PHYSIOLOGICAL AND ANATOMICAL STUDIES ON HEAVY METALS STRESS IN *STROBILANTHES ALTERNATA* (BURM.F.) MOYLAN EX J.R.I.WOOD

Thesis submitted to the University of Calicut
for the Degree of
DOCTOR OF PHILOSOPHY IN BOTANY

KARTHIKA DEVARAJAN

Post graduate and Research Department of Botany
Sree Neelakanda Government Sanskrit College
Pattambi

2022

CERTIFICATE

This is to certify that the thesis entitled "**PHYSIOLOGICAL AND ANATOMICAL STUDIES ON HEAVY METALS STRESS IN *STROBILANTHES ALTERNATA* (BURM.F.) MOYLAN EX J.R.I.WOOD"** submitted by **Karthika Devarajan** in partial fulfillment of the requirements for the Degree of **Doctor of Philosophy** in Botany, **University of Calicut** is a bonafied record of the research work undertaken by her in this Department under my supervision during the period 2018-2022 and that no part thereof has been presented before, for the award of any degree or diploma.

S.N.G.S College **Dr. Hussain K.**

DECLARATION

I hereby declare that the thesis entitled "**PHYSIOLOGICAL AND ANATOMICAL STUDIES ON HEAVY METALS STRESS IN *STROBILANTHES ALTERNATA* (BURM.F.) MOYLAN EX J.R.I.WOOD"** submitted by me in partial fulfilment of the requirements for the Degree of **Doctor of Philosophy in Botany**, **University of Calicut** is the bonafied work carried out by me and no part of the work has formed the basis for the award of any other degree or diploma.

S.N.G.S College

Karthika Devarajan

ACKNOWLEDGEMENT

I record my deep sense of gratitude to my supervising guide **Dr.Hussain K**. Assistant professor, Department of Botany, Sree Neelakanda Govt. Sanskrit College, Pattambi for providing all the necessary facilities and guidance to carry out my work. He is the one who drew me to the broad horizon of Plant Physiology research.

I wish to express my deep sense of gratitude to my teacher, **Dr.Nabeesa Salim**, Professor and former head of the Botany Department, University of Calicut for her valuable guidance and ceaseless motivation throughout my research work that helped me to complete the work in stipulated time.

No statements would be adequate to articulate my deep sense of gratefulness to **Dr.Vivek P.J**., Head of the Department of Botany, SNGS College, Pattambi, for his constant support.

I also express my sincere thanks to **Dr.Sunil John J**.,Principal, Sree Neelakanda Govt.Sanskrit College, Pattambi, for providing necessary facilities required during the entire course of my research.

I specially recall here the names of my beloved teachers, **Dr. Krishnakumar, Dr. Smitha, Dr. Jayakrishnan, Dr. Maju, Dr.Reshmi** and **Dr. Sumaya** for their timely guidance and advice during the work. I also extend my gratitude to all the non teaching staffs in the Department of Botany, for their help.

I am thanlful to my collegues, **Sudheeshna P.K**. and **Fathimath Zuhra P**. for all help, concern, motivation and support throughout the entire period.

I wish to record my heartfelt gratitude to **Ms. Swetha P.R, Ms. Anila P.S., Ms. Sneha Unni, Ms. Firdous, Ms. Anu, Ms. Nishma, Ms. Amrutha, Ms. Malavika** and all friends of BSc.Botany and MSc.Botany for their generous support and encouragement.

I would like to express my sincere thanks to **Dr.A.K.Pradeep**, Herbarium curator, Department of Botany, University of Calicut for the identification of taxa that I have selected for the research work.

I profoundly thank **Dr.Ajay**, Assistant Professor, Department of Zoology, SNGS College, for the immeasurable help he extended. All my collegues of the Department of Botany deserve to be thanked for their constant encouragement and unstinted support.

I also express my deepest gratitude to **Dr.Jos T.Puthur**, Associate professor and Reader, Department of Botany, University of Calicut, for his valuable suggestions during the research work.

I sincerely thank the authorities of the Library at SNGS College, Pattambi for all the kind help and useful advices. I record my sincere gratitude to Kerala Forest Research Institute, Thrissur , Central sophisticated instrument facility, University of Calicut for providing the instrumentation facilities. I express my special gratitude to **Mr.Hari**, Head of Anatomy division, Arya Vaidya Sala, Kottakal for all his support and providing the facilities there.

I wish to thank **Dr.Sameena**, University of Calicut for helping and providing laboratory facilities for analysis.

I express my special thanks to **Mr. Sudheesh**, for the timely supply of chemicals and glasswares.

My mother is a constant support on my work. A formal word will not reveal my indebtedness to my amma, **Mrs.Rekha Devarajan**. She is the one who gives me love, support, help, motivation and all throughout the period. She is the one who equally supported with all her efforts to finish my work.

I express my loving acknowledgement and heartfelt gratitude to my husband, **Mr.Aswin** for his constant encouragement, moral support, love and also financial assistance throughout my work. I thank you for patiently dealing with my frustration.

I am really indebted to my family members, especially my father **Mr..Devarajan**, my sister **Mrs. Deepa Devarajan** and my grandparents for their help, moral support, encouragement and prayers.

Above all, I pour my sincere gratitude to the **Almighty,** for giving me the strength, knowledge, ability and opportunity to undertake the research and to preserve and complete it satisfactorily.

Lastly, I offer my regards to all those who supported in any respect during the completion of this thesis as well as expressing my apology, that I could not mention personally one by one.

CONTENTS

INTRODUCTION

Mineral nutrition is an integral aspect of plant physiology and mineral and metal-ions are particularly important for healthy plant life. Excess or deficient occurrence of mineral ions impose various adverse effect on plant growth, development and productivity. Generally plants exhibit various symptoms in response to metal stress expressing as growth retardation, ultrastructural changes in plant organs, impaired metabolism due to ionic imbalance and water deficiency resulting in loss of turgidity, reduced photosynthetic and respiratory functions. In plants, mineral elements are used as components of structural proteins, cofactors in biochemical reactions, constituents of macromolecules, enzymes and DNA, and also as regulation of electrochemical balance of cellular compartments. Plants develop adaptive and flexible strategies for sensing and responding to fluctuations in element availability to optimize growth and development under a dynamic range of environmental conditions. In addition, once taken up elements must be allocated to different organs, tissues and cell types through light homeostasis mechanisms to ensure metal requirement, storage and remobilization under different conditions.

