Spreng: Botanical, phytochemical, pharmacological and nutritional significance. *Molecules*, **21**: 369-378. https://doi.org/10.3390/molecules21030369

Aryani, R. D., Nugroho, A. P. and Purwestri, Y. A. (2018). Effects of copper on accumulation, plant growth, superoxide dismutase activity and total phenolic compounds in paddy (*Oryza sativa* L. 'Cempo Merah') leaves. In: Sukartiko, C. (Ed.), *Proceeding of the 2nd International Conference on Tropical Agriculture*, Springer Nature Switzerland, 1–8.

Ascensão, L. and Pais, M. S. (1998). The leaf capitate trichomes of *Leonotis leonurus*: Histochemistry, ultrastructure and secretion. *Annals of Botany*, **81**: 263–271. https://doi.org/10.1006/anbo.1997.0548

Ashaari, N. S., Sabri, S., Lai, K. S., Song, A. A. L., Rahim, R. A. and Abdullah, J. O. (2021). Kinetic studies and homology modeling of a dual-substrate linalool/nerolidol synthase from *Plectranthus amboinicus*. *Scientific Reports*, **11**: 1–16. https://doi.org/10.1038/s41598-021-81451-6

Ashraf, M., Ashraf, M. Y., Mehmood, K., Akhtar, J., Hussain, F. and Arshad, M. (2010). Phytoremediation of saline soils for sustainable agricultural productivity. In: Ashraf, M., Ozturk, M. and Ahmad, M. S. A. (Eds.), *Plant Adaptation and*

Phytoremediation, Springer Dordrecht, Netherlands, 335–355. https://doi.org/10.1007/978-90-481-9370-7_15

Asiimwe, S., Borg-Karlsson, A. K., Azeem, M., Mugisha, K. M., Namutebi, A. and Gakunga, N. J. (2014). Chemical composition and toxicological evaluation of the aqueous leaf extracts of *Plectranthus amboinicus* (Lour.) Spreng. *International Journal of Pharmaceutical Science Invention*, **3**: 19–27.

Awad, A. B., Chinnam, M., Fink, C. S. and Bradford, P. G. (2007). Beta-Sitosterol activates Fas signaling in human breast cancer cells. *Phytomedicine*, **14**: 747–754. https://doi.org/10.1016/j.phymed.2007.01.012

Awasthi, J. P., Saha, B., Panigrahi, J., Regon, P., Sahoo, S., Chowra, U. and Siddique, U. (2019). Redox balance, metabolic fingerprint and physiological characterization in contrasting North East Indian rice for aluminum stress tolerance. *Scientific Reports*, **9**: 8681. https://doi.org/10.1038/s41598-019-45158-3

Awasthi, J. P., Saha, B., Regon, P., Sahoo, S., Chowra, U., Pradhan, A. and Siddique, U. (2017). Morpho-physiological analysis of tolerance to aluminum toxicity in rice varieties of North East India. PLOS ONE, **12**: e0176357. https://doi.org/10.1371/journal.pone.0176357

Azevedo, R., Rodriguez, E., Mendes, R. J., Mariz-Ponte, N., Sario, S., Lopes, J. C., Ferreira de Oliveira, J. M. P. and Santos, C. (2018). Inorganic Hg toxicity in plants: A comparison of different genotoxic parameters. *Plant Physiology and Biochemistry*, **125**: 247–254. https://doi.org/10.1016/j.plaphy.2018.02.014

Babangida, S. K., Muhammad, A., Garba, A. and Sallau, A. B. (2021). Antioxidants in bioremediation of chromium (VI) by conventional and nanotechnological approaches: A review. *Toxicological and Environmental Chemistry*, **103**: 162–183.

Babu, S. and Jayaraman, S. (2020). An update on β-sitosterol: A potential herbal nutraceutical for diabetic management. *Biomedicine and Pharmacotherapy*, **131**: 110702.

Bahar, B., Sökmen, B., Hasdemir, A., Yusufoglu, R. and Yanardag, R. (2014). Some monohydroxy tetradecanoic acid isomers as novel urease and elastase inhibitors and as new antioxidants. *Applied Biochemistry and Biotechnology*, **172**: 1358–1364.

Baker, A. J. M. and Walker, P. L. (1989). Physiological responses of plants to heavy metals and the quantification of tolerance and toxicity. *Chemical Speciation and Bioavailability*, **1**: 7–17.

Balasaraswathi, K., Jayaveni, S., Sridevi, J., Sujatha, D., Aaron, K. P. and Rose, C. (2017). Cr-induced cellular injury and necrosis in *Glycine max* L.: Biochemical mechanism of oxidative damage in chloroplast. *Plant Physiology and Biochemistry*, **118**: 653-666.

Bankaji, I., Sleimi, N., López-Climent, M. F., Perez-Clemente, R. M. and Gomez-Cadenas, A. (2014). Effects of combined abiotic stresses on growth, trace element accumulation and phytohormone regulation in two halophytic species. *Journal of Plant Growth Regulation*, **33**: 632–643.

Bao, X., Zhang, Y., Zhang, H. and Xia, L. (2022). Molecular mechanism of β-sitosterol and its derivatives in tumor progression. *Frontiers in Oncology*, **12**: 926975.

Baran, P., Aktaş, K. and Özdemir, C. (2020). Structural investigation of the glandular trichomes of endemic *Salvia smyrnea* L. *South African Journal of Botany*, **76**: 572–578.

Baranitharan, M. and Dhanasekaran, S. (2014). Mosquitocidal efficacies of medicinal plant of *Coleus aromaticus* Benth (Lamiaceae) leaf extracts against Chikungunya vector, *Aedes aegypti* (Linn.) (Diptera: Culicidae). *International Journal of Current Research in Chemistry and Pharmaceutical Science*, **1**: 61-67.

Barcelo, J., Poschenrieder, C. and Gunse, B. (1985a). Effect of chromium VI on mineral element composition of bush beans. *Plant Nutrition*, **8**: 211-217.

Barcelo, J., Poschenrieder, C., Ruano, A. and Gunse, B. (1985b). Leaf water potential in Cr (VI) treated bean plants (*Phaseolus vulgaris* L.). *Plant Physiology Supplements*, **77**: 163-164.

Basberg-Pahlsson, A. M. (1989). Toxicity of heavy metals (Zn, Cu, Cd, Pb) to vascular plants. *Water, Air and Soil Pollution*, **47**: 287-319.

Basile, A., Sorbo, S., Pisani, T., Paoli, L., Munzi, S. and Loppi, S. (2012). Bioaccumulation and ultrastructural effects of Cd, Cu, Pb and Zn in the moss *Scorpiurum circinatum* (Brid.) Fleisch. and Loeske. *Environmental Pollution*, **166**: 208–211.

Basit, F., Bhat, J. A., Han, J., Guan, Y., Jan, B. L., Shakoor, A. and Alansi, S. (2022). Screening of rice cultivars for Cr stress response by using the parameters of seed germination, morpho-physiological and antioxidant analysis. *Saudi Journal of Biological Sciences*, **29**: 3918–3928.

Baslas, R. K. and Kumar, P. (1981). Chemical examination of essential oil of *Coleus aromaticus* Benth. *Journal of the Indian Chemical Society*, **58**: 103–104.

Bates, L. S., Waldren, R. P. and Teare, I. D. (1973). Rapid determination of free proline for water-stress studies. *Plant and Soil*, **39**: 205–207. https://doi.org/10.1007/BF00018060

Batista, M. F., Moscheta, I. S., Bonato, C. M., Batista, M. A., Almeida, O. J. G. A. and Inoue, T. (2013). Aluminium in corn plants: Influence on growth and morphoanatomy of root and leaf. *Brazilian Journal of Soil Science*, **37**: 177–187.

Batool, R., Hameed, M., Ashraf, M., Ahmad, M. S. A. and Fatima, S. (2015). Physio-anatomical responses of plants to heavy metals. In: Öztürk, M., Ashraf, M., Aksoy, A. and Ahmad, M. S. A. (Eds.), *Phytoremediation for Green Energy*, Springer Dordrecht, Netherlands, 79–96.

Bechtaoui, N., Rabiu, M. K., Raklami, A., Oufdou, K., Hafidi, M. and Jemo, M. (2021). Phosphate-dependent regulation of growth and stresses management in plants. *Frontiers in Plant Science*, **12**: 679916.

Bennett, R. D., Heftmann, E. and Winter, B. J. (1969). A function of sitosterol. *Phytochemistry*, **8**: 2325–2328.

Bennicelli, R. Z., Stepniewska, A., Banach, K., Szajnocha and Ostrowski, J. (2004). The ability of *Azolla caroliniana* to remove heavy metals (Hg (II), Cr (III), Cr (IV)) from municipal waste water. *Chemosphere*, **55**: 141–146.

Bera, A. K., Kanta-Bokari, A. K. and Bokaria, K. (1999). Effect of tannery effluent on seed germination, seedling growth and chloroplast pigment content in mung bean (*Vigna radiate* L. Wilczek). *Environmental Ecology*, **17**: 958–961.

Bernal, M., Roncel, M., Ortega, J. M., Picorel, R. and Yruela, I. (2004). Copper effect on cytochrome b559 of photosystem II under photoinhibitory conditions. *Plant Physiology*, **120**: 686-694.

Bernier, M., Popovic, R. and Carpentier, R. (1993). Mercury inhibition at the donor side of photosystem II is reversed by chloride. *FEBS Letters*, **321**: 19–23.

Bhakuni, D. S., Dhar, M. L., Dhar, M. M., Dhawan, B. N. and Mehrotra, B. N. (1969). Screening of Indian plants for biological activity. Part II. *Indian Journal of Experimental Biology*, **7**: 250–262.

Bhalerao, S. A. and Sharma, A. S. (2015). Chromium: As an environmental pollutant. *International Journal of Current Microbiology and Applied Sciences*, **4**: 732–746.

Bharath, P., Gahir, S. and Raghavendra, A. S. (2021). Abscisic acid-induced stomatal closure: An important component of plant defense against abiotic and biotic stress. *Frontiers in Plant Science*, **12**: 615114.

Bhat, P., Hegde, G. and Hegde, G. R. (2012). Ethnomedicinal practices in different communities of Uttara Kannada district of Karnataka for treatment of wounds. *Journal of Ethnopharmacology*, **143**: 501–514.

Bhatt, P., Joseph, G. S., Negi, P. S. and Varadaraj, M. C. (2013). Chemical composition and nutraceutical potential of Indian borage (*Plectranthus amboinicus*) stem extract. *Journal of Chemistry*,**4**: 1-7. https://doi.org/10.1155/2013/320329

Bhattacharya, S. and Sahu, P. K. (2020). Carvacrol: A review of its pharmacological and clinical applications. *Journal of Pharmacy and Pharmacology*, **72**: 1049-1066. doi: 10.1111/jphp.13244

Bhimanagoud, K., Riaz, M., Nagesha, S. N., Nagaraja, M. S., Prashant, D. G., Ondara, Z., Kerima, A., Karosiya, A. and Chavan, M. (2019). Field application of *Bacillus subtilis* isolates for controlling late blight disease of potato caused by *Phytophthora infestans*. *Biocatalysis and Agricultural Biotechnology*, **22**: 101366.

Bishnoi, N. R., Chugh, L. K. and Sawhney, S. K. (1993). Effects of chromium on photosynthesis, respiration and nitrogen fixation in Pea (*Pisum sativum* L.) seedlings. *Journal of Plant Physiology*, **142**: 25–30.

Blokhina, O., Virolainen, E. and Fagerstedt, K. V. (2003). Antioxidants, oxidative damage and oxygen deprivation stress: A review. *Annals of Botany*, **91**: 179–194.

Bochicchio, R., Sofo, A., Terzano, R., Gattullo, C. E., Amato, M. and Scopa, A. (2015). Root architecture and morphometric analysis of *Arabidopsis thaliana* grown in Cd/Cu/Zn-gradient agar dishes: A new screening technique for studying plant response to metals. *Plant Physiology and Biochemistry*, **91**: 20–27.

Bodner, C. C. and Gereau, R. E. (1988). A contribution to Bontoc ethnobotany. *Economic Botany*, **42**: 307–369.

Bojórquez-Quintal, E., Escalante-Magaña, C., Echevarría-Machado, I. and Martínez-Estévez, M. (2017). Aluminum, a friend or foe of higher plants in acid soils. *Frontiers in Plant Science*, **8**: 271596.

Boonyapookana, B., Upatham, E. S., Kruatrachue, M., Pokethitiyook, P. and Singhakaew, S. (2002). Phytoaccumulation and phytotoxicity of cadmium and chromium in duckweed *Wolffia globosa*. *International Journal of Phytoremediation*, **4**: 87–100.

Borges, C. E., Cazetta, J. O., Sousa, F. B. F. D. and Oliveira, K. S. (2020). Aluminum toxicity reduces the nutritional efficiency of macronutrients and micronutrients in sugarcane seedlings. *Science and Agrotechnology*, **44**: e015120.

Bortolin, G. S., Teixeira, S. B., Pinheiro, R. D. M., Ávila, G. E., Carlos, F. S., Pedroso, C. E. da S. and Deuner, S. (2020). Seed priming with salicylic acid minimizes oxidative effects of aluminum on *Trifolium* seedlings. *Journal of Soil Science and Plant Nutrition*, **20**: 2502–2511.

Bos, R., Hendriks, H. and van Os, F. H. (1983). The composition of the essential oil in the leaves of *Coleus aromaticus* Bentham and their importance as a component of the species antiaphthosae. *Pharmaceutisch Weekblad Scientific Edition*, **5**: 129–130.

Bouwmeester, H. J., Davies, J. A. R. and Toxopeus, H. (1995). Enantiomeric composition of carvone, limonene and carveols in seeds of dill and annual and biennial caraway varieties. *Journal of Agriculture and Food Chemistry*, **43**: 3057–3064.

Bowler, C. H., Montagu, M. V. and Inze, D. (1992). Superoxide dismutase and stress tolerance. *Annual Review of Plant Physiology and Plant Molecular Biology*, **43**: 83–116.

Bray, E. A. (2002). Abscisic acid regulation of gene expression during water deficit stress in the era of the *Arabidopsis* genome. *Plant Cell Environment*, **25**: 153–161.

Brdar-Jokanović, M. (2020). Boron toxicity and deficiency in agricultural plants. *International Journal of Molecular Sciences*, **21**: 1424.

Broadhurst, C. L., Chaney, R. L., Angle, J. S., Maucel, T. K., Erbe, E. F. and Murphy, C. A. (2004). Simultaneous hyperaccumulation of nickel, manganese and calcium in Alyssum leaf trichomes. *Environmental Science and Technology*, **38**: 5797–5802.

Brown, D. (1997). Grenada: Isle of spices. *Herbs*, **22**: 6–7.

Bruneton, J. (1987). *Elements of Phytochemistry and Pharmacognosy*. Technical Documentation Editions, Lavoisier Publication, Intercept, Paris, New Jersey, pp.**585**.

Bruneton, J. (1999). *Pharmacognosy, Phytochemistry: Medicinal Plants* (3rd ed.). Technical Documentation Editions, Lavoisier Publication, Intercept, Paris, New Jersey.

Buchanan, B. B., Gruissem, W. and Jones, R. L. (2000). *Biochemistry and Molecular Biology of Plants*. American Society of Plant Physiologists, Rockville, Maryland, United States.

Buchanan, B. B., Gruissem, W. and Jones, R. L. (2015). *Biochemistry and Molecular Biology of Plants* (2nd Edn.). John Wiley and Sons, New York, United States.

Budikova, S. (1999). Structural changes and aluminum distribution in maize root tissues. *Biologia Plantarum*, **42**: 259–266.

Bukhari, S., Shang, S., Zhang, M., Zheng, W., Zhang, G. P., Wang, T., Shamsi, I. and Wu, F. (2016). Genome-wide identification of chromium stress-responsive microRNAs and their target genes in tobacco (*Nicotiana tabacum*) roots. *Environmental Toxicology and Chemistry*, **34**: 2573–2582.

Burkhead, J. L., Gogolin Reynolds, K. A., Abdel-Ghany, S. E., Cohu, C. M. and Pilon, M. (2009). Copper homeostasis. *New Phytologist*, **182**: 799–816.

Cai, S., Xiong, Z., Li, L., Li, M., Zhang, L., Liu, C. and Xu, Z. (2014). Differential responses of root growth, acid invertase activity and transcript level to copper stress in two contrasting populations of *Elsholtzia haichowensis*. *Ecotoxicology*, **23**: 76–91.

Can Baser, K. H. (2008). Biological and pharmacological activities of carvacrol and carvacrol-bearing essential oils. *Current Pharmaceutical Design*, **14**: 3106–3119.

Canini, A., Alesiani, D., D'Arcangelo, G. and Tagliatesta, P. (2007). Gas chromatography-mass spectrometry analysis of phenolic compounds from *Carica papaya* L. leaf. *Journal of Food Composition and Analysis*, **20**: 584–590.

Cano, J. H. and Volpato, G. (2004). Herbal mixtures in the traditional medicine of Eastern Cuba. *Journal of Ethnopharmacology*, **90**: 293–316.

Capdevila, M. and Atrian, S. (2011). Metallothionein protein evolution: A mini assay. *Journal of Biological Inorganic Chemistry*, **16**: 977–989.

Carbajal, D., Casaco, A., Arruzazabala, L., Gonzalez, R. and Fuentes, V. (1991). Pharmacological screening of plant decoctions commonly used in Cuban folk medicine. *Journal of Ethnopharmacology*, **33**: 21–24.

Cardoso, K. B., Oliveira, H. L. M., Melo, U. Z., Fernandez, C. M. M., Campo, C. F. A. A., Gonçalves, J. E. and Gazim, Z. C. (2020). Antioxidant activity of α and β-amyrin isolated from *Myrcianthes pungens* leaves. *Natural Product Research*, **34**: 1777–1781.

Cargnelutti, D., Tabaldi, L. A., Spanevello, R. M., de Oliveira Jucoski, G., Battisti, V., Redin, M., Linares, C. E., Dressler, V. L., de Moraes Flores, E. M., Nicoloso, F. T., Morsch, V. M. and Schetinger, M. R. (2006). Mercury toxicity induces oxidative stress in growing cucumber seedlings. *Chemosphere*, **65**: 999–1006.

Castillo, R. A. M. and Gonzalez, V. P. (1999). *Plectranthus amboinicus* (Lour.) Spreng. *Cuban Journal of Medicinal Plants*, **4**: 110–115.

Chaffai, R., Seybou, T., Marzouk, B. and Ferjani, E. (2009). A comparative analysis of fatty acid composition of root and shoot lipids in *Zea mays* under copper and cadmium stress. *Acta Biologica Hungarica*, **60**: 109–125.

Chai, M., Shi, F., Li, R., Qiu, G., Liu, F. and Liu, L. (2014). Growth and physiological responses to copper stress in a halophyte *Spartina alterniflora* (Poaceae). *Acta Physiologiae Plantarum*, **36**: 745–754.

Chance, B. and Maehly, A. C. (1955). Assay of catalases and peroxidases. *Methods in Enzymology*, **2**: 764–775.

Chandra, P. and Kulshreshtha, K. (2004). Chromium accumulation and toxicity in aquatic vascular plants. *Botanical Review*, **70**: 313–327.

Chandra, R., Bharagava, R. N., Yadava, S. and Mohan, D. (2009). Accumulation and distribution of toxic metals in wheat (*Triticum aestivum* L.) and Indian mustard (*Brassica campestris* L.) irrigated with distillery and tannery effluents. *Journal of Hazardous Materials*, **162**: 1514–1521.

Chang, Y. C., Yamamoto, Y. and Matsumoto, H. (1999). Accumulation of aluminium in the cell wall pectin in cultured tobacco (*Nicotiana tabacum* L.) cells treated with a combination of aluminium and iron. *Plant Cell and Environment*, **22**: 1009–1017.

Chatterjee, J. and Chatterjee, C. (2000). Phytotoxicity of cobalt, chromium and copper in cauliflower. *Environmental Pollution*, **109**: 169–174.

Chauhan, D. K., Yadav, V., Vaculík, M., Gassmann, W., Pike, S., Arif, N., Tripathi, D. K. (2021). Aluminum toxicity and aluminum stress-induced physiological tolerance responses in higher plants. *Critical Reviews in Biotechnology*, **41**: 715–730.

Chebeir, M., Chen, G. and Liu, H. (2016). Emerging investigators series: Frontier review: Occurrence and speciation of chromium in drinking water distribution systems. *Environmental Science: Water Research and Technology*, **2**: 906–914.

Chen, J., Shafi, M., Li, S., Wang, Y., Wu, J., Ye, Z., Peng, D., Yan, W. and Liu, D. (2015). Copper induced oxidative stresses, antioxidant responses and phytoremediation potential of Moso bamboo (*Phyllostachys pubescens*). *Scientific Reports*, **5**: 13554.

Chen, Q., Zhang, X., Liu, Y., Wei, J., Shen, W. and Shen, Z. (2017). Hemin-mediated alleviation of zinc, lead and chromium toxicity is associated with elevated photosynthesis, antioxidative capacity, suppressed metal uptake and oxidative stress in rice seedlings. *Plant Growth Regulation*, **81**: 253–264.

Chen, X., Tong, J., Su, Y. and Xiao, L. (2020). *Pennisetum sinese*: A potential phytoremediation plant for chromium deletion from soil. *Sustainability*, **12**: 3651.

Chen, Y. S., Yu, H. M., Shie, J. J., Cheng, T. J. R., Wu, C. Y., Fang, J. M. and Wong, C. H. (2014). Chemical constituents of *Plectranthus amboinicus* and the synthetic analogs possessing anti-inflammatory activity. *Bioorganic and Medicinal Chemistry*, **22**: 1766–1772.

Chifundera, K. (2001). Contribution to the inventory of medicinal plants from the Bushi area, South Kivu Province, Democratic Republic of Congo. *Fitoterapia*, **72**: 351–368.

Chinmayee, D., Anu, M. S., Mahesh, B., Sheeba, M. A., Mini, I. and Swapna, T. S. (2014). A comparative study of heavy metal accumulation and antioxidant responses in *Jatropha curcas* L. *Journal of Environmental Science, Toxicology and Food Technology*, **8**: 58–67.

Chmielowska-ąk, J. and Deckert, J. (2021). Plant recovery after metal stress - A review. *Plants*, **10**: 450.

Choi, Y. E., Harada, E., Wada, M., Tsuboi, H., Morita, Y., Kusano, T. and Sano, H. (2001). Detoxification of cadmium in tobacco plants: Formation and active excretion of crystals containing cadmium and calcium through trichomes. *Planta*, **213**: 45–50.

Choi, Y. H., Kong, K. R., Kim, Y. A., Jung, K. O., Kil, J. H., Rhee, S. H. and Park, K. Y. (2003). Induction of Bax and activation of caspases during beta-sitosterol-mediated

apoptosis in human colon cancer cells. *International Journal of Oncology*, **23**: 1657–1662.

Chung, W. H. (2017). Unraveling new functions of superoxide dismutase using yeast model system: Beyond its conventional role in superoxide radical scavenging. *Journal of Microbiology*, **55**: 409–416.

Ciamporová, M. (2002). Morphological and structural responses of plant roots to aluminium at organ, tissue and cellular levels. *Biologia Plantarum*, **45**: 161-171.

Clemens, S. (2001). Molecular mechanisms of plant metal tolerance and homeostasis. *Planta*, **212**: 475–486.