Heavy metals are naturally occurring elements which are widely distributed in the Earth's crust, they derive from rocks of volcanic sedimentary or metamorphic origin, but in recent years, the prevalence of heavy metals in areas of agricultural and industrial activities has increased because of human activity. Many heavy metals such as iron, zinc, copper, nickel, cobalt and molybdenum are essential for plant growth and cellular biochemistry being involved in cell functioning, gene regulation, signal transduction etc. and their absence or deficiency inhibit plant growth.

Plants growing in metal enriched soil absorb metals to varying levels, in response to external and internal factors. Considerable interest has been focused on the pattern of absorption, translocation and accumulation, because experimental studies of heavy metal tolerance show subtle differences in absorption and translocation by tolerant and intolerant genotypes.

Perusal of botanical literature reveals data relating to heavy metal uptake, illustrating the scale of differences between species and/or genotypes and between metals in the field and laboratory studies ranging from trace nutrient elements to toxic heavy metals (Foy *et al*., 1978; Lepp, 1981; Fitter and Hay, 1983; Borovik, 1990; Frieland, 1990; Orcutt and Nilsen, 2000; Cseh, 2002; Memon and Schroder, 2009; Solanki and Dhankar, 2011; Shanker, 2019; Babangida *et al*., 2021; Yan *et al*., 2021).

Heavy metals like aluminium, chromium and mercury are non-essential elements for plant growth and are highly reactive and consequently they are toxic to plants at higher concentrations. Aluminium is one of the most abundant and potent toxic element in acidic soil (Foy *et al*., 1978; Kochian, 1995). Toxicity of aluminium is initially imposed as growth inhibition (Kanproth and Foy, 1985, Roy *et al*., 1988; Foy,1978,1992). Primary effect of aluminium is found to be on the root tip and elongation region of the root in general and plant growth in particular (Taylor, 1995). According to Kochian (1995) and Matsumoto (2000), aluminium sensitive plants absorb more aluminium than aluminium tolerant plants and mechanisms behind the aluminium tolerance is mainly aluminium exclusion.

Aluminium is naturally occurring element and is not essential for plant growth, yet can accumulate in plants. Plants commonly contain 0.1-500 mg aluminium / gram dry matter and the addition of low amounts of Al^{3+} to nutrient culture may stimulate plant growth (Taiz *et al*., 2015).

Primary toxicity of aluminium ion is reported to affect root tip and elongation region of plant level (Taylor, 1995; Rengel, 1996). Generally aluminium interfere with cell division in root tips and lateral root formation increases cell wall rigidity (Kochian, 1995; Posehneider *et al*., 2008). In addition to structural changes, aluminium interferes with biochemical aspects such as reduction of DNA synthesis, enzyme inactivation, uptake and transportation of essential elements (Foy, 1992; Kochian, 1995). Cytotoxicity of aluminium in plants has been well documented (Kochian, 1995; Delhaize and Ryan,1995; Kollimier *et al*., 2000, Marienfield *et al*., 2000; Zhang *et al*., 2010; Silva, 2012).

Chromium occurs naturally in the environment and also produced by industrial process like manufacture of dyes and paints, chromium plating, leather tannery etc. (Shanker *et al.,* 2005; Shahid *et al*., 2017). The impact of chromium contamination in the physiology of plants depends on the metal speciation which is responsible for the mobilization, subsequent uptake and resultant toxicity in the plant system (Shanker *et al*., 2005).

Toxicity effect of chromium on plants is observed at multiple levels like inhibited growth of roots and shoots, deranged metabolism, inhibited enzyme activity, mutagenesis and reduced yield (Clijster and Van Assche, 1985; Bishnoi *et al*., 1993; Shanker *et al*., 2005). Toxic effects of chromium have been reported in growth and development (Rout, *et al*., 1997; Iqbal *et al*., 2001). Another important effect of chromium on plants is induction of free radical scavenging enzymes such as catalase, peroxidase and superoxide dismutase which are involved in the detoxification of chromium toxicity (Stoh-and Bagchi, 1995; Prasad, 1998, Shanker *et al*., 2004, 2005).

Mercury is global environmental pollutant present in soil, water, air and biota. Naturally occurring mercury get released to the atmosphere and then exchanged between the soil and water system by processes such as wind, erosion,

degassing of mercury mineralized soil, volcanic erosion and other geothermal activities (Ebinghams *et al*., 1999; Orcutt and Nilsen, 2000) .

Mercury is one of the most toxic heavy metal which is detrimental to plant at trace quantity levels (Woolhouse, 1983; Lenka *et al*., 1993) and toxicity of this metal includes membrane damage (Quarite *et al*., 1997) reaction with thiol groups of metabolites and site competition with metabolites interactions with other elements (Orcutt and Nilsen, 2000; Perfus-Berbeoch *et al*., 2002).

The toxicity of mercury in physiological process of plants has been elucidated mainly on growth retardation, uptake and distribution (Beauford *et al*., 1977; Velasco- Alingsung *et al*., 2005) tolerance mechanism (Lenka *et al*., 1993; Ahmed and Tajmir-Riahi, 1993). Adverse effect of mercury on plant on photosynthesis and chlorophyll synthesis have been reported by Bernier *et al*., (1993); Jain and Puranik, (1993); Shaw, (1995). Phytoremediation potential of *Brassica juncea* to remove mercury has been investigated and explained by Moreno *et al*., (2005, 2008).

Zinc is the most common plant micronutrient and the role of excess and deficient zinc content in plants have been elaborately investigated and reported (Cakmak, 2002; Alloway, 2004). Zinc is an essential component of thousands of proteins in plants, although it is toxic to plants in excess (Taiz *et al*., 2015). Zinc is the only metal represented in all six enzyme classes and the enzymatic function and activity are determined by the binding observations of Zn^{2+} ligand complex and three such binding sites- structural, catalytic and co catalytic have been recognized (Broadley *et al*., 2007) . It is constituent of metaloenzymes or a cofactor for several enzymes such as anhydrases, dehydrogenases, oxidases and peroxidases and plays an important role in regulating nitrogen metabolism, cell multiplication, photosynthesis and auxin synthesis in plants (Parmer *et al*., 2009). Zinc also plays an important role in the synthesis of nucleic acids and proteins

and help in the utilization of phosphorus and nitrogen during seed formation. While zinc is crucial for the above-mentioned processes, high levels of uncomplexed zinc are toxic to plants.

Naturally growing plants and cultivated ones particularly crop plants are exposed to different stresses. Heavy metals are currently of much environmental concern because they are harmful to humans, animals and tend to bioaccumulate and reach food chain. Medicinal plants are used worldwide and very common in many countries, since more than 70% of the global population uses medicinal plants to cure various diseases. However, plants are often sources of exposure to the toxic elements.

Medicinal plants have been employed for the study of heavy metal toxicity on plants under natural conditions and artificial techniques like simulated experimentation. Impact of heavy metals in *Boerhavia diffusa* was investigated histochemically in simulated experiments treating with cadmium, chromium and lead and chromium was found to be more toxic than lead and cadmium (Abdussalam *et al*., 2013). Herbal medicines prepared from 130 plants of different families in Brazil were reported to contain mercury in various concentrations which varied with difference in species level (Caldas and Machado,2004). Kalpana *et al*., (2018) evaluated heavy metals in selected medicinal plants like *Bacopa monneri* and *Centella asiatica* naturally growing in field and exposed to toxic level of zinc in those two species.

Potentially toxic levels of mercury distribution in *Chelidonium majus*, *Crategus monogyna* and *Artemisia absinthium* were reported and bioaccumulation values were at higher levels (Badea, 2015). According to Hussain *et al.,* (2010) and Hussain and Nabeesa-Salim, (2012), bioaccumulation potential of *Bacopa monneiri* is considerably high and since this plant is an

important ingredient of many ayurvedic preparations, the risk factor is very prominent.

Ur-Rehman *et al*., (2019) investigated content of endogenous heavy metals in locally available medicinal plants and found that mercury concentration was very high in *Melia azedarach*. Heavy metal contamination of two species of *Mentha piperita* and *Zataria multiflora* analyzed by Shahkarami *et al*., (2021) and reported more zinc concentration level in both plants.

Strobilanthes alternata (Burm.f.) Moylan ex J.R.I. wood (Synonym: *Hemigraphis colorata*) belongs to the family Acanthaceae. The name of the plant, *Hemigraphis colorata* has been changed to *Strobilanthes alternata* (Burm.f.) Moylan ex J.R.I. Wood as per the new nomenclature. Name change was followed as per the citation, IPNI (2021), International Plant Name Index. Published on the internet, in collaboration with, The Royal Botanical Garden, Kew, Harvard university Herbaria and Libraries and Australian National Botanical Garden.

Strobilanthes alternata is a wild herb grows profusely in almost all terrestrial lands and commonly considered as a weed and due to the color combination of the leaves it is cultivated also as an ornamental plant. The leaf show metallic purple luster on upper surface and a solid dark purple on ventral surface. Leaf is entire, lenses shaped, toothed, scalloped or lobed margin. Flowers bloom irregularly throughout the year in tropics. Flowers appear in terminal 2 to 10 cm long spikes and are white in color with faint purple marks within.

The plant is known by several vernacular names such as Aluminium plant, cementary plant, metal leaf, red flame ivy, waffle plant, Java ivy etc. (Silja *et al*., 2008). Ethnomedicinal plant knowledge of the Mullu kuruma tribe of Wayanad district of Kerala, was reported by Silja *et al.*, (2008). Those authors reported that, the plant is used in folk medicine due to its wound healing activity. The leaves

are ground into a paste and applied on fresh cut wounds. In Kerala, the plant is popular in the name "Murikootti" or "Murianpacha" because of its incredible potency to heal fresh wounds as reported by Subramoniam *et al*., (2001). Those authors demonstrated the effect of *Hemigraphis colorata* on inflammation and wound-healing in mice. Those authors studied the excision wound-healing and anti-inflammatory properties of the plant leaf suspension/paste. The wound contraction and epithelialization were faster in *Hemigraphis colorata* leaf paste applied to mice compared to control. According to Subramoniam *et al.*, *(*2001), wound healing potential of *Hemigraphis colorata* is shown as the drug adhering on the wound and preventing the discharges from the wound within a few hours after the application. Saravanan *et al*., (2010) studied the wound healing activity of *Hemigraphis colorata* and found that the better wound healing in test group may be due to the increase in collagen concentration per unit area and stabilization of fibers. The increased wound contraction and tensile strength may be due to the active constituents present in the extract and support the use of *Hemigraphis colorata* in the topical management of wound healing.

The medicinal properties of *Hemigraphis colorata* was known earlier to folk or tribal people. Anitha *et al*., (2012) investigated the antibacterial activity of *Hemigraphis colorata* against the selected pathogens. The results of the phytochemical screening revealed the presence of phenol, saponins, coumarins, tannins, proteins, carboxylic acid, flavonoids, Xanthoproteins, proteins and alkaloids in the crude aqueous, acetone, benzene, chloroform, ethanol and petroleum ether extracts of this plant leaves and stem. Bhargavi *et al*., (2011) and Pawar and Toppo, (2012) stated that phytochemicals present in this plant provide curative property and crude leaf paste provides excision wound healing activity. A nano encapsulated polyherbal ointment for antiinflammation prepared from the extracts of *Hemigraphis colorata* was formulated and reported by Megha *et al*., (2013) and therein the authors stated that, these plants are having antimicrobial,

anti-inflammatory and antioxidant properties. Therefore, these plants are recommended for the preparation of ointment which can be used for the treatment of dermatological infections.

Investigation reports on *Strobilanthes alternata* regarding the medicinal properties are essentially centered around the wound healing potential of the plant and earlier studies (Subramanian *et al.,* 2001; Anitha *et al*., 2012; Megha *et al*., 2013) repeatedly reported the same aspect with slight modifications. Those authors made attempts to correlate wound healing on the basis of anti-inflammatory, antibacterial and antifungal qualities of Strobilanthes *alternata*. So far, the plant has not yet been significant in any systematic, physiological and biochemical experiments. Notwithstanding, the celebrated property i.e., the wound healing has been well approved and practiced by folk and tribal people since most of them are engaged in field work of farming and are often get exposed to accidental wounds.