Clemens, S. (2006). Toxic metal accumulation, responses to exposure and mechanisms of tolerance in plants. *Biochemistry*, **88**: 1707–1719.

Clijsters, H. and Van Assche, F. (1985). Inhibition of photosynthesis by heavy metals. *Photosynthesis Research*, **7**: 31–40.

Cobbett, C. S. (2000). Phytochelatins and their roles in heavy metal detoxification. *Plant Physiology*, **123**: 825–832.

Coetzee, J. J., Bansal, N. and Chirwa, E. M. N. (2020). Chromium in environment, its toxic effect from chromite-mining and ferrochrome industries and its possible bioremediation. *Expo Health*, **12**: 51–62.

Cosio, C., DeSantis, L., Frey, B., Diallo, S. and Keller, C. (2005). Distribution of cadmium in leaves of *Thlaspi caerulescens*. *Journal of Experimental Botany*, **56**: 765–775.

Costa, G. and Morel, J. L. (1994). Water relations, gas exchange and amino acid content in Cd-treated lettuce. *Plant Physiology and Biochemistry*, **32**: 561–570.

Courbet, G., Gallardo, K., Vigani, G., Brunel-Muguet, S., Trouverie, J., Salon, C. and Ourry, A. (2019). Disentangling the complexity and diversity of crosstalk between sulfur and other mineral nutrients in cultivated plants. *Journal of Experimental Botany*, **70**: 4183–4196.

Crankshaw, D., Walsh, J. and Morrison, J. (2014). The effects of methyl palmitate, a putative regulator from perivascular fat, on the contractility of pregnant human myometrium. *Life Sciences*, **116**: 10.1016/j.lfs.2014.08.018.

Cseh, E. (2002). Metal permeability, transport and efflux in plants. In: Prasad, M. N. V. and Strzalka, K. (Eds.), *Physiology and Biochemistry of Metal Toxicity and Tolerance in Plants*, Springer Dordrecht, Netherlands, 1–36.

Cui, J. L., Luo, C. L., Tang, C. W. Y., Chan, T. S. and Li, X. D. (2017). Speciation and leaching of trace metal contaminants from e-waste contaminated soils. *Journal of Hazardous Materials*, **329**: 150–158.

da Silva de Jesus, D., Machado Martins, F. and Dias de Azevedo Neto, A. (2016). Structural changes in leaves and roots are anatomical markers of aluminum sensitivity in sunflower. *Pesquisa Agropecuaria Tropical*, **46**: 216-220.

DalCorso, G., Manara, A. and Furini, A. (2013). An overview of heavy metal challenge in plants: From roots to shoots. *Metallomics*, **5**: 1117–1132.

Dall'Osto, L., Holt, N. E., Kaligotla, S., Fuciman, M., Cazzaniga, S., Carbonera, D., Frank, H. A., Alric, J. and Bassi, R. (2012). Zeaxanthin protects plant photosynthesis by modulating chlorophyll triplet yield in specific light-harvesting antenna subunits. *Journal of Biological Chemistry*, **287**: 41820–41834.

Daud, M., Mei, L., Variath, M., Ali, S., Li, C., Rafiq, M. and Zhu, S. (2014). Chromium (VI) uptake and tolerance potential in cotton cultivars: Effect on their root physiology, ultramorphology and oxidative metabolism. *BioMed Research International*, 975946.

Davies, K. L., Davies, M. S. and Francis, D. (1991). Zinc-induced vacuolation in root meristematic cells of *Festuca rubra* L. *Plant Cell and Environment*, **14**: 399–406.

Davis, E. M. and Croteau, R. (2000). Cyclization enzymes in the biosynthesis of monoterpenes, sesquiterpenes and diterpenes. In: Leeper, F. J. and Vederas, J. C. (Eds.), *Topics in Current Chemistry: Biosynthesis-Aromatic Polyketides, Isoprenoids, Alkaloids*, Springer-Verlag, Berlin, Germany, pp. 53–95.

De Fillippis, L. F. (1979). The effect of heavy metal compounds on the permeability of Chlorella cells. *Journal of Plant Physiology*, **92**: 39–49.

De Padua, L. S. (1988). *Some Medicinal Plants for Common Ailments*. National Book Store, Inc, India.

De Rosis, F., Anastasio, S. P., Selvaggi, L., Beltrame, A. and Moriani, G. (1985). Female reproductive health in two lamp factories: Effects of exposure to inorganic mercury vapour and stress factors. *British Journal of Industrial Medicine*, **42**: 488–494.

de Souza, A., Wang, J. Z. and Dehesh, K. (2017). Retrograde signals: Integrators of inter organellar communication and orchestrators of plant development. *Annual Review of Plant Biology*, **68**: 85–108.

De, A. K., Sen, A. K., Modak, D. P. and Jana, S. (1985). Studies of toxic effect of Hg (II) on *Pistia stratiotes*. *Water, Air and Soil Pollution*, **24**: 351–360.

Del Bubba, M., Ancillotti, C., Checchini, L., Ciofi, L., Fibbi, D., Gonnelli, C. and Mosti, S. (2013). Chromium accumulation and changes in plant growth, selected phenolics and sugars of wild type and genetically modified *Nicotiana langsdorffii*. *Journal of Hazardous Materials*, **262**: 394–403.

del Real, P., García-Gonzalo, M., Lobo, A. and Pérez-Sanz, A. (2014). Chromium speciation modifies root exudation in two genotypes of *Silene vulgaris*. *Environmental and Experimental Botany*, **107**: 1–6.

Delhaize, E. and Ryan, P. R. (1995). Aluminium toxicity and tolerance in plants. *Plant Physiology*, **107**: 315–321.

Demirevska-Kepova, K., Simova-Stoilova, L., Stoyanova, Z. P. and Feller, U. (2006). Cadmium stress in barley: Growth, leaf pigment and protein composition and detoxification of reactive oxygen species. *Journal of Plant Nutrition*, **29**: 451–468.

Demirevska-Kepova, K., Simova-Stoilova, L., Stoyanova, Z., Hölzer, R. and Feller, U. (2004). Biochemical changes in barley plants after excessive supply of copper and manganese. *Environmental and Experimental Botany*, **52**: 253–266.

Devarajan, K. (2023). Physiological and anatomical studies on heavy metals stress in *Strobilanthes alternata* Burm F Moylan ex J R I Wood (Ph.D. thesis). University of Calicut, Kerala, India.

Devkota, B. and Schmidt, G. H. (2000). Accumulation of heavy metals in food plants and grasshoppers from the Taigetos Mountains, Greece. *Agriculture, Ecosystems and Environment*, **78**: 85–91.

Diaz, J. H. (2016). Chemical and plant-based insect repellents: Efficacy, safety and toxicity. *Wilderness and Environmental Medicine*, **27**: 153–163.

Diaz, J., Bernal, A., Pomar, F. and Merino, F. (2001). Induction of shikimate dehydrogenase and peroxidase in pepper (*Capsicum annuum* L.) seedlings in response to copper stress and its relation to lignification. *Plant Science*, **161**: 179–188.

Dietz, K. J., Baier, M. and Kramer, M. (1999). Free radicals and reactive oxygen species as mediators of heavy metal toxicity in plants. In: Prasad, M. N. V. and Hagemeyer, J. (Eds.), *Heavy Metal Stress in Plants: From Molecules to Ecosystem*, Springer Berlin, Germany, pp. 73–79.

Dipierro, N., Mondelli, D., Paciolla, C., Brunetti, G. and Dipierro, S. (2005). Changes in the ascorbate system in the response of pumpkin (*Cucurbita pepo* L.) roots to aluminium stress. *Journal of Plant Physiology*, **162**: 529–536.

Diwan, H., Khan, I., Ahmed, A. and Iqbal, M. (2010). Induction of phytochelatins and antioxidant defence system in *Brassica juncea* and *Vigna radiata* in response to chromium treatments. *Plant Growth Regulation*, **61**: 97-107.

Dixit, S. K., Rizvi, P. W. and Ashraf, S. (2019). Interactive role of brassinosteroids and calcium ameliorates in response to aluminium toxicity in plants. *International Journal of Trend in Scientific Research and Development*, **3**: 183-203.

Dixit, V., Pandey, V. and Shyam, R. (2001). Differential antioxidative responses to cadmium in roots and leaves of pea (*Pisum sativum* L. cv. Azad). *Journal of Experimental Botany*, **52**: 1101-1109.

Djebali, W., Zarrouk, M., Brouquisse, R., El Kahoui, S., Limam, F., Ghorbel, M. H. and Chaïbi, W. (2005). Ultrastructure and lipid alterations induced by cadmium in tomato (*Lycopersicon esculentum*) chloroplast membranes. *Plant Biology*, **7**: 358-368.

Dörmann, P. (2007). Functional diversity of tocochromanols in plants. *Planta*, **225**: 269-276.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. T. and Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, **28**: 350-356.

Dumanović, J., Nepovimova, E., Natić, M., Kuča, K. and Jaćević, V. (2020). The significance of reactive oxygen species and antioxidant defense system in plants: A concise overview. *Frontiers in Plant Science*, **11**: 552969.

Dutta, S. (1959). Essential oil of *Coleus aromaticus* of Indian origin. *Indian Oil and Soap Journal*, **25**: 120-126.

Economou-Eliopoulos, M., Tsoupas, G. and Kiousis, G. (2013). Exploration for Platinum-group elements (PGE) in various geotectonic settings of Greece. *Journal of the Virtual Explorer*, **45**: 1-35.

Egamberdieva, D., Wirth, S. J., Alqarawi, A. A., Abd Allah, E. F. and Hashem, A. (2017). Phytohormones and beneficial microbes: Essential components for plants to balance stress and fitness. *Frontiers in Microbiology*, **8**: 2104.

Ehrhardt, M., Osterroht, C. and Petrick, G. (1980). Fatty-acid methyl esters dissolved in seawater and associated with suspended particulate material. *Marine Chemistry*, **10**: 67-76.

El Asbahani, A., Miladi, K., Badri, W., Sala, M., Aït Addi, E. H., Casabianca, H., El Mousadik, A., Hartmann, D., Jilale, A., Renaud, F. N. and Elaissari, A. (2015). Essential oils: from extraction to encapsulation. *International Journal of Pharmaceutics*, **483**: 220–243. https://doi.org/10.1016/j.ijpharm.2014.12.069

Eleftheriou, E. P., Adamakis, L. D. S., Panteris, E. and Fatisiou, M. (2015). Chromium induced ultrastructural changes and oxidative stress in roots of *Arabidopsis thaliana*. *International Journal of Molecular Sciences*, **16**: 15852-15871.

Elgayyar, M., Draughon, F. A., Golden, D. A. and Mount, J. R. (2001). Antimicrobial activity of essential oils from plants against selected pathogenic and saprophytic microorganisms. *Journal of Food Protection*, **64**: 1019-1024.

Elloumi, N., Zouari, M., Chaari, L., Jomni, C., Marzouk, B. and Ben Abdallah, F. (2014). Effects of cadmium on lipids of almond seedlings (*Prunus dulcis*). *Botanical Studies*, **55**: 61.

Elzaawely, A., Dang Xuan, T. and Tawata, S. (2007). Changes in essential oil, kava pyrones and total phenolics of *Alpinia zerumbet* (Pers.) B.L. Burtt. and R.M. Sm. leaves exposed to copper sulphate. *Environmental and Experimental Botany*, **59**: 347-353. https://doi.org/10.1016/j.envexpbot.2006.04.007

Emamverdian, A. and Ding, Y. (2017). HMs induced changes on growth, antioxidant enzyme's activity, gas exchange parameters and protein structures in *Sasa kongosanensis* f. aureo-striatus. *Polish Journal of Environmental Studies*, **26**: 585-592.

Emamverdian, A., Ding, Y., Mokhberdoran, F. and Xie, Y. (2015). Heavy metal stress and some mechanisms of plant defense response. *Science World Journal*, 756120.

Epstein, E. (1972). *Mineral Nutrition of Plants, Principles and Perspectives*. John Wiley and Sons, New York, United States.

Ezhilvannan, D. and Sharavanan, P. S. (2023). Effect of Chromium ions on the biochemical parameters of cotton plant, *Gossypium hirsutum* (L.). *Ecology, Environment and Conservation*, **29**: 494-497

Fagria, N. K. (1982). Differential tolerance of rice cultivars to aluminium in nutrient solution. *Pesquisa Agropecuária Brasileira*, **17**: 1-9.

Fahn, A. (1982). *Plant Anatomy* (3rd ed.). Oxford: Pergamon Press, United Kingdom.

Fahn, A. (2000). Structure and function of secretory cells. In D. L. Hallahan and J. C. Gray (Eds.), Plant Trichomes, Academic Press., 37-75.

Falchuk, K. H., Goldwater, L. J. and Vallee, B. L. (1977). The biochemistry and toxicity of mercury. In: Mc Auliffe, C. A. (Ed.), *The Chemistry of Mercury*, Mac Millan, New York, United States, 259-283.

Faleiro, L., Miguel, G., Gomes, S., Costa, L., Venâncio, F. and Teixeira, A. (2005). Antibacterial and antioxidant activities of essential oils isolated from *Thymbra capitata* L. (Cav.) and *Origanum vulgare* L. *Journal of Agricultural and Food Chemistry*, **53**: 8162-8168.

Fan, X., Zhou, X., Chen, H., Tang, M. and Xie, X. (2021). Cross-talks between macro- and micronutrient uptake and signaling in plants. *Frontiers in Plant Science*, **12**: 663477.

Fang, K., Xie, P., Zhang, Q., Xing, Y., Cao, Q. and Qin, L. (2020). Aluminum toxicity-induced pollen tube growth inhibition in apple (*Malus domestica*) is mediated by interrupting calcium dynamics and modification of cell wall components. *Environmental Experimental Botany*, **171**: 103928.

Feil, S. B., Pii, Y., Valentinuzzi, F., Tiziani, R., Mimmo, T. and Cesco, S. (2020). Copper toxicity affects phosphorus uptake mechanisms at molecular and physiological levels in *Cucumis sativus* plants. *Plant Physiology and Biochemistry*, **157**: 138-147.

Fergusson, J. E. (1990). The heavy elements: Chemistry, environmental impact and health effects. Pergamon Press, Oxford, United Kingdom.

Ferreira, P. A. A., Ceretta, C. A., Soriani, H. H., Tiecher, T. L., Soares, C. R. F. S., Rossato, L. V., Nicoloso, F. T., Brunetto, G., Paranhos, J. T. and Cornejo, P. (2015). *Rhizophagus clarus* and phosphate alter the physiological responses of *Crotalaria juncea* cultivated in soil with a high Cu level. *Applied Soil Ecology*, **91**: 37-47.

Fischer, A. and Brodziak-Dopierała, B. (2022). The mercury concentration in spice plants. *Processes*, **10**: 1954.

Fitter, A. H. and Hay, R. K. M. (1983). *Environmental Physiology and Plants*. Academic Press, Elsevier, United States.

Fitz, W. J. and Wenzel, W. W. (2002). Arsenic transformation in the soil-rhizosphere-plant system: Fundamentals and potential application of phytoremediation. *Journal of Biotechnology*, **99**: 259-278.

Fodor, F. (2002). Physiological responses of vascular plants to heavy metals. In: Prasad, M. N. V. and Strzalka, K. (Eds.), *Physiology and Biochemistry of Metal Toxicity and Tolerance in Plants*, Kluwer Academic, Netherlands, 149-177.

Folin, O. and Denis, W. (1915). A colorimetric method for the determination of phenols (and phenol derivatives) in urine. *Journal of Biological Chemistry*, **22**: 305-308.

Foy, C. D. (1984). Physiological effects of hydrogen, aluminium and manganese toxicities in acid soil. In: Adams, F. (Ed.), *Soil Acidity and Liming*, American Society of Agronomy Inc, Washington, 57-97.

Foy, C. D. (1992). Soil chemical factors limiting plant root growth. *Advances in Soil Science*, **19**: 97-131.

Foy, C. D., Chaney, R. L. and White, M. C. (1978). The physiology of metal toxicity in plants. *Annual Review of Plant Physiology*, **29**: 511-566.

Fozia, A., Muhammad, A., Muhammad, A. and Zafar, M. K. (2008). Effect of chromium on growth attributes in sunflower (*Helianthus annuus* L.). *Journal of Environmental Science*, **20**: 1475–1480.

França, F., Lago, E. L. and Marsden, P. D. (1996). Plants used in the treatment of leishmanial ulcers due to *Leishmania* (*Viannia*) *braziliensis* in an endemic area of Bahia, Brazil. *Revista da Sociedade Brasileira de Medicina Tropical*, **29**: 229-232.

Fu, L., Chen, C., Wang, B., Zhou, X., Li, S., Guo, P., Shen, Z., Wang, G. and Chen, Y. (2015). Differences in copper absorption and accumulation between copper-exclusion and copper-enrichment plants: A comparison of structure and physiological responses. *PLoS One*, **10**: e0133424.

Fulekar, M. H. (2016). Phytoremediation of heavy metals by *Helianthus annuus* in aquatic and soil environment. *International Journal of Current Microbiology and Applied Sciences*, **5**: 392-404.

Furini, A. (2012). *Plants and Heavy Metals*. Springer, Dordrecht, Netherlands.

Galloway, J. N. and Cowling, E. B. (1994). Atmospheric chemistry of chromium. *Critical Reviews in Environmental Science and Technology*, **24**: 269-298.

Gálusová, T., Piršelová, B., Rybanský, Ľ., Krasylenko, Y., Mészáros, P., Lehová, A., Štrbáčová, M., Moravčíková, J. and Matušíková, I. (2020). Plasticity of soybean stomatal responses to arsenic and cadmium at the whole plant level. *Polish Journal of Environmental Studies*, **29**: 3569-3580.

Ganesh, K. S., Baskaran, L., Chidambaram, A. and Sundaramoorthy, P. (2009). Influence of chromium stress on proline accumulation in soybean (*Glycine max* L. Merr.) genotypes. *Global Journal of Environmental Research*, **3**: 106–108.

Gangola, M. R., Bharathi, R. and Ramadoss, B. R. (2018). Sugars play a critical role in abiotic stress tolerance in plants. In: S. H., Wani (Ed.), *Biochemical, Physiological and Molecular Avenues for Combating Abiotic Stress Tolerance in Plants*, Academic Press, Elsevier, Netherlands, 17-38.

Gangwar, S. and Singh, V. P. (2011). Indole acetic acid differently changes growth and nitrogen metabolism in *Pisum sativum* L. seedlings under chromium (VI) phytotoxicity: Implication of oxidative stress. *Scientia Horticulturae*, **129**: 321-328.

Garcia, A., Baquedano, F. J., Navarro, P. and Castillo, F. J. (1999). Oxidative stress induced by copper in sunflower plants. *Free Radical Research*, **31**: 45-50.

Gardea-Torresdey, J. L., Peralta-Videa, J. R., Montes, M., de la Rosa, G. and Corral-Diaz, B. (2004). Bioaccumulation of cadmium, chromium and copper by *Convolvulus arvensis* L.: Impact on plant growth and uptake of nutritional elements. *Bioresource Technology*, **92**: 229-235.

Garg, N. and Manchanda, G. (2009). ROS generation in plants: Boon or bane? *Plant Biosystems*, **143**: 81-96.

Gaur, S., Kumar, J., Prasad, S. M., Sharma, S., Bhat, J. A., Sahi, S., Singh, V. P., Tripathi, D. K. and Chauhan, D. K. (2021). Silicon and nitric oxide interplay alleviates copper-induced toxicity in mung bean seedlings. *Plant Physiology and Biochemistry*, **167**: 713-722.

Gautam, V., Kohli, S. K., Kapoor, D., Bhardwaj, R. and Ahmad, P. (2021). Stress protective effect of *Rhododendron arboreum* leaves (MEL) on chromium-treated *Vigna radiata* plants. *Journal of Plant Growth Regulation*, **40**: 423-435.

Ghafoori, M., Majid, N. M., Islam, M. and Luhat, S. (2013). Bioaccumulation of heavy metals by *Dyera costulata* cultivated in sewage sludge contaminated soil. *African Journal of Biotechnology*, **10**.

Giannakoula, A., Therios, I. and Chatzissavvidis, C. (2021). Effect of lead and copper on photosynthetic apparatus in citrus (*Citrus aurantium* L.) plants. The role of antioxidants in oxidative damage as a response to heavy metal stress. *Plants*, **10**: 155-158.

Giannopolitis, C. N. and Ries, S. K. (1977). Superoxide dismutase occurrence in higher plants. *Plant Physiology*, **59**: 309-314.

Gill, R. A., Ali, B., Islam, F., Farooq, M. A., Gill, M. B., Mwamba, T. M., and Zhou, W. (2015). Physiological and molecular analyses of black and yellow seeded *Brassica napus* regulated by 5-aminolivulinic acid under chromium stress. *Plant Physiology and Biochemistry*, **94**: 130-143.

Gill, R. A., Ali, B., Yang, S., Tong, C., Islam, F., Gill, M. B., et al. (2017). Reduced glutathione mediates phenoultrastructure, kinome and transportome in chromium-induced *Brassica napus* L. *Frontiers in Plant Science*, **8**: 2037.

Gill, R. A., Zhang, L., Ali, B., Farooq, M. A., Cui, P. and Yang, S. (2015). Chromium-induced physio-chemical and ultrastructural changes in four cultivars of *Brassica napus* L. *Chemosphere*, **120**: 154–164.

Giri, S. and Singh, A. K. (2017). Human health risk assessment due to dietary intake of heavy metals through rice in the mining areas of Singhbhum copper belt, India. *Environmental Science and Pollution Research*, **24**: 14945-14956.

Githinji, C. W. and Kokwaro, J. O. (1993). Ethnomedicinal study of major species in the family Labiatae from Kenya. *Journal of Ethnopharmacology*, **39**: 197–203.

Gladkov, E. A. and Gladkova, O. V. (2021). Plants and maximum permissible concentrations of heavy metals in soil. *Archivum Technologiae et Scientiae*, **25**: 77–82.

Godbold, D. L. (1994). Aluminium and heavy metal stress: From the rhizosphere to the whole plant. In D. L. Godbold and A. Hüttermann (Eds.), *Effects of acid rain on forest processes*, Wiley-Liss, 231-264.

Godbold, D. L. and Hüttermann, A. (1986). The uptake and toxicity of mercury and lead to spruce seedlings. *Water, Air and Soil Pollution*, **31**: 509-515.

Godbold, D. L. and Kettner, C. (1991). Use of root elongation studies to determine aluminium and lead toxicity in *Picea abies* seedlings. *Journal of Plant Physiology*, **138**: 231-238.

Gong, Q., Wang, L., Dai, T., Zhou, J., Kang, Q., Chen, H., Li, K. and Li, Z. (2019). Effects of copper on the growth, antioxidant enzymes and photosynthesis of spinach seedlings. *Ecotoxicology and Environmental Safety*, **171**: 771-780. https://doi.org/10.1016/j.ecoenv.2019.01.016

González-Mendoza, D., Espadas y Gil, F., Escoboza-Garcia, F., Santamaria, J. M. and Zapata-Perez, O. (2013). Copper stress on photosynthesis of black mangle (*Avicennia germinans*). *Annals of the Brazilian Academy of Sciences*, **85**: 665-670.

Gratão, P. L., Polle, A., Lea, P. J. and Azevedo, R. A. (2005). Making the life of heavy-metal stressed plants a little easier. *Functional Plant Biology*, **32**: 481–494.