Strobilanthes alternata is a naturally growing wild plant, investigations on growth, physiology, behavior of the plant towards abiotic stress like heavy metal toxicity have not yet been studied. So also the mechanism and/or biochemistry, involvement of primary and secondary metabolites in wound healing process in naturally growing plants. Highlight of the proposed study is the elucidation of secondary metabolites, occurrence and their incredible therapeutic property in plants.

The present study is aimed at the elucidation of the physiological and/or biochemical aspects of *Strobilanthes alternata* in general and heavy metal toxicity in particular. Simulated experiments are proposed to unveil the growth performance under nutrient culture technique as well as in growth medium artificially contaminated with known quantities of heavy metals such as aluminium, chromium, mercury and zinc. On the basis of the results of the

preliminary study on *Strobilanthes alternata* the following objectives are taken into consideration.

1. Vegetative propagation of *Strobilanthes alternata* cuttings and cultivation practice in Hoagland nutrient medium and evaluation of propagule performance.
2. Effect of heavy metals like aluminium, chromium, mercury and zinc on the growth and metabolism of the plant by cultivating the rooted propagules in Hoagland nutrient solution artificially contaminated with known quatities of these metals.
3. Standardization of the heavy metal concentration permitting survival of plants and showing about 50% growth retardation under nutrient medium artificially contaminated with known quantities of aluminium, chromium, mercury and zinc.
4. Growth performance evaluation on the basis of morphological data such as root length, stem length, leaf area etc. and evaluation of metal toxicity in terms of tolerance index and stomatal index.
5. Using standard micro technique and staining procedure, anatomical studies to elucidate the impact of each heavy metal in the structure, form and functions of different tissues to correlate the toxicity symptom on various parts-root, stem and leaves of the plant.
6. Confirmation and clarification of structural details using scanning electron microscopy.
7. Evaluation of the primary essential elements and toxic heavy metal distribution under Scanning electron microscope attached with Energy Dispersive X-ray Analysis.
8. Biochemical changes in the primary metabolites and other cell constituents like protein, proline, phenolics, photosynthetic pigments etc.

9. Evaluation of heavy metal toxicity levels and pattern in the terms of the distribution and process of lipid peroxidation, MDA (Malondialdehyde) and carotenoids.
10. Role of antioxidant enzymes-superoxide dismutase and catalase in scavenging the superoxide radicals and other oxidants.
11. Measurement of bioaccumulation potential of *Strobilanthes alternata* by analyzing the absorption and translocation pattern of aluminium, chromium, mercury and zinc in various parts-root, stem and leaves to evaluate the tolerance potential of *Strobilanthes alternata* towards aluminium, chromium, mercury and zinc.
12. The prime and foremost objective of the investigation is the elucidation of the mechanism of wound healing process by qualitative and quantitative analysis the secondary metabolites using Gas Chromatography-Mass Spectrometry (GC-MS) in the leaves of *Strobilanthes alternata*, because the wound healing is presumed to be performed due to the involvement of many secondary metabolites which are functionally bioactive. Another equally important objective is the elucidation of stimulatory/ inhibitory role of aluminium, chromium, mercury and zinc on the synthesis of secondary metabolites and detection of adverse and beneficial effects of the metals if any in improving the medicinal property of *Strobilanthes alternata*.

REVIEW OF LITERATURE

Plants acquire essential and functional elements from the soil, but because of the selection/absorption of elements is imperfect, plants take- up non-essential elements also which are bioavailable. Some of these indirectly accumulated elements are highly toxic to plants. Toxic heavy metals do significant contributions to environmental pollution as a result of anthropogenic activities such as mining, energy-and fuel production, power transmission, intensive agricultural practices, sludge and industrial effluents (Foy *et al*.,1978; Lepp,1981: Salt *et al.*,1998; Orcutt and Nilsen, 2000; Cseh, 2002; Pilon-Smits, 2005; Li *et al*., 2015; Clemens and Ma, 2016; Vareda *et al*., 2019).

Heavy metals are currently of much environmental concern. They are harmful to humans, animals and plants and tend to reach and bioaccumulate in the food chain. A limited number of heavy metal ions are water soluble in physiological conditions and they become bioavailable for plants either being essential or potentially toxic causing risk for life. Many nutrient heavy metals (mainly Fe, Zn, Cu, Ni, Co and Mo) are toxic when present in excess, are essential for plants cellular biochemistry being involved in cell protection for regulation and signal translocation and their absence inhibits plant growth. Availability and toxicity of heavy metals in the environment, responses and adaptive strategies of plants to metal toxicity, bioaccumulation potential and phytoremediation technology etc. have been extensively discussed and excellently reviewed by several authors (Foy *et al*., 1978; Salt *et al*., 1998; Chang *et al*., 2000; Pilon-smits, 2005; Broadley *et al*., 2007; Migocka and Klobus, 2007; Solanki and Dhankhar, 2011; Shahid *et al*., 2017; Wani *et al*., 2018: Babangida *et al*., 2021).

Plants are good bio-indicators as they play a significant role in food chain transfer and in defining environmental health. They are easy to grow and adaptable to environmental stress and also reflect toxicant damage in other

organisms such as animals (Minissi and Lombi, 1997). Heavy metals in soil and plants have received increasing attention in recent years because of the harmful effects on dietary intake (Usha *et al*., 2002; Clemens and Ma, 2016).

The primary toxicity mechanisms of the different metal ions may be different as their chemical properties. An excess of these metals ions or soluble metal chelators may induce a series of biochemical and physiological alterations in plants. The primary characteristic feature of heavy metal stress are membrane damage, alteration of enzyme activities and inhibition of root growth which leads to secondary effects such as disturbance of hormone balance, deficiency of essential nutrients, inhibition of photosynthesis, change in photoassimiliate translocation, alteration in water relations etc. (Breckle,1989; Huang and Cunningham, 1996; Singh, 2005). Plants develop a complex mechanism by which they control the uptake and accumulation of heavy metals (Cobbett and Goldsbrough, 2002). These mechanisms involve chelation and sequestration of metal ions by a particular class of metal binding ligands denominated as phytochelatin (PC) and metallothionines (Cobbett, 2000).