Grill, E., Winnacker, E. L. and Zenk, M. H. (1985). Phytochelatins, the principal heavy metal complexing peptides of higher plants. *Science*, **230**: 674-676.

Gupta, D. K., Corpas, F. J., Palma, J. M. and Corpas, F. (2019). Heavy metal toxicity in plants: From molecules to ecosystems. Springer Nature.

Gupta, N., Ram, H. and Kumar, B. (2016). Mechanism of zinc absorption in plants: Uptake, transport, translocation and accumulation. *Reviews in Environmental Science and Biotechnology*, **15**: 89-109.

Gurib-Fakim, A., Sewraj, M. D., Gueho, J. and Dulloo, E. (1996). Medicinal plants of Rodrigues. *International Journal of Pharmacognosy*, **34**: 2-14.

Gutbrod, P., Yang, W., Grujicic, G. V., Peisker, H., Gutbrod, K., Du, L. F. and Dörmann, P. (2021). Phytol derived from chlorophyll hydrolysis in plants is metabolized via phytenal. *Journal of Biological Chemistry*, **296**: 100-125.

Habeeb, H., Janeeshma, E. and Puthur, J. (2021). Dynamic alterations of metabolites in *Plectranthus amboinicus* (Lour.) Spreng. to encounter drought and Zn toxicity. *Brazilian Journal of Botany*, **44**: 134-142.

Habiba, U., Ali, S. and Rizwan, M. (2019). Alleviative role of exogenously applied mannitol in maize cultivars differing in chromium stress tolerance. *Environmental Science and Pollution Research*, **26**: 5111-5121.

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

Haider, F. U., Wang, X., Farooq, M., Hussain, S., Cheema, S. A. and Ain, N. U. (2022). Biochar application for the remediation of trace metals in contaminated soils: Implications for stress tolerance and crop production. *Ecotoxicology and Environmental Safety*, **230**: 113165.

Hall, G., Pelchat, J., Pelchat, P. and Vaive, J. E. (2002). Sample collection, filtration and preservation protocols for the determination of "total dissolved" mercury in waters. *Analyst*, **127**: 674-680.

Hall, J. L. (2002). Cellular mechanisms for heavy metal detoxification and tolerance. *Journal of Experimental Botany*, **53**: 1-11.

Han, F. X., Sridhar, B. B. M., Monts, D. L. and Su, Y. (2004). Phytoavailability and toxicity of trivalent and hexavalent chromium to *Brassica juncea*. *New Phytologist*, **162**: 489-499.

Handa, N., Kohli, S. K., Thukral, A. K., Bhardwaj, R., Alyemeni, M. N., Wijaya, L. and Ahmad, P. (2018). Protective role of selenium against chromium stress involving metabolites and essential elements in *Brassica juncea* L. seedlings. *Biotechnology*, **8**: 66.

Harborne, J. B. (1980). Plant phenolics. In E. A. Bell and B. V. Charlwood (Eds.), Secondary Plant Products. *Encyclopedia of Plant Physiology*, Springer-Verlag Berlin., **8**: 329-395

Harsha, V. H., Hebbar, S. S., Shripathi, V. and Hegde, G. R. (2003). Ethnomedicobotany of Uttara Kannada District in Karnataka, India - plants in treatment of skin diseases. *Journal of Ethnopharmacology*, **84**: 37-40.

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

Hashem, A., Abd_Allah, E. F., Alqarawi, A. A. and Egamberdieva, D. (2016). Bioremediation of adverse impact of cadmium toxicity on *Cassia italica* Mill by arbuscular mycorrhizal fungi. *Saudi Journal of Biological Sciences*, **23**: 39-47.

Hashem, F. A. and Sahab, A. F. (1999). Chemical response of parsley and mentha herbs to certain stress agents. *Food Chemistry*, **65**: 29-33.

Hattori, M., Nakabayashi, T., Lim, Y. A., Miyashiro, H., Kurokawa, M., Shiraki, K., Gupta, M. P., Correa, M. and Pilapitiya, U. (1995). Inhibitory effects of various Ayurvedic and Panamanian medicinal plants on the infection of Herpes simplex Virus-1 in vitro and in vivo. *Phytotherapy Research*, **9**: 270-276.

Hayes, P. E., Clode, P. L., Guilherme-Pereira, C. and Lambers, H. (2019). Calcium modulates leaf cell-specific phosphorus allocation in Proteaceae from southwestern Australia. *Journal of Experimental Botany*, **70**: 3995-4009.

He, H., Li, Y. and He, L. F. (2019). Aluminum toxicity and tolerance in Solanaceae plants. *South African Journal of Botany*, **123**: 23-29.

He, M. and Ding, N. Z. (2020). Plant Unsaturated Fatty Acids: Multiple Roles in Stress Response. *Frontiers in Plant Science*, **11**: 562785.

Heath, R. L. and Packer, L. (1968). Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. *Archives of Biochemistry and Biophysics*, **125**:189-198.

Heaton, A. C. P., Rugh, C. L. and Wang, N. J. (2005). Physiological responses of Transgenic mer A-Tobacco (*Nicotiana tabacum*) to foliar and root mercury exposure. *Water, Air and Soil Pollution*, **161**: 137-155.

Helena, O. (2012). Chromium as an environmental pollutant, insights on induced plant toxicity. *Journal of Botany*, 375843.

Henriques, F. S. (2010). Changes in biomass and photosynthetic parameters of tomato plants exposed to trivalent and hexavalent chromium. *Plant Biology*, **54**: 583-586.

Henry, J. R. (2000). Phytoremediation of mercury. In An Overview of the Phytoremediation of Lead and Mercury (pp. 44-46). U.S. Environmental Protection Agency.

Hensley, K., Benaksas, E. J., Bolli, R., Comp, P., Grammas, P., Hamdheydari, L., Mou, S., Pye, Q. N., Stoddard, M. F. and Wallis, G. (2004). New perspectives on vitamin E: gamma-tocopherol and carboxyelthylhydroxychroman metabolites in biology and medicine. *Free Radical Biology and Medicine*, **36**: 1-15.

Hieno, A., Ara, N. H. and Yamamoto, Y. Y. (2019). H2O2-mediated biotic and abiotic stress responses in plants. In S. Panda and Y. Yamamoto (Eds.), *Redox Homeostasis in Plants: From Signaling to Stress Tolerance* (pp. 19-42). Springer.

Hojati, M., Modarres-Sanavy, S., Enferadi, S., Majdi, M., Ghanati, F., Farzadfar, S. and Pazoki, A. (2017). Cadmium and copper induced changes in growth, oxidative metabolism and terpenoids of *Tanacetum parthenium*. *Environmental Science and Pollution Research*, **24**: 12261-12272.

Holland, S., Lodwig, E., Sideri, T., Reader, T., Clarke, I. and Gkargkas, K. (2007). Application of the comprehensive set of heterozygous yeast deletion mutants to elucidate the molecular basis of cellular chromium toxicity. *Genome Biology*, **8**: R268.

Horst, W. J., Puschel, A. K. and Schmohl, N. (1997). Induction of callose formation is a sensitive marker for genotypic aluminium sensitivity in maize. *Plant and Soil*, **192**: 23-30.

Horst, W. J., Wang, Y. and Eticha, D. (2010). The role of the root apoplast in aluminium-induced inhibition of root elongation and in aluminium resistance of plants: A review. *Annals of Botany*, **106**: 185-197. doi: 10.1093/aob/mcq053

Hossain, M. A., Piyatida, P., da Silva, J. A. T. and Fujita, M. (2012). Molecular mechanism of heavy metal toxicity and tolerance in plants: Central role of glutathione in detoxification of reactive oxygen species and methylglyoxal and in heavy metal chelation. *Journal of Botany*, 1-12. https://doi.org/10.1155/2012/872875

Hossain, M. N., Tahjib-Ul-Arif, M., Hannan, A., Sultana, N., Akhter, S., Hasanuzzaman, M., Akter, F., Hossain, M. S., Sayed, M. A., Hasan, M. T., Skalicky, M., Li, X. and Restič, M. (2020). Melatonin modulates plant tolerance to heavy metal stress: Morphological responses to molecular mechanisms. *International Journal of Molecular Sciences*, **22**: 11445.

Hossain, M. S., Abdelrahman, M., Tran, C. D., Nguyen, K. H., Chu, H. D., Watanabe, Y. and Tran, L.-S. P. (2019). Insights into acetate-mediated copper homeostasis and antioxidant defense in lentil under excessive copper stress. *Environmental Pollution*, 113544.

Huang, J. W. and Cunningham, S. D. (1996). Lead phytoextraction: Species variation in lead uptake and translocation. *New Phytologist*, **134**: 73-84.

Huang, J. W., Grunes, D. L. and Kochian, L. V. (1992). Aluminum effects on the kinetics of calcium uptake into cells of the wheat root apex. *Planta*, **188**: 414-421.

Huang, J. W., Shaff, J. E., Grunes, D. L. and Kochian, L. V. (1992). Aluminum effects on calcium fluxes at the root apex of aluminum-tolerant and aluminum-sensitive wheat cultivars. *Plant Physiology*, **98**: 230-237.

Huang, W. J., Oo, T. L., He, H. Y., Wang, A. Q., Zhan, J. and Li, C. Z. (2014). Aluminum induces rapidly mitochondria-dependent programmed cell death in Al-sensitive peanut root tips. *Botanical Studies*, **55**: 1-12.

Huang, W., Yang, X., Yao, S., Lwin Oo, T., He, H. and Wang, A. (2014). Reactive oxygen species burst induced by aluminum stress triggers mitochondria-dependent programmed cell death in peanut root tip cells. *Plant Physiology and Biochemistry*, **82**: 76-84.

Huang, W., Yang, X., Yao, S., Oo, T. L., He, H., Wang, A., Li, C. and He, L. (2014b). Reactive oxygen species burst induced by aluminum stress triggers mitochondria-dependent programmed cell death in peanut root tip cells. *Plant Physiology and Biochemistry*, **82**: 76-84. doi: 10.1016/j.plaphy.2014.05.014

Huang, W.-J., Oo, T. L., He, H.-Y., Wang, A.-Q., Zhan, J., Li, C.-Z., Wei, S.-Q. and He, L.-F. (2014a). Aluminum induces rapidly mitochondria-dependent programmed cell death in Al-sensitive peanut root tips. *Botany Studies*, **55**: 67-72.

Huett, D. O. and Menary, R. C. (1980). Aluminium distribution in freeze-dried roots of cabbage, lettuce and kikuyu grass by energy dispersive X-ray analysis. *Australian Journal of Plant Physiology*, **7**: 101-111.

Hullatti, K. K. and Bhattacharjee, P. (2011). Pharmacognostical evaluation of different parts of *Coleus amboinicus* Lour., Lamiaceae. *Pharmacognosy Journal*, **3**: 39-44.

Hussain, K. (2007). Ecophysiological aspects of *Bacopa monnieri* (L.) Pennel. Ph.D. Thesis, University of Calicut, Kerala, India.

Hussain, K., Abdulssalam, A. K., Ratheesh-Chandra, P. and Nabeesa Salim. (2010). Bioaccumulation of heavy metals in *Bacopa monnieri* (L.) Pennell growing under different habitat. *International Journal of Ecology and Development*, **10**: 66-73.

Hussain, S. S., Ali, M., Ahmad, M. and Siddique, K. H. M. (2011). Polyamines: Natural and engineered abiotic and biotic stress tolerance in plants. *Biotechnology Advances*, **29**: 300-311.

Hussain, T., Tan, B., Murtaza, G., Liu, G., Rahu, N., Kalhoro, M. S., Kalhoro, D. H., Adebowale, T. O., Mazhar, M. U. and Rehman, Z. (2020). Flavonoids and type 2 diabetes: Evidence of efficacy in clinical and animal studies and delivery strategies to enhance their therapeutic efficacy. *Pharmacological Research*, **152**: 104629.

Ichihara, K. I. and Fukubayashi, Y. (2010). Preparation of fatty acid methyl esters for gas-liquid chromatography. *Journal of Lipid Research*, **51**: 635-640.

Igwe, K. K., Nwankwo, P. O., Otuokere, I. E., Ijioma, S. N. and Amaku, F. M. (2015). GCMS analysis of phytocomponents in the methanolic extract of *Moringa oleifera* leaves. *Journal of Research in Pharmaceutical Science*, **2**: 1-6.

Inoue, Y., Hada, T., Shiraishi, A., Hirose, K., Hamashima, H. and Kobayashi, S. (2005). Biphasic effects of geranylgeraniol, teprenone and phytol on the growth of *Staphylococcus aureus*. *Antimicrobial Agents and Chemotherapy*, **49**: 1770–1774.

Iqbal, M. Z., Saeeda, S. and Muhammed, S. (2001). Effects of chromium on an important arid tree (*Caesalpinea pulcherima*) at Karachi city, Pakistan. *Ekologia* (Bratislava), **20**: 414-422.

İşkil, R., Surgun-Acar, Y., Çatav, Ş. S., Zemheri-Navruz, F. and Erden, Y. (2022). Mercury toxicity affects oxidative metabolism and induces stress responsive mechanisms in wheat (*Triticum aestivum* L.). *Physiology and Molecular Biology of Plants*, **28**: 911–920.

Ismail, S., Khan, F. and Iqbal, A. (2013). Phytoremediation: Assessing tolerance of tree species against heavy metal (Pb and Cd) toxicity. *Pakistan Journal of Botany*, **45**: 2181-2186.

Jabeen, N., Abbas, Z., Iqbal, M., Rizwan, M., Jabbar, A. and Farid, M. (2016). Glycinebetaine mediates chromium tolerance in Mungbean through lowering of Cr uptake and improved antioxidant system. *Archives of Agronomy and Soil Science*, **62**: 648–662.

Jabeen, R., Ahmad, A. and Iqbal, M. (2009). Phytoremediation of heavy metals: Physiological and molecular mechanisms. *Botany Review*, **75**: 339–364.

Jacobson, T., Navarrete, C., Sharma, S. K., Sideri, T. C., Ibstedt, S. and Priya, S. (2012). Arsenite interferes with protein folding and triggers formation of protein aggregates in yeast. *Journal of Cell Science*, **125**: 5073–5083.

Jain, A. K., Dixit, A. and Mehta, S. C. (2012). Wound healing activity of aqueous extract of leaves and roots of *Coleus aromaticus* in rats. *Acta Poloniae Pharmaceutica-Drug Research*, **69**:1119-1123.

Jain, M. and Puranik, M. (1993). Protective effect of reduced glutathione on inhibition of chlorophyll biosynthesis by mercury in excised greening maize leaf segments. *Indian Journal of Experimental Biology*, **31**: 708-710.

Jain, S. K. and Lata, S. (1996). Amazonian uses of some plants growing in India. *Indigenous Knowledge and Development Monitor*, **4**: 21-23.

Jain, S., Sharma, R. K., Gautam, V., Shrman, K., Shrivastav, A. and Sawarkar, A. (2021). Morphological characterization and enzymatic activity of endophytic bacteria from *Aegle marmelos* leaves. *The Pharma Innovation Journal*, **10**: 406-409.

Jalloh, M. A., Chen, J., Zhen, F. and Zhang, G. (2008). Effect of different N fertilizer forms on antioxidant capacity and grain yield of rice growing under Cd stress. *Journal of Hazardous Materials*, **162**: 1081–1065.

Janeeshma, E., Puthur, J. T. and Ahmad, P. (2021). Silicon distribution in leaves and roots of rice and maize in response to cadmium and zinc toxicity and the associated histological variations. *Physiologia Plantarum*, **173**: 460-471.

Jaskowiak, J., Kwasniewska, J., Milewska-Hendel, A., Kurczynska, E. U., Szurman-Zubrzycka, M. and Szarejko, I. (2019). Aluminum alters the histology and pectin cell wall composition of barley roots. *International Journal of Molecular Sciences*, **20**: 3039.

Jaskowiak, J., Tkaczyk, O., Slota, M., Kwasniewska, J. and Szarejko, I. (2018). Analysis of aluminum toxicity in *Hordeum vulgare* roots with an emphasis on DNA integrity and cell cycle. *PLoS One*, **13**: e0193156.

Javed, M. T., Tanwir, K. and Abbas, S. (2021). Chromium retention potential of two contrasting *Solanum lycopersicum* Mill. Cultivars as deciphered by altered pH dynamics, growth and organic acid exudation under Cr stress. *Environmental Science and Pollution Research*, **28**: 542-554.

Jayaraman, M., Senthilkumar, A. and Venkatesalu, V. (2015). Evaluation of some aromatic plant extracts for mosquito larvicidal potential against *Culex quinquefasciatus*, *Aedes aegypti* and *Anopheles stephensi*. *Parasitology Research*, **114**: 1511-1518.

Jeliazkov (Zheljazkov), V., Craker, L. and Xing, B. (2006). Effects of Cd, Pb and Cu on growth and essential oil contents in dill, peppermint and basil. *Environmental and Experimental Botany*, **58**: 9-16.

Jiang, B., Liu, Y., Zheng, J., Tan, M., Wang, Z. and Wu, M. (2015). Synergetic transformations of multiple pollutants driven by Cr (VI)–sulfite reactions. *Environmental Science and Technology*, **49**: 363-371.

Jiang, L., Shi, G., Ding, Y., Lou, L. and Cai, Q. (2013). Differential responses of two bamboo species (*Phyllostachys auresulcata* 'Spectabilis' and *Pleioblastus chino* 'Hisauchii') to excess copper. *BioEnergy Research*, **6**: 1223-1229.

Jinhua, Z., Keli, Y., Zhonggui, Z., Wusheng, J. and Donghua, L. (2009). Antioxidant response system and chlorophyll fluorescence in chromium (VI)-treated *Zea mays* L. seedlings. *Acta Biologica Cracoviensia=Series Botanica*, **51**: 23-33.

Jobby, R., Jha, P., Yadav, A. K. and Desai, N. (2018). Biosorption and biotransformation of hexavalent chromium [Cr(VI)]: A comprehensive review. *Chemosphere*, **207**: 255-266.

Ju, J., Picinich, S. C., Yang, Z., Zhao, Y., Suh, N., Kong, A. N. and Yang, C. S. (2010). Cancer-preventive activities of tocopherols and tocotrienols. *Carcinogenesis*, **31**: 533-542.

Jung, Y., Ha, M., Lee, J., Ahn, Y. G., Kwak, J. H., Ryu, D. H. and Hwang, G. S. (2015). Metabolite profiling of the response of burdock roots to copper stress. *Journal of Agricultural and Food Chemistry*, **63**: 1309-1317.

Kabata-Pendias, A. and Pendias, H. (2001). *Trace Elements in Soils and Plants* (3rd edn.). CRC Press.

Kabata-Pendias, A. and Szteke, B. (2015). *Trace elements in abiotic and biotic environments*. Taylor and Francis.

Kagi, J. H. R. and Hapke, H. J. (1984). Biochemical interactions of mercury, cadmium and lead. In J. O. Nriagu (Ed.), *Changing metal cycles and human health* ,Springer Verlag. 237-250.

Kahle, H. (1993). Response of roots of trees to heavy metals. *Environmental and Experimental Botany*, **33**: 99-119.

Kalaji, H. M., Schansker, G., Brestic, M., Bussotti, F., Calatayud, A., Ferroni, L.and ąba, W. (2017). Frequently asked questions about chlorophyll fluorescence, the sequel. *Photosynthesis Research*, **132**: 13-66.

Kalesware Muniandy, Zaiton Hassan and Mohd Hafez Mohd Isa (2014). Antimicrobial activity of the ethanolic extract of *Coleus aromaticus* against common wound pathogens. RJPBCS, **5**: 1296-1301.

Kamran, M., Malik, Z., Parveen, A., Huang, L., Riaz, M., Bashir, S., Mustafa, A., Abbasi, G. H., Xue, B. and Ali, U. (2020). Ameliorative effects of biochar on rapeseed (*Brassica napus* L.) growth and heavy metal immobilization in soil irrigated with untreated wastewater. *Journal of Plant Growth Regulation*, **39**: 266-281.

Kamran, M., Wang, D., Alhaithloul, H. A. S.,and Alghanem, S. M. (2021). Jasmonic acid-mediated enhanced regulation of oxidative, glyoxalase defense system and reduced chromium uptake contributes to alleviation of chromium (VI) toxicity in choysum (*Brassica parachinensis* L.). *Ecotoxicology and Environmental Safety*, **208**: 111758.

Karimi, P., Khavari-Nejad, R. A., Niknam, V., Ghahremaninejad, F. and Najafi, F. (2012). The effects of excess copper on antioxidative enzymes, lipid peroxidation, proline, chlorophyll and concentration of Mn, Fe and Cu in Astragalus neo-mobayenii. *The Scientific World Journal*, 615-670.

Kasimanickam, R. K., Kasimanickam, V. R., Rodriguez, J. S., Pelzer, K. D., Sponenberg, P. D. and Thatcher, C. D. (2010). Tocopherol induced angiogenesis in placental vascular network in late pregnant ewes. *Reproductive Biology and Endocrinology*, **8**: 86.

Kaur, S., Kaur, N., Siddique, K. H. M. and Nayyar, H. (2016). Beneficial elements for agricultural crops and their functional relevance in defence against stresses. *Archives of Agronomy and Soil Science*, **62**: 905-920.

Khan, M. A., Ahmad, A. and Mujeeb, M. (2018). Carvacrol attenuates inflammation and oxidative stress in lipopolysaccharide-stimulated macrophages. *International Immunopharmacology*, **56**: 105-114. doi: 10.1016/j.intimp.2018.02.014

Khare, R. S., Shanta, B. and Kanika, K. (2011). *Coleus aromaticus* Benth - a nutritive medicinal plant of potential therapeutic value. *International Journal of Pharma and Bio Sciences*, **2**: 488-500.

Khasim, S. M. (2002). Botanical microtechnique: Principles and practice. Capital Publishing Company.

Khatun, S., Ali, M. B., Hahn, E. and Paek, K. (2008). Copper toxicity in *Withania somnifera*: Growth and antioxidant enzymes responses of in vitro grown plants. *Environmental and Experimental Botany*, **64**: 279-285.

Kimbrough, D. E., Cohen, Y., Winer, A. M., Creelman, L. and Mabuni, C. (1999). A Critical Assessment of Chromium in the Environment. *Critical Reviews in Environmental Science and Technology*, **29**: 1–46. https://doi.org/10.1080/10643389991259164

Kisa, D., Elmastas, M. and Ozturk, L. (2016). Responses of the phenolic compounds of *Zea mays* under heavy metal stress. *Applied Biological Chemistry*, **59**: 813-820.

Kishor, K., P. B., Hima Kumari, P., Sunita, M. S. and Sreenivasulu, N. (2015). Role of proline in cell wall synthesis and plant development and its implications in plant ontogeny. *Frontiers in Plant Science*, **6**: 544.

Kizilay, Z. and Kahraman Cetin, N. (2018). Effect of methyl palmitate on the formation of epidural fibrosis in an experimental epidural fibrosis model. *Journal of Investigative Surgery*, **31**: 469-474.

Klobus, G., Burzynski, M. and Buczek, J. (2002). Heavy metals and nitrogen metabolism. In M. N. V. Prasad and K. Strzalka (Eds.), *Physiology and biochemistry of metal toxicity and tolerance in plants*, Kluwer Academic Publishers,**6**: 179-199.

Koch, M. T., Pawelzik, E. and Kautz, T. (2021). Chloride changes soil-plant water relations in potato (*Solanum tuberosum* L.). *Agronomy*, **11**: 736.