Aluminium

Aluminium is naturally occurring element and is not essential for plants and yet can accumulate in plant tissues. Plants commonly contain 0.1-500 mg aluminium per gram dry matter and the addition of low amounts of Al^{3+} to a nutrient culture may stimulate plant growth (Taiz *et al*., 2015). According to Foy *et al*., (1978); Kochian *et al*., (2005) and Lei *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. Aluminium is toxic for many plants when the concentration is greater than 2-3 ppm with a soil pH 5.5 (Balsberg-Pahlsson,1990). Because of its small size and high charge, aluminium ions have a strong polarizing effect on other atoms. Aluminium does not exist as a free

metal but occurs in combination with oxygen, silicon, fluoride and to a lesser extent with other elements also (Silva, 2012). According to those authors, aluminium is released to the environment mainly by natural processes. Several factors influence aluminium mobility and subsequent transport within the environment. These include chemical speciation, hydrological flow paths, soil water interactions, and the composition of the underlying geological materials. Acid environments caused by acid mine drainage or acid rain can cause an increase in the dissolved aluminium content of the surrounding waters (Kochian,1995).

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,1988; Kochian,1995). A significant correlation between low pH and high Al^{3+} concentration has been shown in acidified fresh water, where this metal may reach levels of 0.3-1.6 mM (Panda and Matsumoto, 2007) and cause serious metabolic derangement in hydrophytes (Ciamporova, 2002).

Aluminium phytotoxicity in higher plants is associated with sites of responses that involve a number of physiological processes. Evidences indicate that Al toxicosis is primarily associated with disruption of root structure physiology and function of plants (Taylor, 1988; Kochian and Shaff, 1991). In neutral or weakly acidic pH, aluminium exists in the form of insoluble aluminosilicate or oxide which are non-toxic to plants. When the soil becomes more acidic, aluminium is solubilized into phytotoxic form. Soluble aluminium can be classified into several groups, free or mononuclear forms of Al^{3+}, polynuclear aluminium and aluminium as a low molecular weight complex (Kochian,1995). Al^{3+} ions are dominant in acidic soil *i.e.*, soil below pH 4.5 and render the most toxic form.

Cytotoxicity of aluminium in plants has been well documented (Kochian,1995; Delhaize and Ryan,1995; Kollmeier *et al*.,2000; Marienfeld *et al.*, 2000; Yan *et al*., 2021). In the cellular cytoplasm, aluminium is either as reversible/irreversible macromolecular complexes. Aluminium ions exist in acidic conditions as polyvalent cations which bind strongly to the negative charges in the cell (Poschenrieder *et al*., 2008; Zhang *et al*., 2010).

It has been reported that the root cap is the site of perception of aluminium injury, based on anatomical studies of maize roots by (Bennett *et al*., 1987). They observed rapid changes in the ultra-structure of the root cap cells including stress induced alterations, in the secretary pathway and suggested that aluminium could indirectly inhibit root growth via an unknown signal transduction pathway involving root cap, apical meristem, hormones and other putative signals.

The primary target of aluminium ion is found to be the root tip and elongation regions at plant level (Liugany *et al*., 1994; Taylor, 1995; Rengel, 1996). Aluminium-sensitive plants absorb more aluminium than aluminium-tolerant plants, thus the chief mechanism behind aluminium tolerance is aluminium exclusion (Kochian, 1995; Matsumoto, 2000). According to Kochian (1995) and Delhaize and Ryan (1995), there are two strategies behind the aluminium tolerance mechanism, exclusion of aluminium from the root apex and internal tolerance once aluminium enters the plant symplasm. The exclusion mechanism involves secretion of aluminium chelating ligands binding of aluminium with the cell wall and mucilage, a plant- induced pH barrier in the rhizosphere , root apoplasm, selective permeability of the plasma membrane and Al^{3+} efflux.

Generally, aluminium interferes with cell division in root tips and lateral roots, increase cell wall rigidity by cross linking pectins, reduces DNA replication by increasing the rigidity of the DNA double helix, fixes phosphorous in less

available forms in soils and on root surfaces, decreases root respiration, interferes with enzyme activity governing sugar phosphorylation and the deposition of cell wall polysaccharides and the uptake, transport and also use of several essential nutrients (Foy ,1992).

Fagria (1982), observed decrease in shoot growth after aluminium treatment in rice and reported decrease in yield, dry weight of shoots and roots, length of roots and plant height. Similar impact due to aluminium toxicity was reported by Pavan and Bingham, (1982) in coffee seedlings. Simon *et al*., (1994) opined that aluminium treatment resulted in reduced leaf area and dry weight of leaf, stem and root in Tomato cultivars grown in nutrient solutions. According to the author, content of calcium, potassium, magnesium, iron and zinc of the roots, stem and leaves were decreased with increasing concentration of aluminium. One of the proposed mechanisms of aluminium toxicity involves aluminium interaction with ion transport systems functioning at root cell plasma membrane (Taylor, 1988; Kochian, 1995).

The initial and dramatic symptom of aluminium toxicity is inhibition of root elongation which can occur within 1-2 hours after exposure to aluminium (Delhaize and Ryan, 1995). Aluminium- injured roots appear characteristically stubby and brittle, root tips and lateral roots thickened and brown. The root system as a whole become coralloid in appearance (Foy, 1984). Ryan *et al*., (1993) suggested that only the terminal 2-3 mm of maize root needed to be exposed to aluminium to cause inhibition of root growth. According to Budikova, (1999) partial root growth inhibition was the impact in maize root tissues due to aluminium treatment and root cap length and root cap area reduction were another effect of aluminium treatment. Aluminium stress leads the inhibition of callose synthesis resulting in the higher tissue damage.

Most evident significance of aluminium toxicity is root growth inhibition which takes place by damage of root apical cells- inducing of root cap cell elongation zone (Poschenrieder *et al*., 2008). As a result of root damage, the toxicity symptoms are manifested in the shoot also and the ultimate consequence is reduced biomass (Mossor-pietraszenska, 2001). An exhaustive review by Silva (2012) revealed the mechanism of aluminium toxicity and resistance in plants. According to the author, potential targets of aluminium toxicity include cell wall, plasma membrane, signal transduction pathways root cytoskeleton and DNA, associated with root growth. Recent advances and prospects of aluminium toxicity in plants is reviewed by Lei *et al*., (2021). Therein the authors proposed that the potential of plants to resist aluminium toxicity by a series of mechanisms. Many physiological functions have been developed to improve the aluminium tolerance by external exclusion which reduce aluminium entry into the cells. Another mechanism is the internal detoxification consisting of antioxidant defense system.

Cakmak and Horst, (1991) found the highest lipid peroxidation in the root tips of soybean at a longer duration of aluminium exposure. Major ROS scavenging enzymes in plants include SOD, APX and CAT (Bowler *et al*., 1992; Willekens *et al*., 1997; Mittler, 2002). 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). Significant difference in stress enzyme activity were observed between the treatments. Both aluminium and zinc treatments showed significantly increased SOD activity in *Lemna minor* plants, but induction of the enzyme was most conspicuous under higher zinc concentration increase in comparison with the control (Radic, *et al*., 2009).

Common responses of shoot and root to aluminium include cellular and structural changes, increased rate of diffusion resistance, reduction of stomatal aperture, decreased photosynthetic rate (Mossor-pietrazecoska, *et al*., 1997). According to Mossor-pietraszecoska, (2001) signal transduction pathway that transmits stress information within the cell and throughout the plant body due to aluminium stress as observed in yellow *Lupine*. A major consequence of aluminium toxicity is inhibition of root growth and this has been reported in numerous species (Ma *et al*.,2002). According to those authors, in roots of rye (*Secale cereale*) exposed to aluminium, cytoskeleton is the cellular target of toxicity due to the alternation in stability, organization and polymerization. According to Kikui *et al*., (2005) physiological and genetic analyses of aluminium tolerance in rice focusing on root growth during germination resulted in root growth inhibition and increase in root diameter.

According to Godbold, (1994) aluminium specifically affects cell division and cell elongation by disruption of DNA synthesis and displacement of calcium in the apoplast of cells subjected to heavy metals. Vazquez *et al*., (1999) put forth the hypothesis that in aluminium tolerant plants aluminium can rapidly cross the plasma membrane. This data clearly contradicts the former conclusion that aluminium mainly accumulates in the apoplast and enters the symplast only after severe cell damage has occurred.

Patch-Clamp study on the physiology of aluminium toxicity and tolerance resulted in the identification and characterization of Al^{3+} induced anion channels in maize (Pineros *et al*., 2001). Plasmamembrane 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, Sasak *et al*., (2004) recorded flow of malate currents from aluminium tolerant plants but not in aluminium sensitive plants.

Chromium

Chromium is the seventh most abundant element that occurs naturally in rocks, soil, plants and animals. Hexavalent chromium has been widely distributed by natural and anthropogenic sources characterized by higher toxicological and solubility properties than the trivalent form. Chromium is used in manufacturing stainless steel, dyes and pigments, leather tanning, paper and pulp production. Chromium is a toxic heavy metal which adversely affect growth and development of plants. Phytotoxicity due to chromium treatment have been investigated by many researchers on important crop plants and also on lower group plants (Panda and Patra,1998,2000; Han *et al*., 2004; Shanker *et al*., 2004, 2005: Panda and Choudhury, 2005). Effect of chromium on plants include alterations in morphology (Moral *et al*., 1995; Samantaray *et al*.,1996; Iqbal *et al*., 2001), biomass production (Shankar *et al*.,2005; Panda, 2007), biochemical pathway (Samantaray *et al*., 1996; Shankar *et al*., 2005), production of reactive oxygen species (Shankar *et al*., 2004), production of enzymic and non-enzymic antioxidants (Panda *et al*., 2003; Rai *et al*., 2004), bioaccumulation potential (Shankar *et al*., 2005; Yadav *et al*., 2005; Ratheeshchandra *et al*., 2010). Involvement of secondary metabolites as a consequence of chromium toxicity has been reported in *Phyllanthus amarus* (Rai and Mehrotra, 2008) and in *Plantago ovate* (kundu *et al*., 2018).

Corradi *et al., (*1993) recorded suppression of lateral roots in *Salvia sclarea* with a diminishing trend with the increase in the dose of chromium. According to those authors, lateral root production was completely inhibited at 10 ppm of Cr(VI) and high dose of Cr (60ppm) added to the plants resulted in the death of plants within three days of treatment in hydroponics as well as pot culture experiments.

Excessive content of chromium in the soil causes severe leaf necrosis and stunting of roots, which led to the death of *Eucalyptus* seedlings and yellowing

of leaves of *Acacia mangium* was also recorded due to excessive chromium and nickel content in the soil (Malajczuk and Dell,1995). Moral *et al*., (1995) and Samantaray *et al*., (1997) reported that chromium toxicity affected root length and damage the architecture of entire root. 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 chromium treatment (Iqbal *et al*., 2001).

Stem length, leaf area and biomass were reported to be inhibited by chromium (Tripathi *et al*., 1999). Treatment of cauliflower with chromium resulted in decreased water potential and reduction in trachea vessels diameter (Chatterjee and Chatterjee, 2000). According to Davies *et al*., (2002) although some crop plants are not affected by low concentration of chromium this metal is toxic to higher plants at 100mM/kg dry weight. According to Shanker *et al*., (2005) biomass production and yield were generally affected by chromium (Cr IV) in many plants. Biomass production was found to be reduced in *Oryza sativa* plants as a consequences of chromium toxicity (Panda,2007).

Chlorophyll synthesis was reported to be inhibited by chromium in *Nymphaea alba* (Vajpayee *et al*., 2000). Inhibition of chlorophyll b synthesis by the influence of chromium is well known in plants (Panda and Chowdhary,2005; Shanker *et al*., 2005). Due to chromium application mung bean plants showed severe stunted growth and leaf chlorosis (Rout *et al*., 1997). According to Samantaray *et al*., (1997) chromium reduces chlorophyll and carotenoid synthesis indirectly by the inhibition of iron and zinc transport to the leaves. Bera *et al*., (1999) studied the effect of chromium on chloroplast pigments content in mung bean and opined that irrespective of the concentration, chlorophyll a, chlorophyll b and total chlorophyll were decreased in six-day-old seedlings. Toxic effect of chromium has been shown by *Ocimum teneflorum* as reduction of photosynthetic pigments and protein (Rai *et al*., 2004). Panda and Choudhury (2005) suggested that oxidative stress induced by chromium-initiated degradation of

photosynthetic pigments causing decline in growth and high concentration of chromium disturbed chlorophyll ultra-structure and affected photosynthesis. Chromium stress has been reported as one of the important factors that affect photosynthesis (Shanker *et al*., 2005).