Kochian, L. V. (1995). Cellular mechanisms of aluminum toxicity and resistance in plants. *Annual Review of Plant Physiology and Plant Molecular Biology*, **46**: 237-260.

Kochian, L. V. and Shaff, J. E. (1991). Investigating the relationship between aluminum toxicity, root growth and root-generated ion currents. In R. I. Wright, V. C. Baligar and R. P. Murrmann (Eds.), *Plant-soil interactions at low pH*, Kluwer Academic, **34**: 769-778.

Kochian, L. V., Pineros, M. A. and Hoekenga, O. A. (2005). The physiology, genetics and molecular biology of plant aluminum resistance and toxicity. *Plant and Soil*, **74**: 175-195.

Kollmeier, M., Felle, H. H. and Horst, W. J. (2000). Genotypical differences in aluminum resistance of maize are expressed in the distal part of the transition zone. Is reducing basipetal auxin flow involved in inhibition of root elongation by aluminum? *Plant Physiology*, **122**: 945-956.

Korpe, D. A. and Aras, S. (2011). Evaluation of copper-induced stress on eggplant (*Solanum melongena* L.) seedlings at the molecular and population levels by use of various biomarkers. *Mutation Research*, **719**: 29-34.

Kotagiri, D. and Kolluru, V. C. (2017). Effect of salinity stress on the morphology and physiology of five different *Coleus* species. *Biomedical and Pharmacology Journal*, **10**: 1639-1649.

Kotas, J. and Stasicka, Z. (2000). Chromium occurrence in the environment and methods of its speciation. *Environmental Pollution*, **107**: 263-283.

Kovacik, J., Babula, P., Hedbavny, J. and Klejdus, B. (2014). Hexavalent chromium damages chamomile plants by alteration of antioxidants and its uptake is prevented by calcium. *Journal of Hazardous Materials*, **273**: 110-117.

Kremer, D., Dunkic, V., Ruscic, M., Matevski, V., Ballian, D., Bogunic, F.and Bezic, N. (2014). Micromorphological traits and essential oil contents of *Micromeria kerneri* Murb. and *M. Juliana* (L.) Benth. (Lamiaceae). *Phytochemistry*, **98**: 128-136.

Kuebel, K. R. and Tucker, A. O. (1988). Vietnamese culinary herbs in the United States. *Economic Botany*, **42**: 413-419.

Kumar, P. S. and Kumar, N. (2020). *Plectranthus amboinicus*: A review on its pharmacological and pharmacognostical studies. *American Journal of Physiology*, **10**: 55-62.

Kumar, P., Tokas, J., Singal, H. R. and R. (2019). Amelioration of chromium VI toxicity in sorghum (*Sorghum bicolor* L.) using glycine betaine. *Scientific Reports*, **9**: 16020.

Kumar, V., Pandita, S., Sidhu, G. P. S., Sharma, A., Khanna, K., Kaur, P. and Setia, R. (2021). Copper bioavailability, uptake, toxicity and tolerance in plants: A comprehensive review. *Chemosphere*, **262**: 127810.

Kumari, R. and Prasad, M. N. V. (2014). Effect of UV-B pretreatment on essential oil components, health sensory secondary metabolites and antioxidant potential of *Coleus aromaticus*. *International Journal of Biological and Pharmaceutical Research*, **5**: 675-688.

Kumari, S., Jamwal, R., Mishra, N. and Singh, D. K. (2020). Recent developments in environmental mercury bioremediation and its toxicity: A review. *Environmental Nanotechnology, Monitoring and Management*, **13**: 100283.

Kupper, H., Gotz, B., Mijovilovich, A., Frithjof C. Kupper and Wolfram Meyer-Klaucke (2009). Complexation and toxicity of copper in higher plants. I. Characterization of copper accumulation, speciation and toxicity in *Crassula helmsii* as a new copper accumulator. *Plant Physiology*, **151**: 702-714.

Küpper, H., Küpper, F. and Spiller, M. (1998). In situ detection of heavy metal substitution chlorophylls in water plants. *Photosynthesis Research*, **58**: 123-133.

Küpper, H., Šetlík, I., Spiller, M., Küpper, F. C. and Prášil, O. (2000). Heavy metal-induced inhibition of photosynthesis: Targets of in vivo heavy metal chlorophyll formation. *Journal of Phycology*, **38**: 429-441.

Kusumoto, I. T., Nakabayashi, T., Kida, H., Miyashiro, H., Hattori, M., Namba, T. and Shimotohno, K. (1995). Screening of various plant extracts used in Ayurvedic medicine for inhibitory effects on Human Immunodeficiency Virus Type 1 (HIV-1) Protease. *Phytotherapy Research*, **9**: 180-184.

Lang, F., Sarvari, E., Szigeti, Z., Fodor, F. and Cseh, E. (1995). Effect of heavy metals on the photosynthetic apparatus in cucumber. In P. Mathis (Ed.), *Photosynthesis: From Light to Biosphere*,Kluwer Academic, **72**: 533-536.

Laporte, D., Rodríguez, F., González, A., Zúñiga, A., Castro-Nallar, E., Sáez, C. A. and Moenne, A. (2020). Copper-induced concomitant increases in photosynthesis, respiration and C, N and S assimilation revealed by transcriptomic analyses in *Ulva compressa* (Chlorophyta). BMC *Plant Biology*, **20**: 25.

Larsen, P. B., Tai, C. Y., Kochian, L. V. and Howell, S. H. (1996). *Arabidopsis* mutants with increased sensitivity to aluminum. *Plant Physiology*, **110**: 743-751.

Lavid, N., Schwartza, A., Varden, O. and Tel-or, E. (2001). The involvement of polyphenols and peroxidase activities in heavy metal accumulation by epidermal glands of water lilly (Nymphaeceaea). *Planta*, **212**: 323-328.

Lee, S. C. and Lee, A. Y. (2019). Antioxidant activity of carvacrol and its role in preventing oxidative stress. *Food Research International*, **119**: 108-115. doi: 10.1016/j.foodres.2019.02.024

Lee, Y. P. and Takahashi, T. (1966). An improved colorimetric determination of amino acids with the use of ninhydrin. *Analytical Biochemistry*, **14**: 71-77.

Leesombun, A., Arpron, K., Thanapakdeechaikul, K., Suwannawiang, J., Mukto, P., Sungpradit, S. and Boonmasawai, S. (2022). Effects of *Coleus amboinicus* L. essential oil and ethanolic extracts on planktonic cells and biofilm formation of *Microsporum canis* isolated from feline dermatophytosis. *Antibiotics*, **11**: 1734.

Lenka, M., Das, B. L., Panda, K. K. and Panda, B. B. (1993). Mercury tolerance of *Chloris barbata* Sw. and *Cyperus rotundus* L. isolated from contaminated sites. *Biologia Plantarum*, **35**: 443-446.

Lepp, N. W. (1981). *Effect of heavy metal pollution on plants*. Vol. 2. Applied Science Publishers.

Levitt, J. (1980). *Responses of plants to environmental stress*. (2nd ed., Vol. 2). Academic Press.

Lewis, D. H. (2019). Boron: The essential element for vascular plants that never was. *New Phytologist*, **221**: 1685-1690.

Li, C., Shi, H., Xu, L., Xing, M., Wu, X. and Bai, Y. (2023). Combining transcriptomics and metabolomics to identify key response genes for aluminum toxicity in the root system of *Brassica napus* L. seedlings. *Theoretical and Applied Genetics*, **136**: 169.

Li, X., Li, Y., Mai, J., Tao, L., Qu, M. and Liu, J. (2018). Boron alleviates aluminum toxicity by promoting root alkalization in transition zone via polar auxin transport. *Plant Physiology*, **177**: 1254-1266.

Liang, J., Huang, X., Yan, J., Li, Y., Zhao, Z. and Liu, Y. (2021). A review of the formation of Cr (VI) via Cr (III) oxidation in soils and groundwater. *Science of the Total Environment*, **774**: 145762.

Liao, P. C., Lai, M. H., Hsu, K. P., Kuo, Y. H., Chen, J., Tsai, M. C., Li, C. X., Yin, X. J., Jeyashoke, N. and Chao, L. K. (2008). Identification of β-Sitosterol as in vitro anti-inflammatory constituent in *Moringa oleifera*. *Journal of Agricultural and Food Chemistry*, **66**: 10748–10759.

Lichtenthaler, H. K. and Wellburn, A. R. (1983). Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *Biochemical Society Transactions*, **11**: 591-592.

Lidon, F. C., Ramalho, J. C. and Barreiro, M. G. (1997). Aluminium modulation of the photosynthetic carbon reduction cycle in *Zea mays*. *Photosynthetica*, **34**: 393-400.

Lim, W., Ham, J., Bazer, F. W. and Song, G. (2019). Carvacrol induces mitochondria-mediated apoptosis via disruption of calcium homeostasis in human choriocarcinoma cells. *Journal of Cellular Physiology*, **234**: 1803–1815.

Lima, M. A., Oliveira, F. F. M., Gomes, G. A., Lavor, P. L., Santiago, G. M., Nagao-Dias, A. T., Arriaga, A. M., Lemos, T. L. and Carvalho, M. G. (2011). Evaluation of larvicidal activity of the essential oils of plant species from Brazil against *Aedes aegypti* (Diptera: Culicidae). *African Journal of Biotechnology*, **10**: 11716-11720.

Lin, J. M. and Pehkonen, S. O. (1995). Chromium in atmospheric particles: A review. *Journal of Environmental Engineering*, **121**: 737-745.

Lin, Y. R., Xu, F., Li, Z. H., Yang, L. H., Li, B. and Deng, G. G. (2005). Analysis of volatile composition in the perfume of *Ficus Carica* Lam. produced by fermentation. *Biotechnology*, **15**: 54-56.

Lindberg, S. and Greger, M. (2002). Plant genotypic differences under metal deficient and enriched conditions. In M. N. V. Prasad and K. Strzałka (Eds.), *Physiology and Biochemistry of Metal Toxicity and Tolerance in Plants*, **34**: 361-376.

Liu, D., Zou, J., Wang, M. and Jiang, W. (2008). Hexavalent chromium uptake and its effects on mineral uptake, antioxidant defence system and photosynthesis in *Amaranthus viridis* L. *Bioresource Technology*, **99**: 2628–2636.

Liu, J. J., Wei, Z. and Li, J. H. (2014). Effects of copper on leaf membrane structure and root activity of maize seedling. *Botanical Studies*, **55**: 1-6.

Liu, L., Li, W., Song, W. and Guo, M. (2018). Remediation techniques for heavy metal-contaminated soils: Principles and applicability. *Science of the Total Environment*, **633**: 206-219.

Liu, S. D., Song, M. H., Yun, W., Lee, J. H., Kim, H. B. and Cho, J. H. (2020). Effect of carvacrol essential oils on growth performance and intestinal barrier function in broilers with lipopolysaccharide challenge. *Animal Production Science*, **60**: 545-552.

Liu, Y. J., Tao, J. Y., Cao, J., Zeng, Y. P., Li, X. and Ma, J. (2020). The beneficial effects of aluminum on the plant growth in *Camellia japonica*. *Journal of Soil Science and Plant Nutrition*, **20**: 1799–1809.

Liugany, M., Massot, N., Wissemeier, A. H., Poschenrieder, C., Horst, W. J. and Barcelo, J. (1994). Aluminium tolerance of maize cultivars as assessed by callose production and root elongation. *Journal of Plant Nutrition and Soil Science*, **157**: 447-451.

Lomonte, C., Sgherri, C., Baker, A. J. M., Kolev, S. D. and Navari-Izzo, F. (2010). Antioxidative response of *Atriplex codonocarpa* to mercury. *Environmental and Experimental Botany*, **69**: 9-16.

Lopes, C., Azevedo, A. and Dias, J. (2013). Flooding assessment under sea level rise scenarios: Ria de Aveiro case study. *Journal of Coastal Research*, 766–771.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry*, **193**: 265-275.

Lu, D., Huang, Q., Deng, C. and Zheng, Y. (2018). Phytoremediation of Copper Pollution by Eight Aquatic Plants. *Polish Journal of Environmental Studies*, **27**: 175-181.

Lu, H. L., Dong, G., Hua, H., Zhao, W. R., Li, J. Y. and Xu, R. K. (2020). Method for initially selecting Al-tolerant rice varieties based on the charge characteristics of their roots. *Ecotoxicology and Environmental Safety*, **187**: 109-122.

Lukhoba, C. W., Simmonds, M. S. and Paton, A. J. (2006). *Plectranthus*: A review of ethnobotanical uses. *Journal of Ethnopharmacology*, **103**: 1-24.

Lux, A., Lukačová, Z., Vaculík, M., Švubová, R., Kohanová, J., Soukup, M., Martinka, M. and Bokor, B. (2020). Silicification of root tissues. *Plants*, **9**: 111.

Luyckx, M., Hausman, J. F., Lutts, S. and Guerriero, G. (2017). Impact of silicon in plant biomass production: Focus on bast fibres, hypotheses and perspectives. *Plants*, **6**: 37.

Lwalaba, J. L. W., Louis, L. T., Zvobgo, G., Richmond, M. E. A., Fu, L., Naz, S., Mwamba, M., Mundende, R. P. M. and Zhang, G. (2020). Physiological and molecular mechanisms of cobalt and copper interaction in causing phytotoxicity to two barley genotypes differing in Co tolerance. *Ecotoxicology and Environmental Safety*, **187**: 109866.

Ma, J., Lv, C., Xu, M., Chen, G., Lv, C. and Gao, Z. (2016). Photosynthesis performance, antioxidant enzymes and ultrastructural analyses of rice seedlings under chromium stress. *Environmental Science and Pollution Research*, **23**: 1768–1778.

Mahmud, J. A., Hasanuzzaman, M., Nahar, K., Rahman, A., Hossain, M. S. and Fujita, M. (2007). γ-aminobutyric acid (GABA) confers chromium stress tolerance in *Brassica*

juncea L. by modulating the antioxidant defense and glyoxalase systems. *Ecotoxicology*, **26**: 675-690.

Mahmud, K., Weitz, H., Kritzler, U. and Burslem, D. (2024). External aluminium supply regulates photosynthesis and carbon partitioning in the Al-accumulating tropical shrub *Melastoma malabathricum*. *PLoS ONE*, **19**: e0297686.

Maitani, T., Kubota, H., Satoh, K. and Yamada, T. (1996). The composition of metals bound to class III metallothionein (phytochelatin and its desglycyl peptide) induced by various metals in root cultures of *Rubia tinctorium*. *Plant Physiology*, **110**: 1145-1150.

Maiti, S., Ghosh, N., Mandal, C., Das, K., Dey, N. and Adak, M. K. (2012). Responses of the maize plant to chromium stress with reference to antioxidation activity. *Brazilian Journal of Plant Physiology*, **24**: 78-81.

Majeed, A., Muhammad, Z. and Siyar, S. (2019). Assessment of heavy metal induced stress responses in pea (*Pisum sativum* L.). *Acta Ecologica Sinica*, **39**: 284–288.

Maksymiec, W. (2007). Signaling responses in plants to heavy metal stress. *Acta Physiologiae Plantarum*, **29**: 177-187.

Malar, S., Sahi, S., Favas, P. and Perumal, V. (2014). Mercury heavy-metal-induced physiochemical changes and genotoxic alterations in water hyacinths [*Eichhornia crassipes* (Mart.)]. *Environmental Science and Pollution Research*, **21**: 8732-8741.

Mallavarapu, G. R., Rao, L. and Ramesh, S. (1999). Essential oil of *Coleus aromaticus* Benth. from India. *Journal of Essential Oil Research*, **11**: 742-744.

Mangabeira, P. A., Ferreira, A. S., de Almeida, A. A. F., Fernandes, V. F., Lucena, E. and Souza, V. L., (2011). Compartmentalization and ultrastructural alterations induced by chromium in aquatic macrophytes. *Biometals*, **24**: 1017–1026.

Mangathayaru, K., Pratap, D. V. V., Thirumurgan, D., Patel, P. S., David, D. J. and Karthikeyan, J. (2005). Essential oil composition of *Coleus amboinicus* Lour. *Indian Journal of Pharmaceutical Sciences*, **67**: 122-123.

Manivannan, N., Subirana, M. A., Boada, R., Marini, C., Llugany, M., Valiente, M. and Simonelli, L. (2023). Mercury speciation in selenium-enriched wheat plants hydroponically exposed to mercury pollution. *Scientific Reports*, **13**: 21132.

Manjamalai, A., Tom Alexander and Erlin Grace, V. M. (2012). Bioactive evaluation of the essential oil of *Plectranthus amboinicus* by GC-MS analysis and its role as a drug for microbial infections and inflammation. *International Journal of Pharmacy and Pharmaceutical Science*, **4**: 205-211.

Marienfeld, S., Schmohl, N., Klein, M., Schroder, W. H., Kuhn, A. J. and Horst, W. J. (2000). Localisation of aluminium in root tips of *Zea mays* and *Vicia faba*. *Journal of Plant Physiology*, **156**: 666-671.

Marques, D. M., Júnior, V. V., da Silva, A. B., Mantovani, J. R., Magalhães, P. C. and de Souza, T. C. (2018). Copper toxicity on photosynthetic responses and root morphology of *Hymenaea courbaril* L. (Caesalpinioideae). *Water, Air and Soil Pollution*, **229**: 1-14.

Marrugo-Negrete, J., Durango-Hernández, J., Pinedo-Hernández, J., Enamorado-Montes, G. and Díez, S. (2016). Mercury uptake and effects on growth in *Jatropha curcas*. *Journal of Environmental Sciences*, **48**: 120-125.

Marschner, H. (1983). General introduction to the mineral nutrition of plants. In A. Lauchli and R. L. Bieleski (Eds.), *Encyclopedia of Plant Physiology* Springer Verlag, **15**: 5-60.

Martins, J. P. R., de Vasconcelos, L. L., de Souza Braga, P. C., Rossini, F. P., Conde, L. T., Rodrigues, L. C. A., Falqueto, A. R. and Gontijo, A. B. P. L. (2020). Morphophysiological responses, bioaccumulation and tolerance of *Alternanthera tenella* Colla (Amaranthaceae) to excess copper under in vitro conditions. *Plant Cell Tissue and Organ Culture*, **143**: 303-318.

Martins, J. P. R., Martins, A. D., Pires, M. F., Braga Junior, R. A., Reis, R. O., Dias, G. D. M. G. and Pasqual, M. (2016). Anatomical and physiological responses of *Billbergia zebrina* (Bromeliaceae) to copper excess in a controlled microenvironment. *Plant Cell, Tissue and Organ Culture*, **126**: 43-57.

Masciarelli, E., Visconti, U., Ficociello, B. and Casorri, L. (2017). Minireview on chromium in agricultural plants and consequences for human nutrition. *Italian Journal of Occupational and Environmental Hygiene*, **8**: 53-60.

Matsumoto, H. (2000). Cell biology of aluminium toxicity and tolerance in higher plants. *International Review of Cytology*, **200**: 1-46.

Mattagajasingh, S., Misra, B. and Misra, H. (2008). Carcinogenic chromium (VI)-induced protein oxidation and lipid peroxidation: Implications in DNA-protein crosslinking. *Journal of Applied Toxicology*, **28**: 987-997. https://doi.org/10.1002/jat.1364

Mauro, C., de Silva, C. P., Missima, J., Ohnuki, T., Rinaldi, R. B. and Frota, M. (2008). Comparative anatomical study of vegetative organs of small boldo, *Plectranthus ornatus* Codd. and malvariço, *Plectranthus amboinicus* (Lour.) Spreng. – Lamiaceae. *Revista Brasileira de Farmacognosia*, **18**: 608-613.

Mazaheri-Tirani, M., Dadkhah, N. and Koohi, M. (2021). The Role of Copper Oxide Nanomaterials on *Ruta graveolens* Physiological Response and IRT1 and CAT Gene Expression. *Plant Molecular Biology Reporter*, **40**: 1-12.

McNeilly, T. (1994). Metal toxicity. In A. R. Yeo and T. J. Flowers (Eds.), Soil Mineral Stresses: Approaches to Crop Improvement, Springer-Verlag, **32**: 145-174.

Medda, S. and Mondal, N. K. (2017). Chromium toxicity and ultrastructural deformation of *Cicer arietinum* with special reference to root elongation and coleoptile growth. *Annals of Agrarian Science*, **15**: 396-401.

Meidner, H. and Mansfield, T. A. (1968). Physiology of Stomata. McGraw Hill.

Mellem, J. J., Baijnath, H. and Odhav, B. (2012). Bioaccumulation of Cr, Hg, As, Pb, Cu and Ni with the ability for hyperaccumulation by *Amaranthus dubius*. *African Journal of Agricultural Research*, **7**: 591-596.

Melo, C., Morais, T., Tomé, A., Brito, G., Helena, C. and Santos, F. (2011). Anti-inflammatory effect of α, β-amyrin, a triterpene from *Protium heptaphyllum*, on cerulein-induced acute pancreatitis in mice. *Inflammation Research*, **60**: 673-681.

Memon, A. R. and Schroder, P. (2009). Implications of metal accumulation mechanisms to phytoremediation. *Environmental Science and Pollution Research*, **16**: 162-175.

Men, C. K. and Ghazi, R. M. (2018). Phytoremediation of chromium (VI) using *Colocasia esculenta* in laboratory scale constructed wetlands. *Journal of Tropical Resources and Sustainable Science*, **6**: 45-49.

Meng, L., Wang, B., Zhao, X., Ponce, K., Qian, Q. and Ye, G. (2017). Association mapping of ferrous, zinc and aluminum tolerance at the seedling stage in indica rice using MAGIC populations. *Frontiers in Plant Science*, **8**: 1-15.

Meng, Q., Zou, J., Zou, J. H., Jiang, W. S. and Liu, D. H. (2007). Effect of Cu2+ concentration on growth, antioxidant enzyme activity and malondialdehyde content in garlic (*Allium sativum* L.). *Acta Biologica Cracoviensia Botanica*, **49**: 95-101.

Mengel, K. (2007). Potassium. In A. V. Barker and D. J. Pilbeam (Eds.), *Handbook of Plant Nutrition*, Taylor and Francis, **24**: 91-120.

Mengel, K. and Kirkby, E. A. (2001). *Principles of Plant Nutrition*, Springer Science and Business Media,**1**: 23-34.

Micera, M., Botto, F., Geddo, F., Antoniotti, S., Bertea, C. M., Levi, R., Gallo, M. P. and Querio, G. (2020). Squalene: More than a stem toward sterols. *Antioxidants*, **9**: 688-698.

Michalak, A. (2006). Phenolic compounds and their antioxidant activity in plants growing under heavy metal stress. *Polish Journal of Environmental Studies*, **15**: 523-530.

Milivojevic, D. B., Stojanovic, D. D. and Drinic, S. D. (2000). Effects of aluminium on pigments and pigment-protein complexes of soybean. *Plant Biology*, **43**: 595-597.

Minkina, T., Rajput, V. and Fedorenko, G. (2020). Anatomical and ultrastructural responses of *Hordeum sativum* to the soil spiked by copper. *Environmental Geochemistry and Health*, **42**: 45–58.

Mir, A., Pichtel, J. and Hayat, S. (2021). Copper: uptake, toxicity and tolerance in plants and management of Cu-contaminated soil. *BioMetals*, **34**: 80-94.