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). The authors opined that possibly due to the spatial localization in the specific sub cellular compartment in the root cells. Samantaray and Das (1997) reported the accumulation of chromium by mung bean plants up to 70 ppm in their roots when the plants were grown in chromate my waste. According to Pulford *et al*., (2001) chromium is poorly translocated to aerial parts and held predominating in the roots.

Distribution and bioaccumulation of cadmium and chromium are reported to be species specific in *Vigna* (Ratheesh Chandra *et al*., 2010). According to those authors both metals exert specific influences on the anatomy of root and stem and accumulation of chromium was very high both in root and stem compared to cadmium.

Chromium treatment is known to induce increased production of biomolecules such as glutathione and phytochelatins which may confer resistance or tolerance to chromium. Chromium has been demonstrated to stimulate formation of free radicals and reactive oxygen species such as super oxide radicals, hydrogen peroxide and hydroxyl radicals either by direct electron transfer involving metal cations or as a consequence of metal mediated inhibition of metabolic reactions and their presence cause oxidative damage to the biomolecules such as lipids, proteins and nucleic acids (Stohs and Bagchi,1995; Kanazawa *et al*., 2000). Pea plant exposed to chromium resulted in an increased activity of superoxide dismutase in root tissues (Dixit *et al*., 2002). In an excellent review on chromium toxicity in plants Panda and Choudhury, (2005) suggested

that decreased activity of catalase is an important effect of chromium. Inhibition of catalase activity due to chromium exposure was reported in wheat seedlings (Panda and Patra, 2000; Panda *et al*., 2003). Panda and Choudhury, (2005) further reported that chromium affects catalytic activities of antioxidant enzymes like superoxide dismutase, catalase, peroxidase and glutathione reductase.

According to Rai *et al*., (2004), hyperactivity of superoxide dismutase, guaiacol peroxidase and catalase have been reported in *Ocimum tenuiiflorum* treated with chromium salt as a measure of protecting the plant from chromium stress. Increased activity of catalase and peroxidase due to the exposure of two cultivars of *Brassica napus* to chromium revealed that these enzymes are increased proportional to the chromium concentration. But more activity of both the enzyme occurred in more resistant cultivars (Hosseini *et al*., 2007).

Panda (2007) opined that chromium induces oxidative stress in the root cells of rice seedlings. According to the author chromium translocation take place via membrane transporters like sulfate carriers. Generation of ROS like H_2O_2 and O_2^- indicative of lipid peroxidation and resultant increase of MDA also occur in plants due to chromium stress (Panda *et al*., 2003). Pandey *et al*., (2009) explains the production of ROS in *Pisum sativum* due to Chromium toxicity. Pea plants were exposed to different concentrations of chromium to investigate oxidative stress in isolated chloroplasts.

Synthesis of metallothionines (a metal ion binding protein) is reported as a means to detoxify chromium stress in plants. Yu *et al*., (2019) reported the involvement of metallothionines in metal chelation and ROS scavenging in rice seedlings under chromium treatment either Cr (VI) or Cr (III) at different effective concentrations. Results showed that concentration of chromium in all tissues was higher in the root than the shoot in both chromium treatments. Both chromium exposure resulted in enhancement of metallothionines synthesis in tissues. Results of PCR analysis on *Oryza sativa* confirmed that ten specific

metallothionines genes involved for regulating ROS removal and were expressed differently in plant tissues as well as chromium variants.

Involvement of ROS in chromium toxicity due to 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), *Tetrana qataranse* (Usman *et al*., 2019). Wakeel *et al*., (2020) exhaustively reviewed the role of chromium induced ROS in phytotoxicity in terms of ROS synthesis, enzymatic antioxidant system, lipid peroxidation, DNA damage and gene toxicity, ultrastructural changes in cellular and subcellular levels and photosynthetic changes in plants. Those authors empathetically stated that chromium induced ROS accumulation by altering the enzymatic antioxidant system and associated cytotoxic, genotoxic, ultrastructural and photosynthetic changes in plants.

Abid *et al*., (2019) reported that chromium enters the environment as Cr (III) and Cr (VI) latter being more toxic and highly mobile. The extent of damage caused by chromium on plants depends on its bioavailability, mobilization and subsequent accumulation in the tissues. The mechanism of chromium tolerance in plants is distinct and species specific (Abid *et al.,* 2019). Toxic effect of chromium can be mitigated in plants via special mechanisms that alleviate metal toxicity. The mechanisms involve hyperaccumulation of substantial amount of chromium in plants tissues, altered metabolic pathways and oxidative defense (Gill *et al*., 2015). Those authors further suggest that high levels of chromium in plants induces changes in morphology and physiology of plants due to enhanced generation of ROS.

Several studies showed that uptake of Cr (VI) is an active process and get transported by sulphate and phosphate carriers present in plants whereas

accumulation of Cr (III) is passive with zero energy requirements (Cervantes *et al*., 2001; Shanker *et al*., 2005; de Oliviera *et al*., 2014, 2016). Since chromium does not have any essential role in the metabolism of plants, no specific mechanism has been reported on plant chromium uptake in plants (Oliveira, 2012). Earlier, it has been suggested that mostly chromium is taken up by plants via carriers specific for the absorption of essential ions for plant metabolism and the ability of plants uptake chromium depends on plant type and chromium species (Gardea-Torresdey *et al*., 2004; Juneja and Prakash, 2005; Shukla *et al*., 2007). Since chromium is the least mobile element in plants the concentration of chromium in roots sometimes reach 100 times higher than the shoots (Shanker *et al*., 2005; Shukla *et al*., 2007). Caldelas *et al*., (2012) found the highest concentration of chromium in the cell wall of roots is the cytoplasm and intercellular spaces of rhizome in *Iris pseudacorus*. Liu *et al*., (2009) reported that cell wall fraction contained major portion chromium in the roots of *Leersia hexandra*.