Miras-Moreno, B., Almagro, L., Pedreño, M. A. and Ferrer, M. A. (2014). Accumulation and tolerance of cadmium in a non-metallicolous ecotype of *Silene vulgaris* Garcke (Moench). *Anales de Biología*, **36**: 33-41.

Mishra, B., Sangwan, R. S. and Mishra, S. (2014). Effect of cadmium stress on inductive enzymatic and nonenzymatic responses of ROS and sugar metabolism in multiple shoot cultures of Ashwagandha (*Withania somnifera* Dunal). *Protoplasma*, **251**: 1031–1045.

Mishra, S. and Dubey, R. S. (2005). Heavy metal toxicity induced alterations in photosynthetic metabolism in plants. *Photosynthetica*, **43**: 491-497.

Mishra, S., Srivastava, S., Tripathi, R. D., Govindarajan, R., Kuriakose, S. V. and Prasad, M. N. V. (2006). Phytochelatin synthesis and response of antioxidants during cadmium stress in *Bacopa monnieri* L. *Plant Physiology and Biochemistry*, **44**: 25-37.

Moccia, S., Siano, F., Russo, G. L., Volpe, M. G., La Cara, F., Pacifico, S., Piccolella, S. and Picariello, G. (2020). Antiproliferative and antioxidant effect of polar hemp

extracts (*Cannabis sativa* L., Fedora cv.) in human colorectal cell lines. *International Journal of Food Science and Nutrition*, **71**: 410–423.

Mohanty, M. and Patra, H. K. (2020). Phytoassessment of in situ weed diversity for their chromium distribution pattern and accumulation indices of abundant weeds at South Kaliapani chromite mining area with their phytoremediation prospective. *Ecotoxicology and Environmental Safety*, **194**: 110399.

Mondal, N. K., Das, C. and Datta, J. K. (2015). Effect of mercury on seedling growth, nodulation and ultrastructural deformation of *Vigna radiata* (L.) Wilczek. *Environmental Monitoring and Assessment*, **187**: 241.

Montgomery, R. (1957). Determination of glycogen. *Archives of Biochemistry and Biophysics*, **67**: 378-386.

Moon, E., Lee, Y. M. and Lee, O. H. (1999). A novel angiogenic factor derived from *Aloe vera* gel: β-sitosterol, a plant sterol. *Angiogenesis*, **3**: 117–123.

Moral, R., Palacios, G., Gomes, I., Pedreo, J. N. and Mataix, J. (1994). Distribution and accumulation of heavy metals (Cd, Ni and Cr) in tomato plant. *Fresenius Environmental Bulletin*, **3**: 395-399.

Moral, R., Pedreno, J. N., Gomes, I. and Mataix, J. (1995). Effects of chromium on the nutrient element content and morphology of tomato. *Journal of Plant Nutrition*, **18**: 815-822.

Morallo-Rejesus, B., Maini, H. A., Ohsawa, K. and Yamamoto, I. (1990). Insecticidal actions of several plants to *Callosobruchus chmensis* L. In K. Fujii, A. M. R. Gatehouse, C. D. Johnson, R. Mitchel and T. Yoshida (Eds.), *Bruchids and Legumes: Economics, Ecology and Coevolution*, Springer,**8**: 91-100.

Moreno, F. N., Anderson, C. W. N., Robinson, B. H. and Stewart, R. B. (2004a). Mercury analysis of plant and soil samples using the hybrid-generation AAS method. In L. D. Currie and J. A. Hanly (Eds.), *Tools for Nutrient and Pollutant Management*, Fertilizer and Lime Research Centre, **34**: 425-433.

Moreno, F. N., Anderson, C. W. N., Stewart, R. B. and Robinson, B. H. (2004b). Phytoremediation of mercury-contaminated mine tailings by induced plant-mercury accumulation. *Environmental Practice*, **6**: 165-175.

Moreno, F. N., Anderson, C. W. N., Stewart, R. B. and Robinson, B. H. (2005a). Mercury volatilization and phytoextraction from base-metal mine tailings. *Environmental Pollution*, **136**: 341-352.

Moreno, F. N., Anderson, C. W. N., Stewart, R. B. and Robinson, B. H. (2008). Phytofiltration of mercury-contaminated water: Volatilization and plant-accumulation aspects. *Environmental Experimental Botany*, **62**: 78-85.

Moreno, F. N., Anderson, C. W. N., Stewart, R. B., Robinson, B. H., Ghomshei, M. and Meech, J. A. (2005). Induced plant uptake and transport of mercury in the presence of sulphur-containing ligands and humic acid. *New Phytologist*, **166**: 445-454.

Morina, F., Jovanovic, L., Mojovic, M., Vidovic, M., Pankovic, D., Veljovic and Jovanovic, S. (2010). Zinc induced oxidative stress in *Verbascum thapsus* is caused by an accumulation of reactive oxygen species and quinhydrone in the cell wall. *Plant Physiology*, **140**: 209–224.

Morton, J. F. (1992). Country borage (*Coleus amboinicus* Lour.): A potent flavoring and medicinal plant. *Journal of Herbs Spices and Medicinal Plants*, **1**: 77-90.

Mossor-Pietraszewska, T. (2001). Effect of aluminium on plant growth metabolism. *Acta Biochimica Polonica*, **48**: 673-686.

Muddarisna, N., Krisnayanti, B. D., Utami, S. and Handayanto, E. (2013). Phytoremediation of Mercury-Contaminated Soil Using Three Wild Plant Species and Its Effect on Maize Growth. *Science Education*, **1**: 27-32.

Muhammad, N., Zvobgo, G. and Zhang, G.-P. (2019). A review: The beneficial effects and possible mechanisms of aluminum on plant growth in acidic soil. *Journal of Integrative Agriculture*, **18**: 1518–1528.

Muhammad, Z., Siddiqui, K., Athar, M., Shafiq, M., Farooqi, Z.-u.-R. and Kabir, M. (2015). Effect of Mercury on Seed Germination and Seedling Growth of Mungbean (*Vigna radiata* (L.) Wilczek). *Journal of Applied Science and Environment Management*, **19**: 191–199.

Muniandy, K., Hassan, Z. and Hafez, M. I. S. A. (2014). Antimicrobial activity of the ethanolic extract of *Coleus aromaticus* against common wound pathogens. *Research Journal of Pharmaceutical, Biological and Chemical Science*, **5**: 1296- 1312.

Munoz, J. F., Dopico, B., Labrador, E. (1998). A cDNA encoding a proline-rich protein from *Cicer arietinum*. Changes in expression during development and abiotic stresses. *Physiologia Plantarum*, **102**: 582-590.

Mustafa, G., Sakata, K. and Komatsu, S. (2016). Proteomic analysis of soybean root exposed to varying sizes of silver nanoparticles under flooding stress. *Journal of Proteomics*, **148**: 113–125.

Muthukumaran, M. and Rao, A. (2013). Starch metabolism during leaf senescence in two rice varieties on exposure to aluminium. *Nature Environment and Pollution Technology*, **12**: 703-708.

Muthukumarana, R. and Dharmadasa, R. M. (2014). Pharmacognostical investigation of *Plectranthus hadiensis* (Forssk.) Schweinf. Ex Sprenger and *Plectranthus amboinicus* (Lour.) Spreng. *World Journal of Agricultural Research*, **2**: 240-246.

Mysliwa-Kurdziel, B. and Strzalka, K. (2002). Influence of metals on biosynthesis of photosynthetic pigments. In M. N. V. Prasad and K. Strzalka (Eds.), *Physiology and Biochemistry of Metal Toxicity and Tolerance in Plants*, Kluwer Academic Publishers, **21**: 201-227.

Nagarajan, M. and Ganesh, K. S. (2015). Toxic effects of chromium on growth of some paddy varieties. *International Letters of Natural Science*, **35**: 36–44.

Nair, P. M. G. and Chung, I. M. (2014). Impact of copper oxide nanoparticles exposure on *Arabidopsis thaliana* growth, root system development, root lignification and molecular level changes. *Environmental Science and Pollution Research*, **21**: 12709–12722. https://doi.org/10.1007/s11356-014-2994-1

Nair, P. M. G. and Chung, I. M. (2015). Study on the correlation between copper oxide nanoparticles induced growth suppression and enhanced lignification in Indian mustard (*Brassica juncea* L.). *Ecotoxicology and Environmental Safety*, **113**: 302-313.

Nakano, T. and Suarez, M. (1970). Studies on the neutral constituents of the bark of *Dipteryx odorata*. *Planta Medica*, **18**: 79-83.

Nath, S., Chaudhury, J., Pahan, K. and Mandal, A. (1993). Mercury and organomercurial resistance in rhizobial strains. *Indian Journal of Experimental Biology*, **31**:50-53.

Navari-Izzo, F., Cestone, B., Cavallini, A., Natali, L., Giordani, T. and Quartacci, M. F. (2006). Copper excess triggers phospholipase D activity in wheat roots. *Phytochemistry*, **67**: 1232-1242.

Neenu, S. and Karthika, K. S. (2019). Aluminium toxicity in soil and plants. *Harit Dhara*, **2**: 15-19.

Negreanu-Pirjol, B. S., Ticuta, S., Rodica and Popoviciu, D. R. (2019). Bioaccumulation and effects of aluminium on plant growth in three culture plant species. *Revista de Chimie*, **70**: 602-604. doi: 10.37358/RC.19.2.6964

Nematshahi, N., Lahouti, M. and Ganjeali, A. (2012). Accumulation of chromium and its effect on growth of (*Allium cepa* cv. Hybrid). *European Journal of Experimental Biology*, **2**: 969–974.

Neuwinger, H. D. (2000). African traditional medicine: A dictionary of plant use and applications. Medpharm Scientific Publishers Stuttgart, **12**: 406-408.

Nichols, P. B., Couch, J. D. and Al-Hamdani, S. (2000). Selected physiological responses of *Salvinia minima* to different chromium concentrations. *Aquatic Botany*, **68**: 313-319.

Noctor, G. and Foyer, C. H. (1998). Ascorbate and glutathione: Keeping active oxygen under control. *Annual Review of Plant Physiology and Plant Molecular Biology*, **49**: 249-279.

Ofoe, R., Gunupuru, L. R., Wang-Pruski, G., Fofana, B., Thomas, R. H. and Abbey, L. J. P. S. (2022). Seed priming with pyroligneous acid mitigates aluminum stress and promotes tomato seed germination and seedling growth. *Plant Stress*, **4**: 100083.

Ofoe, R., Thomas, R. H., Asiedu, S. K., Wang-Pruski, G., Fofana, B. and Abbey, L. (2023). Aluminum in plants: Benefits, toxicity and tolerance mechanisms. *Frontiers in Plant Science*, **13**: 1085998.

Okekeogbu, I., Ye, Z., Sangireddy, S. R., Li, H., Bhatti, S., Hui, D., Zhou, S., Howe, K. J., Fish, T. and Yang, Y. (2014). Effect of aluminum treatment on proteomes of radicles of seeds derived from Al-treated tomato plants. *Proteomes*, **2**: 169-190.

Olkhovych, O., Volkogon, M., Taran, N., Batsmanova, L. and Kravchenko, I. (2016). The effect of copper and zinc nanoparticles on the growth parameters, contents of ascorbic acid and qualitative composition of amino acids and acylcarnitines in *Pistia stratiotes* L. (Araceae). *Nanoscale Research Letters*, **11**: 1-9.

Olmedo, P., Zepeda, B., Rojas, B., Silva-Sanzana, C., Delgado-Rioseco, J., Fernández, K., Balic, I., Arriagada, C., Moreno, A. A., Defilippi, B. G. and Campos-Vargas, R. (2021). Cell wall calcium and hemicellulose have a role in the fruit firmness during storage of blueberry (*Vaccinium* spp.). *Plants*, **10**: 553-560.

Omidifar, N., Nili-Ahmadabadi, A., Nakhostin-Ansari, A., Lankarani, K. B., Moghadami, M., Mousavi, S. M., Hashemi, S. A., Gholami, A., Shokripour, M. and Ebrahimi, Z.

(2021). The modulatory potential of herbal antioxidants against oxidative stress and heavy metal pollution: Plants against environmental oxidative stress. *Environmental Science and Pollution Research*, **28**: 61908-61918.

Orcen, N., Nazarian, G. and Gharibkhani, M. (2013). The responses of stomatal parameters and SPAD value in Asian tobacco exposed to chromium polish. *Journal of Environmental Studies*, **22**: 1441-1447.

Orcutt, D. M. and Nilsen, E. T. (2000). Physiology of Plants Under Stress: Soil and Biotic factors. John Wiley and Sons, Inc.

Ortega-Villasante, C., Rellán-Álvarez, R., del Campo, F. F., Carpena-Ruiz, R. O. and Hernández, L. E. (2005). Cellular damage induced by cadmium and mercury in *Medicago sativa*. *Journal of Experimental Botany*, **56**: 2239-2251.

Ozyigit, I. and Akinci, S. (2009). Effects of some stress factors (aluminum, cadmium, and drought) on stomata of Roman nettle (*Urtica pilulifera* L.). *Notes of the Botanical Garden of the Agricultural Botany Institute of Cluj-Napoca*, **37**: 108-115.

Paiva, L. B., de Oliveira, J. G., Azevedo, R. A., Ribeiro, D. R., da Silva, M. G. and Vitória, A. P. (2009). Ecophysiological responses of water hyacinth exposed to Cr3+ and Cr6+. *Environmental and Experimental Botany*, **65**: 403-409.

Palani, K., Balasubramanian, K. and Kalaivani, R. A. (2018). Study on aluminium contamination in Mettur soil and its subsequent uptake by medicinal plants. *Oriental Journal of Chemistry*, **34**: 3129-3133.

Palani,K., Balasubramanian, K. and R. A. Kalaivan (2018). Study on aluminium contamination in Mettur soil and its subsequent uptake by medicinal plants. *Oriental Journal of Chemistry*, **34**: 3129-3133.

Panda, S. K. (2007). Chromium-mediated oxidative stress and ultrastructural changes in root cells of developing rice seedlings. *Plant Physiology*, **164**: 1419-1428.

Panda, S. K. and Choudhury, S. (2005). Chromium stress in plants. *Brazilian Journal of Plant Physiology*, **17**: 95-102.

Panda, S. K. and Matsumoto, H. (2007). Molecular physiology of aluminium toxicity and tolerance in plants. *The Botanical Review*, **73**: 326-347.

Panda, S. K. and Patra, H. K. (2000). Does Cr (III) produce oxidative damage in excised wheat leaves? *Journal of Plant Biology*, **27**: 105-110.

Panda, S. K., Chaudhury, I. and Khan, M. H. (2003). Heavy metals induce lipid peroxidation and affect antioxidants in wheat leaves. *Biologia Plantarum*, **46**: 289-294.

Pandey, V., Dixit, V. and Shyam, R. (2009). Chromium effects on ROS generation and detoxification in pea (*Pisum sativum*) leaf chloroplasts. *Protoplasma*, **236**: 85-95.

Pantoja, O. (2021). Recent advances in the physiology of ion channels in plants. *Annual Review of Plant Biology*, **72**: 463-495.

Papazoglou, E. G., Alexopoulou, E., Papadopoulos, G. K. and Economou-Antonaka, G. (2020). Tolerance to drought and water stress resistance mechanisms of castor bean. *Agronomy*, **10**: 1580.

Parekh, D., Puranik, R. M. and Srivastava, H. S. (1990). Inhibition of chlorophyll biosynthesis by cadmium in greening maize leaf segments. *Biochemie und Physiologie Pflanzen*, **186**: 239-242.

Parida, A. K. and Das, A. B. (2005). Salt tolerance and salinity effects on plants: A review. *Ecotoxicology and Environmental Safety*, **60**: 324-349.

Park, J. H. (2020). Contrasting effects of Cr (III) and Cr (VI) on lettuce grown in hydroponics and soil: Chromium and manganese speciation. *Environmental Pollution*, **266**: 115073.

Park, M. J., Gwak, K. S., Yang, I., Choi, W. S. and Jo, H. J. (2016). Eugenol and thymol inhibit biofilm formation and the virulence of Escherichia coli O157:H7. *Biofouling*, **32**: 1259-1269.

Parmer, N. G., Vithalani, S. D. and Chanda, S. V. (2002). Alteration in growth and peroxidase activity by heavy metals in *Phaseolus* seedlings. *Acta Physiologia Plantarum*, **24**: 89-95.

Paton, A. J., Mwanyambo, M., Govaerts, R. H., Smitha, K., Suddee, S. and Phillipson, P. B.(2019). Nomenclatural changes in *Coleus* and *Plectranthus* (Lamiaceae): A tale of more than two genera. *PhytoKeys*, **129**: 1-158.

Pavan, M. A. and Bingham, F. T. (1982). Toxicity of aluminium to coffee seedlings grown in nutrient solution. *Soil Science Society of America Journal*, **46**: 993-997.

Paz-Ferreiro, J., Lu, H., Fu, S., Mendez, A. and Gasco, G. (2014). Use of phytoremediation and biochar to remediate heavy metal polluted soils: A review. *Solid Earth*, **5**: 2155-2179.

Pegel, K. H. (1997). The importance of sitosterol and sitosterolin in human and animal nutrition. *South African Journal of Science*, **93**: 263-268.

Peñarrubia, L., Andrés-Colás, N., Moreno, J. and Puig, S. (2010). Regulation of copper transport in *Arabidopsis thaliana*: A biochemical oscillator? *Journal of Biological Inorganic Chemistry*, **15**: 29.

Peng, D., Shafi, M., Wang, Y., Li, S., Yan, W., Chen, J., Ye, Z. and Liu, D. (2015). Effect of Zn stresses on physiology, growth, Zn accumulation and chlorophyll of *Phyllostachys pubescens*. *Environmental Science and Pollution Research*, **22**: 14983-14992.

Peralta, J. R., Gardea-Torresdey, J. L., Tiemann, K. J., Arteaga, S. and Rascon, E. (2001). Uptake and effects of five heavy metals on seed germination and plant growth in alfalfa (*Medicago sativa* L.). *Bulletin of Environmental Contamination and Toxicology*, **66**: 727-734.

Pilon-Smits, E. (2005). Phytoremediation. *Annual Review of Plant Biology*, **56**: 15-39.

Pineros, M. A. and Kochian, L. V. (2001). A patch-clamp study on the physiology of aluminium toxicity and aluminium tolerance in maize. Identification and characterization of Al3+ induced an ion channels. *Plant Physiology*, **125**: 292-305.

Piršelová, B. and Ondrušková, E. (2020). Effect of Cadmium Chloride and Cadmium Nitrate on Growth and Mineral Nutrient Content in the Root of Faba Bean (*Vicia faba* L.). *Plants*, **10**: 1007.

Pivetta, C. P., Chitolina, S. F., Dartora, N., de Pelegrin, C. M. G., Dos Santos, M. V., Cassol, F. and Batista, L. S. (2023). Copper exposure leads to changes in chlorophyll content and secondary metabolite profile in *Lantana fucata* leaves. *Functional Plant Biology*, **50**: 571-584.

Pochodylo, A. L. and Aristilde, L. (2017). Molecular dynamics of stability and structures in phytochelatin complexes with Zn, Cu, Fe, Mg and Ca: Implications for metal detoxification. *Environmental Chemistry Letters*, 1-6.

Polle, A., Otter, T. and Seifert, F. (1994). Apoplastic peroxidases and lignification in needles of Norway spruce (Piceaabies L.). *Plant Physiology*, **106**: 53-60.

Poschenrieder, C., Gunse, B., Corrales, I. and Barcelo, J. (2008). A glance into aluminium toxicity and resistance in plants. *Science of the Total Environment*, **400**: 356-368.

Posmyk, M. M., Kontek, R. and Janas, K. M. (2009). Antioxidant enzymes activity and phenolic compounds content in red cabbage seedlings exposed to copper stress. *Ecotoxicology and Environmental Safety*, **72**: 596-602.

Pradas Del Real, A. E., Pérez-Sanz, A., Lobo, M. C. and McNear, D. (2014). The Chromium Detoxification Pathway in the Multimetal Accumulator *Silene vulgaris. Environmental Science and Technology*, **48**: 11235-11243.

Pradhan, A., Kumari, S., Dash, S., Biswal, D. P., Dash, A. K. and Panigrahi, K. C. (2017). Heavy metal absorption efficiency of two species of Mosses (*Physcomitrella patens* and *Funaria hygrometrica*) studied in mercury treated culture under laboratory condition. IOP Conference Series: *Materials Science and Engineering*, **225**: 012225.

Pradhan, D., Sukla, L. B., Sawyer, M. and Rahman, P. K. S. M. (2017). Recent bioreduction of hexavalent chromium in wastewater treatment: A review. *Journal of Industrial Engineering and Chemistry*, **55**: 1-20.

Prado, C., Ponce, S. C., Pagano, E., Prado, F. E. and Roas, M. (2016). Differential physiological responses of two *Salvinia* species to hexavalent chromium at a glance. *Aquatic Toxicology*, **175**: 213-221.

Prado, C., Rodríguez-Montelongo, L., González, J. A., Pagano, E. A., Hilal, M. and Prado, F. E. (2010). Uptake of chromium by *Salvinia minima*: Effect on plant growth, leaf respiration and carbohydrate metabolism. *Journal of Hazardous Materials*, **177**: 546-553.

Prakash, V. (2017). Terpenoids as source of anti-inflammatory compounds. *Asian Journal of Pharmaceutical Clinical Research*, **10**: 68-76.

Prakasia, P. P. and Nair, A. S. (2015). Chemical fingerprint of essential oil components from fresh leaves of *Glycosmis pentaphylla* (Retz.) Correa. *The Pharma Innovation*, **3**: 50.

Prasad, D. D. K. and Prasad, A. R. K. (1987). Effect of lead and mercury on chlorophyll synthesis in mung bean seedlings. *Phytochemistry*, **26**: 881-883.

Prasad, M. N. V. (1997). Trace metals. In M. N. V. Prasad (Ed.), *Plant Ecophysiology*, John Wiley and Sons, Inc,207-249.

Prasad, M. N. V. (1998). Metal biomolecules complexes in plants: Occurrence, functions and applications. *Analusis*, **26**: 25-27.

Prasad, S. M., Singh, J. B., Rai, L. C. and Kumar, H. D. (1991). Metal induced inhibition of photosynthetic electron transport chain of *Cyanobacterium Nostoc* muscorum. FEMS *Microbiology Letters*, **82**: 95-100.

Prathyusha, I. V. S. N. and Chaitanya, K. V. (2019). Effect of water stress on the physiological and biochemical responses of two different *Coleus* (*Plectranthus*) species. *Biologia Futura,* **70**: 312-322.

Priyadarshini, B. and Sujatha, B. (2011). Cadmium-induced plant stress investigated by scanning electron microscopy in *Cajanus cajan*. *Journal of Pharmaceutical Research,* **4**: 938-941.

Prudent, D., Perineau, F., Bessiere, J. M., Michel, G. M. and Baccou, J. C. (1995). Analysis of the essential oil of wild oregano from Martinique (*Coleus aromaticus* Benth.): Evaluation of its bacteriostatic and fungistatic properties. *Journal of Essential Oil Research,* **7**: 165-173.

Pucher, G. W., Leavenworth, C. S. and Vickery, H. B. (1948). Determination of starch in plant tissue. *Annals of Chemistry,* **20**: 850-853.

Pulford, I. D., Watson, C. and McGregor, S. D. (2001). Uptake of chromium by trees: Prospects for phytoremediation. *Environmental Geochemistry and Health*, **23**: 307-311.

Purohit, S., Varghese, T. M. and Kumari, M. (2003). Effect of chromium on morphological features of tomato and brinjal. *Indian Journal of Plant Physiology*, **8**: 17-82.

Qadir, M., Hussain, A., Hamayun, M., Shah, M., Iqbal, A., Husna and Murad, W. (2020). Phytohormones producing rhizobacterium alleviates chromium toxicity in *Helianthus annuus* L. by reducing chromium uptake and strengthening antioxidant system. *Chemosphere*, **258**: 127386.

Qing, X., Zhao, X., Hu, C., Wang, P., Shi, H., Jia, F. and Qu, C. (2015). *Selenium* alleviates chromium toxicity by preventing oxidative stress in cabbage (*Brassica campestyris* L. ssp. Pekinensis) leaves. *Ecotoxicology and Environmental Safety,* **114**: 179-189.

Quiñones, M. A., Fajardo, S., Fernández-Pascual, M., Lucas, M. M. and Pueyo, J. J. (2021). Nodulated white lupin plants growing in contaminated soils accumulate unusually high mercury concentrations in their nodules, roots and especially cluster roots. *Horticulturae*, **7**: 302.

Radic, S., Cvjetko, P., Glavas, K., Roje, V., Pevalek-Kozlina, B. and Pavlica, M. (2009). Oxidative stress and DNA damage in broad bean (*Vicia faba* L.) seedlings induced by thallium. *Environmental Toxicology and Chemistry,* **28**: 189-196.

Rahman, M. M., Rahman, M. M., Islam, K. S. and Chong-ling, Y. (2010). Effect of chromium stress on antioxidative enzymes and malondialdehyde content activities in leaves and roots of mangrove seedlings *Kandelia candel* (L.) Druce. *Journal of Forest and Environmental Science*, **26**: 171-179.

Rahman, R. and Upadhyaya, H. (2021). Aluminium toxicity and its tolerance in plants: A review. *Journal of Plant Biology*, **64**: 101-121.

Rai, S., Singh, P. K., Mankotia, S., Swain, J. and Satbhai, S. B. (2021). Iron homeostasis in plants and its crosstalk with copper, zinc and manganese. *Plant Stress*, **1**: 100008.

Rai, V. (2002). Role of amino acids in plant responses to stresses. *Biologia Plantarum*, **45**: 481-487. doi: 10.1023/A:1022308229759

Rai, V. and Mehrotra, S. (2008). Chromium induced changes in ultramorphology and secondary metabolites of *Phyllanthus amarus* Schum and Thonn-A hepatoprotective plant. *Environmental Monitoring and Assessment*, **147**: 307-315.

Rai, V., Vajpayee, P., Singh, S. N. and Mehrotra, S. (2004). Effect of chromium accumulation on photosynthetic pigments, oxidative stress defense system, nitrate reduction, proline level and eugenol content of *Ocimum tenuiflorum* L. *Plant Science*, **167**: 1159-1169.

Raicht, R. F., Cohen, B. I., Fazzini, E. P., Sarwal, A. N. and Takahashi, M. (1980). Protective effect of plant sterols against chemically induced colon tumors in rats. *Cancer Research*, **40**: 403-405.

Rajab, M. S., Cantrell, C. L., Franzblau, S. G. and Fischer, N. H. (1998). Antimycobacterial activity of (E)-phytol and derivatives: A preliminary structure-activity study. *Planta Medica*, **64**: 2-4.

Rajeswara Rao, B. R., Kaul, P. N., Mallavarapu, G. R. and Ramesh, S. (2000). First observation of little leaf disease and its impact on the yield and composition of the essential oil of rose-scented geranium (*Pelargonium* sp.). *Flavour and Fragrance Journal*, **15**: 137-140.

Ramalingam, S., Packirisamy, M., Karuppiah, M., Vasu, G., Gopalakrishnan, R., Gothandam, K. and Thiruppathi, M. (2020). Effect of β-sitosterol on glucose homeostasis by sensitization of insulin resistance via enhanced protein expression of PPRγ and glucose transporter 4 in high fat diet and streptozotocin-induced diabetic rats. *Cytotechnology*, **72**: 357-366.

Rangani, J., Panda, A., Patel, M. and Parida, A. K. (2018). Regulation of ROS through proficient modulations of antioxidative defense system maintains the structural and functional integrity of photosynthetic apparatus and confers drought tolerance in the facultative halophyte *Salvadora persica* L. *Journal of Photochemistry and Photobiology B: Biology*, **189**: 214-233.

Rani, S., Singh, V., Sharma, M. K. and Sisodia, R. (2021). GC-MS based metabolite profiling of medicinal plant - *Catharanthus roseus* under cadmium stress. *Plant Physiology Reports*, 1-12.

Ranjan, A., Sinha, R., Sharma, T. R., Pattanayak, A. and Singh, A. K. (2021). Alleviating aluminum toxicity in plants: Implications of reactive oxygen species signaling and crosstalk with other signaling pathways. *Physiologia Plantarum*, **173**: 1765-1784.

Rascio, N. and Navari-Izzo, F. (2011). Heavy metal hyperaccumulating plants: How and why do they do it? And what makes them so interesting? *Plant Science*, **180**: 169-181.

Raskin, I. and Ensley, B. D. (2000). Phytoremediation of toxic metals using plants to clean up the environment. John Wiley and Sons Inc.

Ratheesh Chandra, P., Abdussalam, A. K., Nabeesa-salim and Puthur, J. T. (2010). Distribution of bio-accumulated Cd and Cr in two *Vigna* species and the associated histological variations. *Journal of Stress Physiology and Biochemistry*, **6**: 4-12.

Rauser, W. E. (1990). Phytochelatins. *Annual Review of Biochemistry*, **59**: 61-86.

Razaq, M., Zhang, P., Shen, H. and Salahuddin, S. (2017). Influence of nitrogen and phosphorous on the growth and root morphology of Acer mono. *PLoS One*, **12**: e0171321.

Reale, L., Ferranti, F., Mantilacci, S., Corboli, M., Aversa, S., Landucci, F., Baldisserotto, C., Ferroni, L., Pancaldi, S. and Venanzoni, R. (2016). Cyto-histological and morpho-physiological responses of common duckweed (*Lemna minor* L.) to chromium. *Chemosphere*, **145**: 98-105.

Reckova, S., Tuma, J., Dobrev, P. and Vankova, R. (2019). Influence of copper on hormone content and selected morphological, physiological and biochemical parameters of hydroponically grown *Zea mays* plants. *Plant Growth Regulation*, **89**: 191–201.

Reddy, G. N. and Prasad, M. N. V. (1992). Cadmium induced peroxidase activity and isozymes in *Oryza sativa*. *Biochemistry Archives*, **8**: 101-106.

Reed, R. H. and Gadd, G. M. (1990). Metal tolerance in eukaryotic and prokaryotic algae. In A. J. Shaw (Ed.), *Heavy Metal Tolerance in Plants: Evolutionary Aspects*, CRC Press,105-118.

Rehman, M., Liu, L., Wang, Q., Saleem, M. H., Bashir, S., Ullah, S. and Peng, D. (2019). Copper environmental toxicology, recent advances and future outlook: A review. *Environmental Science and Pollution Research*, **26**: 18003-18016.

Reichman, S. M. (2002). The responses of plants to metal toxicity: A review focusing on copper, manganese and zinc. *Australian Minerals and Energy Environment Foundation*, 1-54.

Ren, H., Li, Y. N., Zhao, F. F., Pu, X. J., Wei, L. J., Lv, X., Zhu, F. and Lin, H. H. (2016). The role of autophagy in alleviating damage of aluminum stress in *Arabidopsis thaliana*. *Plant Growth Regulation*, **79**: 167-175.

Rengel, Z. (1996). Uptake of aluminium by plant cells. *New Phytologist*, **134**: 389-409.

Renner, H. W. (1990). In vivo effects of single or combined dietary antimutagens on mutagen-induced chromosomal aberrations. *Mutation Research*, **244**: 185–188.

Retief, E. (2000). Lamiaceae (Labiatae). In O. A. Leistner (Ed.), *Seed Plants of Southern Africa*, National Botanical Institute, 323-334.

Rezakhanlo, A. and Talebi, S. M. (2010). Trichomes morphology of *Stachys lavandulifolia* Vahl. (Labiatae) of Iran. *Procedia - Social and Behavioral Sciences*, **2**: 3755-3763.

Riaz, M., Wu, X., Yan, L., Hussain, S., Aziz, O., Shah, A. and Jiang, C. (2018). Boron supply alleviates Al-induced inhibition of root elongation and physiological characteristics in rapeseed (*Brassica napus* L.). *Journal of Plant Interactions*, **13**: 270-276.

Riaza, M., Yana, L., Wua, X., Hussain, S., Aziz, O. and Jiang, C. (2018). Mechanisms of organic acids and boron induced tolerance of aluminum toxicity: A review. *Ecotoxicology and Environmental Safety*, **165**: 25–35.

Roberts, K., Gurney-Smith, M. and Hills, G. J. (1972). Structure, composition and morphogenesis of the cell wall of *Chlamydomonas reinhardi*. I. Ultrastructure and preliminary chemical analysis. *Journal of Ultrastructure Research*, **40**: 599–613.

Rodriguez, E., Azevedo, R., Fernandes, P. and Santos, C. A. (2011). Cr (VI) induces DNA damage, cell cycle arrest and polyploidization: A flow cytometric and comet assay study in *Pisum sativum*. *Chemical Research in Toxicology*, **24**: 1040–1047.

Rodriguez, E., Santos, C., Azevedo, R., Moutinho-Pereira, J., Correia, C. and Dias, M. C. (2012). Chromium (VI) induces toxicity at different photosynthetic levels in pea. *Plant Physiology and Biochemistry*, **53**: 94-100.

Rodríguez-Alonso, J., Sierra, M. J. and Lominchar, M. Á. (2019). Effects of mercury on the germination and growth of *Quercus ilex* L. seedlings. *Environmental Science and Pollution Research*, **26**:30930-30940.

Rogowska, A. and Szakiel, A. (2021). Enhancement of phytosterol and triterpenoid production in plant hairy root cultures - simultaneous stimulation or competition? *Plants*, **10**: 2028.

Roja, G., Pol, B. B., Subbaraman, A. S., Chintalwar, G. J. and Eapen, S. (2006). Accumulation of essential oils in tissue cultures of *Coleus amboinicus*. *Journal of Herbs, Spices and Medicinal Plants*, **11**: 37-41.

Rosa, M., Prado, C., Podazza, G., Interdonato, R., González, J. A., Hilal, M. and Prado, F. E. (2009). Soluble sugars. *Plant Signaling and Behavior*, **4**: 388-393.

Roshan, P., Naveen, M., Manjul, P. S., Gulzar, A., Anita, S. and Sudarshan, S. (2010). *Plectranthus amboinicus* (Lour) Spreng: An overview. *Pharmaceutical Research*, **4**: 1-15.

Rout, G. R. and Sahoo, S. (2015). Role of iron in plant growth and metabolism. *Reviews in Agricultural Sciences*, **3**:1-24.

Rout, G. R., Sanghamitra, S. and Das, P. (1997). Differential chromium tolerance among eight mung bean cultivars grown in nutrient culture. *Journal of Plant Nutrition*, **20**: 341-347.

Rout, G., Samantaray, S. and Das, P. (2001). Aluminum toxicity in plants: A review. *Agronomie*, **21**: 3-21.

Rout, J. R. and Sahoo, S. L. (2013). Antioxidant enzyme gene expression in response to copper stress in *Withania somnifera* L. *Plant Growth Regulation*, **71**: 95-99.

Rout, J. R., Sahoo, S. L., Das, R., Ram, S. S., Chakraborty, A. and Sudarshan, M. (2017). Changes in antioxidant enzyme activities and elemental profiling of *Abutilon indicum* L. subjected to copper stress. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences*, **87**: 1469-1478.

Rout, O. P., Acharya, R., Mishra, S. K. and Sahoo, R. (2012). Pathorchur (*Coleus aromaticus*): A review of the medicinal evidence for its phytochemistry and pharmacology properties. *International Journal of Applied Biology and Pharmaceutical Technology*, **3**: 348-355.

Rucińska-Sobkowiak, R. (2016). Water relations in plants subjected to heavy metal stresses. *Acta Physiologiae Plantarum*, **38**: 1–13.

Rucińska-Sobkowiak, R., Nowaczyk, G., Krzesłowska, M., Rabęda, I. and Jurga, S. (2013). Water status and water diffusion transport in lupine roots exposed to lead. *Environmental and Experimental Botany*, **87**: 100-109.

Ruehr, N. K., Offermann, C. A., Gessler, A., Winkler, J. B., Ferrio, J. P. and Buchmann, N. (2009). Drought effects on allocation of recent carbon: From beech leaves to soil CO2 efflux. *New Phytologist*, **184**: 950–961.

Ruixin, S., Junjie, Z., Weiyu, S., Yongchao, W., Yulou, T., Zikai, L., Wei, S., Hao, W., Guo, J., Yanjun, M., Guozhang, K., Krishna, J. and Qinghua, Y. (2022). Mercury stress tolerance in wheat and maize is achieved by lignin accumulation controlled by nitric oxide. *Environmental Pollution*, **307**: 119488. https://doi.org/10.1016/j.envpol.2022.119488

Ryan, P. R., Di Tomasso, J. M. and Kochian, L. V. (1993). Aluminium toxicity in roots: An investigation of spatial sensitivity and the role of the root cap. *Journal of Experimental Botany*, **44**: 437-446.

Ryan, P. R., Shaff, J. E. and Kochian, L. V. (1992). Aluminium toxicity in roots: Correlation among ionic currents, ion fluxes and root elongation in aluminium-sensitive and aluminium-tolerant cultivars. *Plant Physiology*, **99**: 1193-1200.

Ryan, P. R., Skerrett, M., Findlay, G. P., Delhaize, E. and Tyerman, S. D. (1997). Aluminium activates an anion channel in the apical cells of wheat roots. *Proceedings of the National Academy of Sciences of the USA*, **94**: 6547-6552.

Ryu, K., Choi, J. Y., Chung, S. and Kim, D. H. (2011). Anti-scratching behavioral effect of the essential oil and phytol isolated from *Artemisia princeps* Pamp. in mice. *Planta Medica*, **77**: 26-30. doi: 10.1055/s-0030-1250456

Sade, H., Meriga, B., Surapu, V., Gadi, J., Sunita, M. S. L., Suravajhala, P. and Kavi Kishor, P. B. (2016). Toxicity and tolerance of aluminum in plants: Tailoring plants to suit acid soils. *BioMetals*, **29**:187–210.

Saeed, N. M., El-Demerdash, E., Abdel-Rahman, H. M., Algandaby, M. M., Al-Abbasi, F. A. and Abdel-Naim, A. B. (2012). Anti-inflammatory activity of methyl palmitate and ethyl palmitate in different experimental rat models. *Toxicology and Applied Pharmacology*, **264**: 84–93.

Safari, F., Akramian, M., Salehi-Arjmand, H. and Khadivi, A. (2019). Physiological and molecular mechanisms underlying salicylic acid-mitigated mercury toxicity in lemon balm (*Melissa officinalis* L.). *Ecotoxicology and Environmental Safety*, **183**: 109542. doi: 10.1016/j.ecoenv.2019.109542

Sagardoy, R., Vázquez, S., Florez-Sarasa, I., Albacete, A., Ribas-Carbo, M., Flexas, J., Abadía, J. and Morales, F. (2010). Stomatal and mesophyll conductances to CO2 are the main limitations to photosynthesis in sugar beet (*Beta vulgaris*) plants grown with excess zinc. *New Phytologist*, **187**: 145-158.

Sahu, G. K., Upadhyay, S. and Sahoo, B. B. (2012). Mercury induced phytotoxicity and oxidative stress in wheat (*Triticum aestivum* L.) plants. *Physiology and Molecular Biology of Plants*, **18**: 21-31.

Saidu, K., Onah, J., Orisadipe, A., Olusola, A., Wambebe, C. and Gamaniel, K. (2000). Antiplasmodial, analgesic and anti-inflammatory activities of the aqueous extract of

the stem bark of *Erythrina senegalensis*. *Journal of Ethnopharmacology*, **71**: 275-280.

Saikia, D., Parihar, S., Chanda, D., Ojha, S., Kumar, J., Chanotiya, C., Shanker, K. and Negi, A. S. (2010). Antitubercular potential of some semisynthetic analogues of phytol. *Bioorganic and Medicinal Chemistry Letters*, **20**: 508-512. https://doi.org/10.1016/j.bmcl.2009.11.107

Saleem, M. H., Afzal, J., Rizwan, M., Shah, Z. U. H., Depar, N. and Usman, K. (2022). Chromium toxicity in plants: Consequences on growth, chromosomal behavior and mineral nutrient status. *Turkish Journal of Agriculture and Forestry*, **46**: 371-389.

Salt, D. E., Smith, R. D. and Raskin, I. (1998). Phytoremediation. *Annual Review of Plant Physiology and Plant Molecular Biology*, **49**:643-668.

Samad, R., Rashid, P. and Karmoker, J. L. (2019). Anatomical responses of rice (*Oryza sativa* L.) to aluminium toxicity. *Journal of Bangladesh Academy of Sciences*, **43**: 123-131.

Samad, R., Rashid, P. and Karmoker, J. L. (2020). Aluminium toxicity on the activities of antioxidant enzymes in rice (*Oryza sativa* L.) and chickpea (*Cicer arietinum* L.) seedlings grown in hydroponic culture. *Bangladesh Journal of Botany*, **49**: 619-624.

Samad, R., Rashid, P. and Karmoker, J. L. (2021). Anatomical changes in chickpea (*Cicer arietinum* L.) under aluminium stress condition. *Dhaka University Journal of Biological Science*, **30**: 187-196.

Samantary, S. and Das, P. (1997). Accumulation and distribution of chromium, nickel and iron in the mung bean plant. *Fresenius Environmental Bulletin*, **6**: 633-641.

Sameena, P. P. (2023). Copper-induced functional and structural modulations in *Ricinus communis* L. and stress amelioration by exogenous application of cytokinins (Ph.D. Thesis, University of Calicut, Kerala, India).

Sameena, P. P. and Puthur, J. T. (2021). Differential modulation of photosynthesis and defense strategies towards copper toxicity in primary and cotyledonary leaves of *Ricinus communis* L. *Journal of Photochemistry and Photobiology*, **8**:100059.

Sampanpanish, P., Pongsapich, W. and Khaodhiar, S. (2006). Chromium removal from soil by phytoremediation with weed plant species in Thailand. *Water, Air and Soil Pollution*, **171**: 191-206. doi: 10.1007/s11270-005-9005-4

Sandmann, G. and Boger, P. (1983). The enzymological function of heavy metals and their role in electron transfer processes of plants. In A. Lauchli and R. L. Bieleski (Eds.), *Encyclopedia of Plant Physiology (New Series): Inorganic Plant Nutrition* .**15**: 563-596.

Santos, C. M. P., Salvadori, M. S., Mota, V. G., Costa, L. M. and Almeida, A. A. C. O. (2013). Antinociceptive and antioxidant activities of phytol in vivo and in vitro models. *Neuroscience Journal*, Article ID 949452.

Santos, F. A. and Rao, V. S. (2017). Carvacrol exhibits analgesic and antispasmodic activities in mice. *European Journal of Pharmacology*, **794**: 157-164. doi: 10.1016/j.ejphar.2016.12.024

Santos, F. A., Serra, C. G., Bezerra, R. J., Figueredo, F. G., Matias, F. F. and Menezes, I. R. (2016). Antibacterial activity of *Plectranthus amboinicus* Lour (Lamiaceae) essential oil against *Streptococcus* mutans. *European Journal of Integrative Medicine*, **8**: 293-297.

Saradhi, A. and Saradhi, P. P. (1991). Proline accumulation under heavy metal stress. *Plant Physiology*, **138**: 554-558.

Sarath, N. G., Shackira, A. M., El-Serehy, H. A., Hefft, D. I. and Puthur, J. T. (2022). Phytostabilization of arsenic and associated physio-anatomical changes in *Acanthus ilicifolius* L. *Environmental Pollution*, **298**: 118828.

Sarkunan, V., Biddappa, C. C. and Nayak, S. K. (1984). Physiology of aluminum toxicity in rice. *Current Science*, **53**: 822-824.

Sasaki, T., Yamamoto, Y., Ezaki, B., Katsuhara, M. and Ahnis, J. (2004). A wheat gene encoding an aluminum-activated malate transporter. *The Plant Journal*, **37**: 645-653.

Saud, S., Wang, D., Fahad, S., Javed, T., Jaremko, M., Abdelsalam, N. R. and Ghareeb, R. Y. (2022). The impact of chromium ion stress on plant growth, developmental physiology and molecular regulation. *Frontiers in Plant Science*, **13**: 994785.

Schmitt, M., Watanabe, T. and Jansen, S. (2016). The effects of aluminium on plant growth in a temperate and deciduous aluminium-accumulating species. *AoB PLANTS*, **8**: plw065. https://doi.org/10.1093/aobpla/plw065

Schuster, E. (1991). The behavior of mercury in the soil with special emphasis on complexation and adsorption processes - A review of the literature. *Water, Air and Soil Pollution*, **56**: 667-680.

Selvakumar, P., Edhayanaveena, B. and Prakash, S. D. (2012). Studies on the antidandruff activity of the essential oil of *Coleus amboinicus* and *Eucalyptus globulus*. *Asian Pacific Journal of Tropical Disease*, **2**:715-719.

Seneviratne, M., Rajakaruna, N., Rizwan, M., Madawala, H. M. S. P., Ok, Y. S. and Vithanage, M. (2019). Heavy metal-induced oxidative stress on seed germination and seedling development: A critical review. *Environmental Geochemistry and Health*, **41**: 1813–1831.

Senthilkumar, A. and Venkatesalu, V. (2010). Chemical composition and larvicidal activity of the essential oil of *Plectranthus amboinicus* (Lour.) Spreng against *Anopheles stephensi*: A malarial vector mosquito. *Parasitology Research*, **107**: 1275–1278.

Sfaxi-Bousbih, A., Chaoui, A. and El Ferjani, E. (2010). Unsuitable availability of nutrients in germinating bean embryos exposed to copper excess. *Biological Trace Element Research*, **135**: 295–303.

Shabbir, Z., Sardar, A., Shabbir, A., Abbas, G., Shamshad, S., Khalid, S., Natasha, N., Murtaza, G., Dumat, C. and Shahid, M. (2020). Copper uptake, essentiality, toxicity, detoxification and risk assessment in soil-plant environment. *Chemosphere*, **259**: 127436.

Shackira, A. M. and Puthur, J. T. (2017). Enhanced phytostabilization of cadmium by a halophyte - *Acanthus ilicifolius* L. *International Journal of Phytoremediation*, **19**: 319–326.

Shackira, A. M. and Puthur, J. T. (2019). Cd^{2+} influences metabolism and elemental distribution in roots of *Acanthus ilicifolius* L. *International Journal of Phytoremediation*, **21**: 1–12.

Shahid, M., Shamshad, S., Rafiq, M., Khalid, S., Bibi, I., Niazi, N. K., Dumat, C. and Rashid, M. I. (2017). Chromium speciation, bioavailability, uptake, toxicity and detoxification in soil-plant system: A review. *Chemosphere*, **178**: 513–533.

Shanker, A. K., Cervantes, C., Loza-Tavera, H. and Avudainayagam, S. (2005). Chromium toxicity in plants. *Environment International*, **31**: 739–753.

Shanker, A. K., Djanaguiraman, M., Sudhagar, R., Chandrashekar, C. N. and Pathmanabhan, G. (2004). Differential antioxidative response of ascorbate glutathione pathway enzymes and metabolites to chromium speciation stress in green gram (*Vigna radiata* (L.) R. Wilczek, cv CO4) roots. *Plant Science*, **166**: 1035–1043.

Sharawy, M. H., El-Agamy, D. S., Shalaby, A. A. and Ammar, El-S. M. (2013). Protective effects of methyl palmitate against silica-induced pulmonary fibrosis in rats. *International Immunopharmacology*, **16**: 191–198.

Sharifi-Rad, J., Sureda, A., Tenore, G. C., Daglia, M., Sharifi-Rad, M., Valussi, M., Tundis, R., Sharifi-Rad, M., Loizzo, M. R., Ademiluyi, A. O., Sharifi-Rad, R., Ayatollahi, S. A. and Iriti, M. (2017). Biological activities of essential oils: From plant chemoecology to traditional healing systems. *Molecules*, **22**: 70.

Sharma, A., Kumar, V., Shahzad, B., Ramakrishnan, M., Singh Sidhu, G. P., Bali, A. S., Handa, N., Kapoor, D., Yadav, P., Khanna, K., Bakshi, P., Rehman, A., Kohli, S. K., Khan, E. A., Parihar, R. D., Yuan, H., Thukral, A. K., Bharadwaj, R. and Zheng, B. (2020). Photosynthetic response of plants under different abiotic stresses: A review. *Journal of Plant Growth Regulation*, **39**:509–531.

Sharma, D. C., Chatterjee, C. and Sharma, C. P. (1995). Chromium accumulation and its effects on wheat (*Triticum aestivum* L. cv HD 2204) metabolism. *Plant Science*, **111**: 145–151.

Sharma, I. and Ahmad, P. (2004). Catalase: A versatile antioxidant in plants. In P. Ahmad (Ed.), *Oxidative damage to plants*, Srinagar: S.P. College, 131-148.

Sharma, P., Jha, A. B., Dubey, R. S. and Pessarakli, M. (2012). Reactive oxygen species, oxidative damage and antioxidative defense mechanism in plants under stressful conditions. *Journal of Botany*, 1-26. doi: 10.1155/2012/217037

Sharma, P., Singh, S. P., Parakh, S. K. and Tong, Y. W. (2022). Health hazards of hexavalent chromium Cr (VI)) and its microbial reduction. *Bioengineered*, **13**: 4923–4938.

Sharma, S. K., Goloubinoff, P. and Christen, P. (2000). Non-native proteins as newly-identified targets of heavy metals and metalloids. In G. Banfalvi (Edn.), *Cellular effects of heavy metals*, 263-274.

Shaw, B. P. (1995). Effect of mercury and cadmium on the activities of antioxidative enzymes in the seedlings of *Phaseolus aureus*. *Plant Biology*, **37**: 587-596.

Shaw, B. P. and Rout, N. P. (1998). Age-dependent responses of *Phaseolus aureus* Roxb. to inorganic salts of mercury and cadmium. *Acta Physiologiae Plantarum*, **20**: 85-90.

Shekar, C. C., Sammaiah, D., Shasthree, T. and Reddy, K. J. (2011). Effect of mercury on tomato growth and yield attributes. *International Journal of Pharma and Bio Sciences*, **2**: 364.

Sheldon, A. R. and Menzies, N. W. (2005). The effect of copper toxicity on the growth and root morphology of Rhodes grass (*Chloris gayana* Knuth.) in resin buffered solution culture. *Plant and Soil*, **278**: 341-349.

Shi, W. G., Liu, W., Yu, W., Zhang, Y., Ding, S., Li, H., Mrak, T., Kraigher, H. and Luo, Z. B. (2019). Abscisic acid enhances lead translocation from the roots to the leaves and alleviates its toxicity in *Populus canescens*. *Journal of Hazardous Materials*, **362**: 275-285.

Shibata, M., Tsuyama, M., Takami, T., Shimizu, H. and Kobayashi, Y. (2004). Accumulation of menaquinones with incompletely reduced side chains and loss of α-tocopherol in rice mutants with alterations in the chlorophyll moiety. *Journal of Experimental Botany*, **55**: 1989-1996. doi: 10.1093/jxb/erh218

Shinwari, K. I., Jan, M., Shah, G., Khattak, S. R., Urehman, S. and Daud, M. K. (2015). Seed priming with salicylic acid induces tolerance against chromium (VI) toxicity in rice (*Oryza sativa* L.). *Pakistan Journal of Botany*, **47**: 161–170.

Shiojiri, K., Ozawa, R. and Yamashita, K. I. (2017). Weeding volatiles reduce leaf and seed damage to field-grown soybeans and increase seed isoflavones. *Scientific Reports*, **7**: 41508.

Shiyab, S. (2019). Morphophysiological effects of chromium in sour orange (*Citrus aurantium* L.). *HortScience*, **54**: 829–834.

Sihag, S. and Joshi, U. (2016). Chromium toxicity affects structural and non-structural carbohydrate value of *Sorghum bicolor* (L.). *International Journal of Current Research and Academic Review*, **4**: 171-182.

Silva, S. (2012). Aluminium toxicity targets in plants. *Journal of Botany*,**3**: 1-8.

Sinduja, M. S., Avudainayagam, S., Davamani, V. and Suganthi, R. (2018). Uptake of Mercury by Marigold and *Amaranthus* on Spiked Soil. *Madras Agricultural Journal*, **105**: 346-351.

Singh, A. K. (2001). Effect of trivalent and hexavalent chromium on spinach (*Spinaceia oleracea* L.). *Environmental Ecology*, **19**: 807-810.

Singh, D. and Aeri, V. (2013). Phytochemical and pharmacological potential of *Acanthus ilicifolius*. *Journal of Pharmacy and Bioallied Sciences*, **5**: 17-20.

Singh, D., Sharma, N. L., Singh, C. K., Sarkar, S. K., Singh, I. and Dotaniya, M. L. (2020). Effect of chromium (VI) toxicity on morpho-physiological characteristics, yield and yield components of two chickpea (*Cicer arietinum* L.) varieties. *PLoS ONE*, **15**: e0243032.

Singh, D., Sharma, N. L., Singh, C. K., Yerramilli, V., Narayan, R., Sarkar, S. K. and Singh, I. (2021). Chromium (VI)-induced alterations in physio-chemical parameters, yield and yield characteristics in two cultivars of mungbean (*Vigna radiata* L.). *Frontiers in Plant Science*, **12**: 735129.

Singh, G., Singh, O. P., Prasad, Y. R., Lamposona, M. P. and Catalan, C. (2002). Studies on essential oils: Chemical and insecticidal investigations on leaf oil of *Coleus amboinicus* (Lour). *Flavour and Fragrance Journal*, **17**: 440-442.

Singh, H. P., Mahajan, P., Kaur, S., Batish, D. R. and Kohli, R. K. (2013). Chromium toxicity and tolerance in plants. *Environmental Chemistry Letters*, **11**:229-254.

Singh, S., Tripathi, D. K., Singh, S., Sharma, S., Dubey, N. K. and Chauhan, D. K. (2017). Toxicity of aluminium on various levels of plant cells and organism: A review. *Environmental and Experimental Botany*, **137**: 177-193.

Singh, V. P. (2005). *Toxic metals and environmental issues*. Sarup and Sons.

Sinha, S. (1999). Accumulation of Cu, Cd, Cr, Mn and Pb from artificially contaminated soil by *Bacopa monnieri*. *Environmental Monitoring and Assessment*, **57**:253-264.

Sinha, V., Pakshirajan, K. and Chaturvedi, R. (2018). Chromium tolerance, bioaccumulation and localization in plants: An overview. *Journal of Environmental Management*, **206**: 235-244.

Sivarajan, V. V. and Balachandran, I. (1994). Ayurvedic drugs and their plant sources. Oxford: IBH Publishing Co Pvt. Ltd.

Skinner, K., Wright, N. and Porter-Goff, E. (2007). Mercury uptake and accumulation by four species of aquatic plants. *Environmental Pollution*, **145**: 234-237.

Slama, I., Abdelly, C., Bouchereau, A., Flowers, T. and Savoure, A. (2015). Diversity, distribution and roles of osmoprotective compounds accumulated in halophytes under abiotic stress. *Annals of Botany*, **115**: 433-447.

Smirnov, O., Kosyan, A., Kosyk, O. and Taran, N. (2014). Buckwheat stomatal traits under aluminium toxicity. *Modern Phytomorphology*, **6**: 15-18.

Snehalatha V. R. and Mannarakkal, J. K. (2016). Effect of mercuric chloride on some morpho-physiological parameters of *Centella asiatica* (L.) urb. *South Indian Journal of Biological Sciences*, **2**: 30-34.

Soares, C., Carvalho, M. E., Azevedo, R. A. and Fidalgo, F. (2019). Plants facing oxidative challenges - A little help from the antioxidant networks. *Environmental and Experimental Botany*, **161**: 4-25.

Solanki, R. and Dhankhar, R. (2011). Biochemical changes and adaptive strategies of plants under heavy metal stress. *Biologia*, **66**: 195-204.

Soni, H., Nayak, G., Patel, S. S., Mishra, K., Singhai, A. K., Swarnkar, P. and Pathak, A. K. (2011). Synergistic effect of polyherbal suspension of *Punica granatum* and *Coleus aromaticus* in evaluation of wound healing activity. *Journal of Herbal Medicine and Toxicology*, **5**: 111-115.

Sreedharren, B., Jaiganesh, K. P., Kannappan, N. and Sulochna, N. (2010). Pharmacognostic studies on *Plectranthus amboinicus* Lour. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, **1**: 413-424.

Srivastava, D., Tiwari, M., Dutta, P., Singh, P., Chawda, K., Kumari, M. and Chakrabarty, D. (2021). Chromium stress in plants: Toxicity, tolerance and phytoremediation. *Sustainability*, **13**:4629.

Sruthi, P. and Puthur, J. T. (2019). Characterization of physiochemical and anatomical features associated with enhanced phytostabilization of copper in *Bruguiera cylindrica* (L.) Blume. *International Journal of Phytoremediation*, **21**: 1423-1441.

Stefanov, K. L., Popova, I., Kamburova, E., Pancheva, Kimenov, G., Kuleva, L. and Popov, S. (1993). Lipid and sterol changes in *Zea mays* caused by lead ions. *Phytochemistry*, **33**: 47-51.

Steffens, J. C. (1990). The heavy metal-binding peptides of plants. *Annual Review of Plant Physiology and Plant Molecular Biology*, **41**: 553-575.

Su, T., Wang, P., Li, H., Zhao, Y., Lu, Y., Dai, P., Ren, T., Wang, X., Li, X., Shao, Q., Zhao, D., Zhao, Y. and Ma, C. (2018). The *Arabidopsis* catalase triple mutant reveals important roles of catalases and peroxisome-derived signaling in plant development. *Journal of Integrative Plant Biology*, **60**: 591-607.

Subrahmanyam, D. (2008). Effects of chromium toxicity on leaf photosynthetic characteristics and oxidative changes in wheat (*Triticum aestivum* L.). *Photosynthetica*, **46**: 339-345.

Sujkowska-Rybkowska, M. and Borucki, W. (2014). Localization of hydrogen peroxide accumulation and diamine oxidase activity in pea root nodules under aluminum stress. *Micron*, **57**: 13-22.

Sun, L. L., Zhang, M. S., Liu, X. M., Mao, Q. Z., Shi, C. and Kochian, L. V. (2020). Aluminium is essential for root growth and development of tea plants (*Camellia sinensis*). *Journal of Integrative Plant Biology*, **62**: 984-997.

Sun, L., Cao, X., Tan, C., Deng, Y., Cai, R., Peng, X. and Bai, J. (2020). Analysis of the effect of cadmium stress on root exudates of *Sedum plumbizincicola* based on metabolomics. *Ecotoxicology and Environmental Safety*, **205**: 111152.

Sundarraj, S., Ramar, T., Sreevani, V., Kaveri, K., Gunasekaran, P., Achiraman, S. and Kannan, S. (2012). gamma-Sitosterol from *Acacia nilotica* L. induces G2/M cell cycle arrest and apoptosis through c-Myc suppression in MCF-7 and A549 cells. J*ournal of Ethnopharmacology*, **141**: 803-809. doi: 10.1016/j.jep.2012.03.014

Sung, M. W. and Kwon, B. K. (1980). Effects of Pb surplus and P deficiency on ATP content in plant leaves. *Korean Journal of Botany*, **23**: 37-44.

Sunitha, K. S., Haniffa, M. A., James Milton, M. and Arthi Manju. (2010). *Coleus aromaticus* Benth act as an immunostimulant in *Channa marulius* Hamilton. *International Journal of Biological Technology*, **1**: 55-59.

Suresh, P. and Vasudeva, N. (2018). Antimicrobial activity of carvacrol against foodborne pathogens. *Food Microbiology*, **69**: 137-144. doi: 10.1016/j.fm.2017.09.012

Suseela, M. R., Sinha, S., Singh, S. and Saxena, R. (2002). Accumulation of chromium and scanning electron microscopic studies in *Scirpus lacustris* treated with metal and tannery effluent. *Bulletin of Environmental Contamination and Toxicology*, **68**:540-548.

Svoboda, K. P. and Greenaway, R. I. (2003). Investigation of volatile oil glands of *Satureja hortensis* L. (summer savory) and phytochemical comparison of different varieties. *International Journal of Aromatherapy*, **13**: 196-202.

Swapna, K. S., Nabeesa-Salim, Ratheesh Chandra and Puthur, J. T. (2015). Structural changes in response to bioaccumulation of iron and mercury in *Chromolaena odorata* (L.) King and Robins. *Environmental Monitoring and Assessment*, **187**: 551.

Tadaiesky, L. B., da Silva, B. R., Batista, B. L. and Lobato, A. K. D. S. (2021). Brassinosteroids trigger tolerance to iron toxicity in rice. *Physiologia Plantarum*, **171**: 371-387.

Taiz, L., Zeiger, E., Moller, I. M. and Murphy, A. (2015). Plant physiology and development (6th Edn.). Sinauer Associates, Inc. Publishers.

Tang, J., Xu, J., Wu, Y., Li, Y. and Tang, Q. (2012). Effects of high concentration of chromium stress on physiological and biochemical characters and accumulation of chromium in tea plant (*Camellia sinensis* L.). *African Journal of Biotechnology*, **11**: 2248-2255.

Taylor, G. J. (1988). The physiology of aluminium tolerance. In H. Sigel (Ed.), *Aluminium and its role in biology*, Marcel Dekker, 165-198.

Taylor, G. J. (1991). Current views of the aluminium stress response: The physiological basis of tolerance. *Current Topics in Plant Biochemistry and Physiology*, **10**: 57-93.

Taylor, G. J. (1995). Overcoming barriers to understanding the cellular basis of aluminium resistance. *Plant and Soil*, **171**: 89-103.

Thilagavathi, T., Arvindganth, R., Vidhya, D. and Dhivya, R. (2015). Preliminary phytochemical screening of different solvent mediated medicinal plant extracts evaluated. *International Research Journal of Pharmacy*, **6**: 246-248.

Thomas, J. C., Malick, F. K., Endreszl, C., Davies, E. C. and Murray, K. S. (1998). Distinct responses to copper stress in the halophyte *Mesembryanthemum crystallinum*. *Physiologia Plantarum*, **102**: 360-368.

Thor, K. (2019). Calcium - nutrient and messenger. *Frontiers in Plant Science*, **10**: 440.

Tohidi, Z., Baghizadeh, A. and Enteshari, S. (2009). The effects of aluminum and phosphorus on *Brassica napus*. *American-Eurasian Journal of Agriculture and Environmental Science*, **6**: 137-142.

Tohidi, Z., Baghizadeh, A. and Enteshari, S. (2015). The effects of aluminum and phosphorous on some of physiological characteristics of *Brassica napus*. *Journal of Stress Physiology and Biochemistry*, **11**: 16-28.

Too, E., Were, B., Onkware, A., Ringo, J., Carlsson, A., Ouma, E., Geleta, M. and Gudu, S. (2014). Response of selected sorghum (*Sorghum bicolor* L. Moench) germplasm to aluminium stress. *African Journal of Agricultural Research*, **9**: 1651-1662.

Tripathi, N., Kumar, S., Singh, R., Singh, C. J., Singh, P. and Varshney, V. (2013). Isolation and identification of gamma-sitosterol by GC-MS from roots of *Girardinia heterophylla*. *Oriental Journal of Chemistry*, **29**: 705-707. doi: 10.13005/ojc/290245

Turner, A. P. (1994). The responses of plants to heavy metals. In S. M. Ross (Ed.), *Toxic metals in soil-plant systems*, John Wiley and Sons Ltd, 154-187.

Turner, G. W., Gershenzon, J. and Croteau, R. B. (2000). Distribution of peltate glandular trichomes on developing leaves of peppermint. *Plant Physiology*, **124**: 655-664.

Ukwubile, C., Ahmed, A., Katsayal, U., Ya'u, J. and Mejida, S. (2019). GC-MS analysis of bioactive compounds from *Melastomastrum capitatum* (Vahl) Fern. leaf methanol extract: An anticancer plant. *Scientific African*, **3**: e00059. doi: 10.1016/j.sciaf.2019.e00059

Usman, K., Al-Ghouti, M. A. and Abu-Dieyeh, M. H. (2019). The assessment of cadmium, chromium, copper and nickel tolerance and bioaccumulation by shrub plant *Tetraena qataranse*. *Scientific Reports*, **9**: 1-11.

Usoro, O. and Mousa, S. (2010). Vitamin E forms in Alzheimer's disease: A review of controversial and clinical experiences. *Critical Reviews in Food Science and Nutrition*, **50**: 414-419. doi: 10.1080/10408390802304222

Vajpayee, P., Tripathi, R. D., Rai, U. N., Ali, M. B. and Singh, S. N. (2001). Chromium accumulation reduces chlorophyll biosynthesis, nitrate reductase activity and protein content of *Nymphea alba*. *Chemosphere*, **41**: 1075-1082.

Vallejo, M. C. G., Moujir, L., Burillo, J., Guerra, L. L., Gonzalez, M., Penate, R. D., San Andres, L., Luis, J. G., Blanco, F. L. and de Galarreta, C. M. R. (2006). Chemical composition and biological activities of the essential oils of *Salvia Canariensis*. *Flavour and Fragrance Journal*, **21**: 277-281.

Van Assche, F. and Clijsters, H. (1990). Effect of metals on enzyme activity in plants. *Plant Cell and Environment*, 13: 195-206.

Vardar, F. and Ünal, M. (2007). Aluminum toxicity and resistance in higher plants. *Advances in Molecular Biology*, **1**: 1-12.

Vassilev, A., Lidon, F., Ramalho, J. C., Doceumatos, M. and Dagraca, M. (2003). Effects of excess Cu on growth and photosynthesis of barley plants. Implication with a screening test for Cu tolerance. *Journal of Central European Agriculture*, **4**: 225-235.

Vazquez, M. D., Poschenrieder, C. H. and Barcelo, J. (1987). Chromium (VI) induced structural changes in bush bean plants. *Annals of Botany*, **59**: 427-438.

Vazquez, M. D., Poschenrieder, C., Corrales, I. and Barcelo, J. (1999). Change in apoplastic aluminium during the initial growth response to aluminium by roots of a tolerant maize variety. *Plant Physiology*, **119**: 435-444.

Velasco-Alinsug, M. P., Rivero, G. C. and Quibuyen, T. A. O. (2005). Isolation of mercury-binding peptides in vegetative parts of *Chromolaena odorata*. *Journal of Biosciences*, **60**: 252-259.

Verma, R. S., Padalia, R. C. and Chauhan, A. (2012). Essential oil composition of *Coleus aromaticus* Benth. from Uttarakhand. *Journal of Essential Oil-Bearing Plants*, **15**: 174-179.

Verma, S. and Dubey, R. S. (2003). Lead toxicity induces lipid peroxidation and alters the activities of antioxidant enzymes in growing rice plants. *Plant Science*, **164**:645-655.

Vernay, P., Gauthier-Moussard, C. and Hitmi, A. (2007). Interaction of bioaccumulation of heavy metal chromium with water relation, mineral nutrition and photosynthesis in developed leaves of *Lolium perenne* L. *Chemosphere*, **68**: 1563-1575.

Viehweger, K. (2014). How plants cope with heavy metals. *Botany Studies*, **55**: 35-47.

Vijayarengan, P. and Jose, M. D. (2014). Changes in growth, pigments and phytoremediating capability of four plant species under copper stress. *International Journal of Environmental Biology*, **4**: 119-126.

Vitorello, V. A., Capaldi, F. R. and Stefanuto, V. A. (2005). Recent advances in aluminum toxicity and resistance in higher plants. *Brazilian Journal of Plant Physiology*, **17**: 129-143.

Wakeel, A., Xu, M. and Gan, Y. (2020). Chromium-induced reactive oxygen species accumulation by altering the enzymatic antioxidant system and associated cytotoxic, genotypic, ultrastructural and photosynthetic changes in plants. *International Journal of Molecular Sciences*, **21**: 728.

Wang, F., Du, C., Chen, J., Shi, L. and Li, H. (2021). A new method for determination of pectin content using spectrophotometry. *Polymers*, **13**: 2847.

Wang, H. O. and Zhong, G. R. (2011). Effect of organic ligands on accumulation of copper in hyperaccumulator and non-accumulator *Commelina communis*. *Biological Trace Element Research*, **143**: 489-499.

Wang, P., Yu, W., Zhang, J., Rengel, Z., Xu, J., Han, Q. and Tang, L. (2016). Auxin enhances aluminum-induced citrate exudation through upregulation of GmMATE and activation of the plasma membrane H+-ATPase in soybean roots. *Annals of Botany*, **118**: 933-940.

Wang, Q., Li, Z., Feng, X., Wang, A., Li, X., Wang, D. and Fan, L. (2021). Mercury accumulation in vegetable *Houttuynia cordata* Thunb. from two different geological areas in southwest China and implications for human consumption. *Scientific Reports*, **11**: 1-12.

Wang, R., Gao, F., Guo, B. Q., Huang, J. C., Wang, L.,and Zhou, Y. J. (2013). Short-term chromium-stress-induced alterations in the maize leaf proteome. *International Journal of Molecular Sciences*, **14**: 11125-11144.

Wang, S., Ren, X., Huang, B., Wang, G., Zhou, P. and An, Y. (2016). Aluminium-induced reduction of plant growth in alfalfa (*Medicago sativa*) is mediated by interrupting auxin transport and accumulation in roots. *Scientific Reports*, **6**: 30079.

Wang, Y., Branicky, R., Noë, A. and Hekimi, S. (2018). Superoxide dismutases: Dual roles in controlling ROS damage and regulating ROS signaling. *Journal of Cell Biology*, **217**:1915-1928.

Warriner, R. and Burrell, R. (2005). Infection and the chronic wound: A focus on silver. *Advances in Skin and Wound Care*, **18**: 2-12.

Watanabe, T., Jansen, S. and Osaki, M. (2005). The beneficial effect and the role of citrate in Al accumulation in *Melastoma malabathricum*. *New Phytologist*, **165**: 773-780.

Wei, Y., Han, R., Xie, Y., Jiang, C. and Yu, Y. (2021). Recent advances in understanding mechanisms of plant tolerance and response to aluminum toxicity. *Sustainability*, **13**: 1782.

Whelan, W. J. (1955). Starch, glycogen, fructosans and similar polysaccharides. In M. N. V. Tracey (Ed.), *Modern methods of plant analysis*, Springer-Verlag**., 3**: 684-699.

Wilkins, D. A. (1978). The measurement of tolerance to edaphic factors by means of root growth. *New Phytologist*, **80**: 623-633.

Winkel-Shirley, B. (2002). Biosynthesis of flavonoids and effects of stress. *Current Opinion in Plant Biology*, **5**: 218-223.

Wong, M. H. and Bradshaw, A. D. (1982). A comparison of the toxicity of heavy metals, using root elongation of rye grass, *Lolium perenne*. *New Phytologist*, **91**: 255-261.

Wu, Y.-M., Wang, Y.-Y., Zhou, Y.-F., Meng, X., Huang, Z.-R., Chen, L.-S. and Yang, L.-T. (2019). Analysis of interacting proteins of aluminum toxicity response factor ALS3 and CAD in citrus. *International Journal of Molecular Sciences*, **20**: 4846.

Xu, G., Fan, X. and Miller, A. (2012). Plant nitrogen assimilation and use efficiency. *Annual Review of Plant Biology*, **63**:153–182.

Xu, J., Zhang, J., Lv, Y., Xu, K., Lu, S., Liu, X. and Yang, Y. (2020). Effect of soil mercury pollution on ginger (*Zingiber officinale* roscoe): Growth, product quality, health risks and silicon mitigation. *Ecotoxicology and Environmental Safety*, **195**: 110472.

Xu, Z. R., Cai, M. L., Chen, S. H., Huang, X. Y., Zhao, F. J. and Wang, P. (2021). High-affinity sulfate transporter Sultr1;2 is a major transporter for Cr (VI) uptake in plants. *Environmental Science and Technology*, **55**: 1576-1584.

Xue, Z. C., Gao, H. Y. and Zhang, L. T. (2013). Effects of cadmium on growth, photosynthetic rate and chlorophyll content in leaves of soybean seedlings. *Biologia Plantarum*, **57**: 587-590.

Xun, Y., Feng, L., Li, Y. and Dong, H. (2017). Mercury accumulation in plant *Cyrtomium macrophyllum* and its potential for phytoremediation of mercury-polluted sites. *Chemosphere*, **189**: 161-170.

Yadav, A., Yadav, S. S., Singh, S. and Dabur, R. (2022). Natural products: Potential therapeutic agents to prevent skeletal muscle atrophy. *European Journal of Pharmacology*,925: 174995.

Yadav, S., Shukla, O. P. and Rai, U. N. (2005). Chromium pollution and bioremediation. *Environews Newsletter of ISEB India*, **11**: 1-4.

Yamin, L., Jianzhong, L., Yongbo, S. and Feng, Z. (2011). Effects of aluminum stress on the stomata characteristics and photosynthesis of *Scutellaria baicalensis* Georgi seedlings. *Medicinal Plant*, **2**: 5-8.

Yan, L., Riaz, M., Liu, J., Yu, M. and Cuncang, J. (2021). The aluminium tolerance and detoxification mechanisms in plants; recent advances and prospects. *Critical Reviews in Environmental Science and Technology*, 1-38.

Yan, L., Riaz, M., Liu, Y., Zeng, Y. and Jiang, C. (2019). Aluminum toxicity could be mitigated with boron by altering the metabolic patterns of amino acids and

carbohydrates rather than organic acids in trifoliate orange. *Tree Physiology*, **39**: 1572–1582.

Yan, X., Wang, J., Song, H., Peng, Y., Zuo, S., Gao, T., Duan, X., Qin, D. and Dong, J. (2020). Evaluation of the phytoremediation potential of dominant plant species growing in a chromium salt–producing factory wasteland, China. *Environmental Science and Pollution Research*, **27**:657–7671.

Yang, H. M., Zhang, X. Y. and Wang, G. X. (2004). Effects of heavy metals on stomatal movements in broad bean leaves. *Russian Journal of Plant Physiology*, **51**: 464-468.

Yang, T. Y., Cai, L. Y., Qi, Y. P., Yang, L. T., Lai, N. W. and Chen, L. S. (2019). Increasing nutrient solution pH alleviated aluminum-induced inhibition of growth and impairment of photosynthetic electron transport chain in *Citrus sinensis* seedlings. *BioMed Research International*, 9058715.

Yap, C. K., Tan, W. S., Wong, K. W., Ong, G. H., Cheng, W. H., Nulit, R., Ibrahim, M. H., Chew, W., Edward, F. B., Okamura, H., Al-Mutairi, K. A., Al-Shami, S. A., Sharifinia, M., Mustafa, M., Leong, W. J. and You, C. F. (2021). Antioxidant enzyme activities as biomarkers of Cu and Pb stress in *Centella asiatica*. *Stresses*, **1**: 253-265.

Yaseen, G., Ahmad, M., Zafar, M., Sultana, S., Kayani, S., Andrade Cetto, A. and Shaheen, S. (2015). Traditional management of diabetes in Pakistan: Ethnobotanical investigation from traditional health practitioners. *Journal of Ethnopharmacology*, **174**: 91–117.

Yıldız, M. O., Çelik, H. and Caglayan, C. (2022). Neuroprotective effects of carvacrol against cadmium-induced neurotoxicity in rats: Role of oxidative stress, inflammation and apoptosis. *Metabolic Brain Disease*, **37**: 1259–1269.

Yoganarasimhan, S. N. (2000). Medicinal plants of India, Tamil Nadu. In V. Srinivasan and N. Kosal Ram (Eds.), Cyber Media.

Yoon, J., Cao, X., Zhou, Q. and Ma, Q. L. (2006). Accumulation of Pb, Cu and Zn in native plants growing on a contaminated Florida site. *Science of the Total Environment*, **368**: 456-464.

Yruela, I. (2005). Copper in plants. *Brazilian Journal of Plant Physiology*, **17**: 145–156.

Yruela, I. (2009). Copper in plants: Acquisition, transport and interactions. *Functional Plant Biology*, **36**: 409-430.

Yu, F. R., Lian, X. Z., Guo, H. Y., McGuire, P. M., Li, R. D., Wang, R. and Yu, F. H. (2005). Isolation and characterization of methyl esters and derivatives from *Euphorbia kansui* (Euphorbeaceae) and their inhibitory effects on the human SCG-7901 cells. *Journal of Pharmacy and Pharmaceutical Sciences*, **8**:528-535.

Yu, X., Lin, Y. and Zhang, Q. (2019). Metallothioneins enhance chromium detoxification through scavenging ROS and stimulating metal chelation in *Oryza sativa*. *Chemosphere*, **220**: 300-313.

Zaheer, I. E., Ali, S., Saleem, M. H., Noor, I.,and El-Esawi, M. A. (2020). Iron–lysine mediated alleviation of chromium toxicity in spinach (*Spinacia oleracea* L.) plants

in relation to morpho-physiological traits and iron uptake when irrigated with tannery wastewater. *Sustainability*, **12**: 6690.

Zeid, I. M. (2001). Responses of *Phaseolus vulgaris* to chromium and cobalt treatments. *Biologia Plantarum*, **44**:111-115.

Zemanová, V., Pavlík, M., Pavlíková, D. and Kyjaková, P. (2015). Changes in the contents of amino acids and the profile of fatty acids in response to cadmium contamination in spinach. *Plant Soil Environment*, **61**:285–290.

Zenda, T., Liu, S., Dong, A. and Duan, H. (2021). Revisiting sulphur - the once neglected nutrient: Its roles in plant growth, metabolism, stress tolerance and crop production. *Agriculture*, **11**: 626.

Zhang, A., Klun, J., Wang, S., Carroll, J. and Debboun, M. (2009). Isolongifolenone: A novel sesquiterpene repellent of ticks and mosquitoes. *Journal of Medical Entomology*, 46(1), 100-106. doi: 10.1603/033.046.0113

Zhang, F. Q., Wang, Y. S., Lou, Z. P. and Dong, J. D. (2007). Effect of heavy metal stress on antioxidative enzymes and lipid peroxidation in leaves and roots of two mangrove plant seedlings (*Kandelia candel* and *Bruguiera gymnorrhiza*). *Chemosphere*, **67**: 44-50.

Zhang, H., Hu, L. Y., Li, P., Li, P., Hu, K. D., Jiang, C. X. and Luo, J. P. (2010). Hydrogen sulfide alleviated chromium toxicity in wheat. *Biologia Plantarum*, **54**: 743-747.

Zhang, H., Zhang, S., Meng, Q., Zou, J., Jiang, W. and Liu, D. (2009). Effects of aluminum on nucleoli in root tip cells, root growth and the antioxidant defense system in *Vicia faba* L. *Acta Biologica Cracoviensia Series Botanica*, **51**:99-106.

Zhang, W. H., Ryan, P. R. and Tyerman, S. D. (2001). Malate permeable channels and cation channels activated by aluminum in the apical cells of wheat roots. *Plant Physiology*, **125**: 1459-1472.

Zhang, W., Yu, T., Yang, X. and Li, H. (2020). Speciation, transformation and accumulation of manure-derived Cu and Zn in the soil-rice system. *Soil and Sediment Contamination*, **29**: 43-52.

Zhang, X. H., Zhang, E. H. and Lang, D. Y. (2011). Autotoxic compounds from rhizosphere soil of *Humulus lupulus* L. extracts: Identification and biological activity. *Agronomy*, **103**: 695. doi: 10.2134/agronj2010.0425

Zhang, X., Wu, J. and Liu, J. (2019). Mechanisms of heavy metal tolerance in plants: A review. *Ecotoxicology and Environmental Safety*, **174**:556-566.

Zhang, Y., Yang, L. and Kucherlapati, M. (2019). Global impact of somatic structural variation on the DNA methylome of human cancers. *Genome Biology*, **20**: 209-215.

Zhang, Y., Zhang, J., Guo, J. L., Zhou, F. L. and Singh, S., Xu, X. (2019). F-box protein RAE1 regulates the stability of the aluminum-resistance transcription factor STOP1 in *Arabidopsis*. *Proceedings of the National Academy of Sciences*, **116**: 319-327.

Zhao, Y., Hu, C., Wang, X., Qing, X., Wang, P. and Zhang, Y. (2019). Selenium alleviated chromium stress in Chinese cabbage (*Brassica campestris* L. ssp. Pekinensis) by regulating root morphology and metal element uptake. *Ecotoxicology and Environmental Safety*, **173**: 314-322.

Zhao, Y., Peralta-Videa, J. R., Lopez-Moreno, M. L., Saupe, G. B. and Gardea-Torresdey, J. L. (2011). Use of plasma-based spectroscopy and infrared microspectroscopy techniques to determine the uptake and effects of chromium (III) and chromium (VI) on *Parkinsonia aculeata*. *International Journal of Phytoremediation*, **13**: 17–33.

Zheljazkov, V. D. and Nielsen, N. E. (1996). Effect of heavy metals on peppermint and cornmint. *Plant and Soil*, **178**: 59-66.

Zhong, C., Cao, X., Hu, J., Zhu, L., Zhang, J., Huang, J. and Jin, Q. (2017). Nitrogen metabolism in adaptation of photosynthesis to water stress in rice grown under different nitrogen levels. *Frontiers in Plant Science*, **8**: 1079.

Zhou, J. T., Wan, H. X., He, J. L., Lyu, D. G. and Li, H. F. (2017). Integration of cadmium accumulation, subcellular distribution and physiological responses to understanding cadmium tolerance in apple rootstocks. *Frontiers in Plant Science*, **8**: 966.

Zhou, S., Okekeogbu, I., Sangireddy, S., Ye, Z., Li, H. and Bhatti, S. (2016). Proteome modification in tomato plants upon long-term aluminum treatment. *Journal of Proteome Research*, **15**: 1670–1684. https://doi.org/10.1021/acs.jproteome.5b01081

Zhou, Z. S., Huang, S. Q., Guo, K., Mehta, S. K., Zhang, P. C. and Yang, Z. M. (2007). Metabolic adaptations to mercury-induced oxidative stress in roots of *Medicago sativa* L. *Journal of Inorganic Biochemistry*, **101**: 1-9. https://doi.org/10.1016/j.jinorgbio.2006.09.023

Zlobin, I., Kholodova, V., Rakhmankulova, Z. and Kuznetsov, V. V. (2015). *Brassica napus* responses to short-term excessive copper treatment with a decrease of photosynthetic pigments, differential expression of heavy metal homeostasis genes including activation of gene NRAMP4 involved in photosystem II stabilization. *Photosynthesis Research*, **125**: 141-150. https://doi.org/10.1007/s11120-015-0094-9

Zong, H., Liu, J., Wang, F. and Song, N. (2020). Root morphological response of six peanut cultivars to chromium (VI) toxicity. *Environmental Science and Pollution Research*, **27**: 18403–18411. https://doi.org/10.1007/s11356-020-08464-4

Zurayk, R., Sukkariyah, B., Baalbaki, R. and Ghanem, A. (2001). Chromium phytoaccumulation from solution by selected hydrophytes. *International Journal of Phytoremediation*, **3**: 335-350. https://doi.org/10.1080/20015891108566

PRESENTATIONS AND PUBLICATIONS

Presentations

Sudheeshna P. K. & Hussain K. (2023). "Structural changes in response to bioaccumulation of mercury in *Plectranthus amboinicus* lour. spreng" in two day international scientific conference on environmental research: issues, challenges and strategies for sustainable development on 1& 2 December,organized by Eurasian Academy of Environmental Sciences at Karwar, Karnataka.

Sudheeshna P. K. & Hussain K. (2021). "structural and functional changes induced by copper toxicity in *Plectranthus amboinicus* lour. spreng" in three days National webinar of Plant Physiology "Frontiers of Plant Physiology for Climate Smart Agriculture" on 9-11 December, organized by ICARNational Institute of Abiotic Stress Management, Pune and Indian Society for Plant Physiology, New Delhi.

Sudheeshna P.K. & Hussain K. (2020). 'Alterations in the antioxidative functions in *Plectranthus amboinicus* lour. spreng exposed to increasing levels of copper' in the international webinar on plant functional biology – doctrina-11, organized by the Department of Post graduate studies and Research in Botany & IQAC, Sir Syed College, Taliparamba, kannur, kerala, in association with Kerala State Higher Education Council, June 05-07.

Sudheeshna P.K. & Hussain K. (2022). "Plectranthus amboinicus lour. spreng; morphological and phytochemical responses to chromium toxicity" in three days International conference on advanced biology 2022, on 23-25th February 2022, organized by Inter University Centre for Evolutionary and Integrative Biology, University of Kerala, Trivandrum.

Sudheeshna P.K. & Hussain K. (2020)."Structural and functional changes induced by copper toxicity in *Plectranthus amboinicus* lour. spreng" in two day international conference on plant physiology and biochemistry on 19& 20 January 2020, organized by Fathima Mata National College, Kollam.

Sudheeshna P.K. & Hussain K. (2023)."Structural and functional changes induced by copper toxicity in *Plectranthus amboinicus* lour. spreng" in two day National seminar on new approaches in seed biological research on 16& 17 November 2023,organized by Kerala State Biodiversity Board at Sree Krishna College Guruvayur.

Sudheeshna P.K. & Hussain K. (2023). "Physiological aspects of Mercury toxicity in *Coleus amboinicus* Lour." in two day National seminar on phytochemistry and molecular taxonomy of flowering plants organized by SERB & Dept. of science and technology held at Vimala college, Thrissur on 18 and 19 January 2024.

Workshop/Conference Attented

Two days Research Facility Training Programme –DST-SERB organized by VSR lab-school of biology, IISER-Thiruvananthpuram during June 29 and 30th 2023.

International Workshop on Physiological and Molecular Markers for Abiotic Stress Tolerance in Plants organized by University of Calicut during October 31 to November 4 ,2023.

National workshop on Biological Techniques with emphasis to Histology, Histochemistry and Biochemistry organized by MES Kalladi College,Mannarkad during 27th February to March 1st 2018.

Achievements

Participated and presented a poster on "Structural changes in response to bioaccumulation of mercury in *Plectranthus amboinicus*" in two day International conference emerging trends in plant science research on march 6 &7, 2023 organized by Catholicate College and Kerala Academy of Sciences - First prize

Participated and presented a poster on "Response of *Plectranthus amboinicus* lour. spreng to chromium toxicity" in two day International conference on multidisciplinary research on 14 & 15 January, 2020 organized by St. Albert's College Ernakulam- First prize

PHARMACOLOGICAL PROFILING OF PLECTRANTHUS AMBOINICUS (LOUR.)SPRENG

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Abstract : *Plectranthus amboinicus* is a semi-succulent perennial plant belongs to the family Lamiaceae. This plant is extensively used in Indian traditional Ayurvedic system as well as tribal medicines for different treatments. The present investigation was carried out to determine the chemical constituents from *Plectranthus amboinicus* leaves by GC-MS technique. Twelve compounds were isolated, in that the most abundant compound is 5-isopropyl -2-methylphenol(55.88%). Then comes phytol(16.79%), squalene(9.09%), gamma sitosterol(4.07%). The activities of the identified compounds includes hypocholesterolemic, hepato protective, analgesic, antidiabetic, lubricant and sedative. So the use of this plant to treat many diseases in Ayurvedic medicines is justified.

Index terms **:** *Plectranthus amboinicus* , GC-MS analysis, bioactive components.

I. INTRODUCTION

Plectranthus amboinicus, once identified as *coleus amboinicus*, is a semisucculent perennial plant in the family Lamiaceae. This plant is with a pungent oregano like flavor and odour. It is widely cultivated and naturalized elsewhere in the tropics where it is used as a traditional medicine, spice and an ornamental plant. Its leaves are extensively used in Indian traditional system as well as tribal medicines for the treatment of bronchitis, asthma, diarrhea, fever etc. The leaves used for the treatment of coughs, sore throats and nasal congestion but also for a range of other problems such as infections and rheumatism.

Many pharmacological properties have been reported including urolithiasis (Baskar and Varalakhsmi.,1992), antiepileptic(Buznego and Perez–saad.,1999), antitumour and anti mutagens (Annapurani and priya.,1999), neuro pharmacological (Perez saad *et al*., 2003), radio protective effect (Rao *et al*., 2006), antibacterial and antifungal properties (Prudent *et al*., 1995).

Plants are a rich source of secondary metabolites with remarkable biological activities. The secondary metabolites are prominent and significant source with a variety of properties. Nowadays, the demand for natural or medicinal plant products are high. GC-MS is the best technique used to identify the bioactive components. It's an analytical method that combines the features of gas chromatography and mass spectrometry to identify different substances within a test sample. The major advantage of GC-MS is analysing small and volatile molecules. They used to separate complex mixtures, identify unknown peaks and determine trace levels of contamination.

The aim of the present study is to identify and determine the bioactive components present in the leaves of *Plectranthus amboinicus* using GC-MS technique. And this study may provide an insight in its use in Ayurvedic medicines.

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Morphological and phytochemical responses of *Coleus Amboinicus* Lour. towards chromium toxicity

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Abstract
Chromium is a potential toxic heavy metal which does not have any essential metabolic function in plants. Chromium is a natural pollutant occurring in soil and water imposing adverse effects on plant growth. In addition to imparting growth retardation, many plants are capable of accumulating Cr ions from soil/water. *Coleus amboinicus* is an important medicinal plant extensively used in traditional and Ayurvedic medicines. Toxic effects of Cr on *C.amboinicus* cultivated in Hoagland medium artificially contaminated with 150µM $K_2Cr_2O_7$ resulted in growth retardation. Growth rate and Cr toxicity levels are assessed in terms of shoot length, root length, leaf area and Tolerance index. Secondary metabolites analysis using GC-MS and the impact of Cr on growth pattern and distribution of secondary metabolites are discussed.

Keywords: *Coleus amboinicus*, chromium, tolerance index, GC-MS, secondary metabolites

Introduction

Heavy metals are major inorganic environmental contaminants that are toxic to the living system. Heavy metals inclusive of chromium (Cr), are toxic to plants and impart morphological, physiological and molecular symptoms of toxicity (Pradhan *et al*., 2017). In addition to cytotoxic effects mutagenic and carcinogenic impacts also have been reported due to Cr (Sankar *et al*., 2005; Kovacik *et al*., 2013).

Chromium in the soil/water adversely affect growth and development of plants. Effect of Cr on plants include alterations in morphological (Moral *et al*., 1995; Samantara *et al*., 1996; Iqbal *et al*., 2001) [12, 5], biomass production (Sankar *et al*., 2005; Panda, 2007) [14], biochemical (Samantara *et al*., 1996; Sankar *et al*., 2005), bioaccumulation potential (Sankar *et al*., 2005; Yadav *et al*., 2005; Ratheeshchandra *et al*., 2010; Kowacik *et al*., 2013; Pradhan *et al*., 2017) [28], production of Reactive oxygen species (Sankar *et al*., 2004), production of enzymic and non-enzymic antioxidants (Panda *et al*., 2003; Rai *et al*., 2004) [16].

Coleus amboinicus Lour. is a potential medicinal herb belonging to the family Lamiaceae. It is distributed in tropical and warm regions of Asia, Africa, and Australia. *Coleus amboinicus* is a large fleshy succulent perennial herb with aromatic pubescence and inherent medicinal power due to the presence of phytochemicals such as flavonoids, esters, phenolics and terpenoids (Arumugam *et al*. 2016) [1]. According to those authors, these phytochemicals attribute antibacterial, antifungal, antioxidant, anti-inflammatory, analgesic, antiepileptic, allelopathic, antihelminthic and larvicidal, properties to the plant.

The objectives of the present study is the elucidation of growth and morphological adaptations of *C.amboinicus* towards the toxicity of Chromium. Since *C.amboinicus* is a medicinal plant containing large number of secondary metabolites (Arumugam *et al*., 2016) [1], effect of Cr toxicity on the distribution of secondary metabolites and resultant impact in the medicinal property of the plant is proposed to undertake.

Materials and Methods

Healthy twigs of *C.amboinicus*, approximately 15cm length consisting of about 8-10 leaves were collected from the Botanical Garden, SNGS College, Pattambi. Cuttings were grown in water for root initiation. Rooted propagules were grown in Hoagland nutrient medium artificially contaminated by 150µM $K_2Cr_2O_7$. This optimal concentration was determined by trial-and-error method by different concentrations of $K_2Cr_2O_7$ and 150µM of Cr is found to be the optimum concentration in which the plants survived but exhibited significant growth retardation. Rooted propagules of *C.amboinicus* were cultivated for 20 days and samples were collected at an intervals of 4 days and used for the studies.

Morphological measurements

Growth of plants were assessed in terms of root length, shoot length and leaf area. The sampled propagules were washed, blotted and length of root, shoot and leaf area were measured manually using a graduated scale and graph paper. Measurements of all parameters using minimum five propagules were recorded each sampling.

Tolerance index

Tolerance index percentage was calculated according to the method of Turner (1994) [27] comparing root length of experimental and control plants.

GC-MS analysis

Fresh leaves of *C.amboinicus* were collected after 20 days of growth then shade dried and powdered. Five g of powder was taken in duplicates which was subjected to extraction using Methanol in Soxhlet apparatus. After running several cycles, the extract obtained was concentrated and volume was noted and was used for GC-MS analysis. GC-MS was performed using Shimadzu GC-MS, with model number QP2010S, at Kerala Forest Research Institute, Thrissur, Kerala.