According to Singh *et al*., (2013) chromium uses channels of iron (Fe) and Sulphur(S) for upward translocation which causes a competition among these metals. Moreover presence of Fe in the growth medium reduces Cr(VI) translocation by plants , which can be due to possible competition between the two metals for carrier channels or also due to precipitation of Fe oxides with chromium (Mallick *et al*., 2010). For phytoremediation, hyperaccumulator plants are used to extract metals from contaminated site and hyperaccumulator plants can accumulate high metal concentration in the above ground tissue having bioaccumulation and translocation factor more than one. Plants that accumulate and translocate chromium are less in number and chromium hyperaccumulators can accumulate more than 1000 mg Cr/Kg in shoot tissues (Redondo-Gomez *et al*., 2011). Some of the chromium hyperaccumulators include *Sparklina*

arrentinensis, Pulchea indica, (Sampanpanish *et al*., 2006), *Amaranthes blubies* (Mellem *et al*., 2012).

Chromium has been well reported to include noxious effects to several physiological and biological effects, consequently plant yield and bioavailability (Shanker *et al*., 2005; Singh *et al*., 2013). Those authors further stated that chromium induced -decrease in plant development, growth and yield can be due to reduced water and nutrient uptake, decrease in cell division, enhanced production of ROS substitution of essential nutrients and oxidative damage to metabolites.

Different strategies developed by plants against chromium toxicity include chelation of chromium with ligands, reduction of Cr(VI) to Cr(III), compartmentation of chromium 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).Antioxidant enzymes work in conjugation with each other to scavenge ROS. CAT generally localize in peroxisomes is an important antioxidant enzymes that governs the scavenging of H_2O_2 to O_2 and H_2O. In plants chromium can either activate or suppress CAT activity. Chromium induced increase in CAT activity has been reported in several plant species such as *Gossipium hirsutum* (Daud *et al*., 2014), *Triticum aestivum* (Address *et al*., 2015) etc.

SOD is an important enzyme which plays central role in scavenging ROS. This enzyme dismutase $2O_2^-$ radicals to O_2 and H_2O_2, and thereby controls steady state level of O_2^- in plant cells (Shahid *et al*., 2014). Chromium induces the activation of SOD and this may be due to an increase in O_2^- levels or direct action of SOD. Increase in SOD activity as a result of chromium toxicity has been reported in *Oryza sativa* (Panda, 2007), *Gossipium hirsutum* (Daud *et al*.,2014), *Brassica compestrus* (Qing *et al*.,2015).

Several physiological studies revealed the role of phytochelatins in the homeostasis and detoxification of toxic metals including chromium. Phytochelatins are among the most important plant protein molecules involved in detoxification of chromium in the plants (Shanker *et al*.,2005; Singh *et al*.,2013). Phytochelatins bind chromium and other heavy metals in the cytosol followed by their sequestration into vacuole (Liu *et al*.,2009). According to Diwan *et al.*, *(*2010), chromium toxicity induces phytochelatins both in shoot and root of the plants as reported chromium mediated induction of the protein in species *Vigna radiate* and *Brassica gentia* which plays an important role in chromium speciation effect.

According to Mahmoud and Samah (2017) Cr (VI) is a strong oxidizing agent and highly toxic to plants while Cr (III) is less toxic and it required in small quantities in biological systems for carbohydrate, lipid and protein metabolism. According to Shahid *et al*., (2017) chromium is a potentially toxic heavy metal which does not have any metabolic function in plants. An excellent review by Shahid *et al*., (2017) traces recent studies highlights on biogeochemistry of chromium in the soil plant systems.

Chromium (VI) is a strong oxidizing agent and highly toxic to plants (Mahmoud and Samah, 2017) while Cr (III) is less toxic and is required in small quantities in biological system for carbohydrate, lipid and protein metabolism (Bakiyaraj *et al*., 2014). Johnson *et al*., (2018) reported the reduction potential of *Coriandrum sativum* to reduce Cr (VI). FTIR analysis on this plant revealed the involvement of phenols, alcohols, X-hydroxy acids and flavonoids in the reduction of Cr (VI) to Cr (III). An exhaustive review by Babangida *et al*., (2021) explains and discusses important sources, distribution, chemistry, toxicity levels and bioremediational practices of Cr (VI). Reduction of Cr (VI) to Cr (III) using chemical reductants or chromium reduction has received much attention in recent

years (Silvio *et al.*,2016; Karthik *et al*.,2017). A major disadvantage of chemical reduction is the addition of secondary sources of contaminants. Hence an alternative treatment for Cr (VI) reduction was proposed recently (Ali and Ghasemzadeh, 2011; Navnidhi *et al*.,2018). Antioxidants is considered as one of the best group of compounds for chromium reduction. Ghasemzadeh and Jaafar, (2011) recommended that phenolics can be considered as an efficient reducing agent for Cr (VI) remediation. According to Navnidhi *et al*., (2018) phenolics and flavonoids occurring commonly in edible and nonedible plants containing antioxidants and their activities are mainly due to redox property. Application of antioxidants and flavonoid molecules is very reasonable owing to the fact that such molecules are biodegradable. Therefore antioxidant compounds with high potential to donate protons is beneficial in converting Cr (VI) to Cr (III). Efficient reduction of Cr (VI) to Cr (III) has been reported in plants like sugarcane, *Moringa oliefera* and mango leaves in acidic pH. The utilization of antioxidants in Cr (VI) reduction compared to the use of chemical reductants have been practiced for Cr (VI) remediation. The natural antioxidants and plant extracts are liable to degradation by microorganisms or by physical process. Advance in nanoscale technology suggested that current problems involving Cr pollution could be resolved by using antioxidant biomaterial which provides benign alternatives to the use of chemical reductants, for the conversion of Cr(VI) to Cr (III) to alleviate chromium toxicity (Mamadou and Savage, 2005; Amit *et al*., 2011). (Mamadou and Savage, 2005; Amit *et al*., 2011). Nanoparticles are having the potential to provide environmental protection against toxic contaminants. They also provide ability and controlled release their content for long last effectiveness. Therefore nanotechnology has been employed in the field of organic reductants. Numerous reports have been published on Cr (VI) reduction using micro and nanoparticles with great success (Babangida *et al*.,2021).

Plant species such as, *Phragmitis karka, Scirpus lacustris* and *Bacopa monnieri* exhibit high potential to absorb, translocate and concentrate chromium in their tissues (Yadav *et al*., 2005). Those authors further stated that about 99% of the absorbed chromium is retained in the root tissue because most plants show low chromium concentration in the shoot tissue even when grown in chromium rich soil. So the food chain is well protected against the chromium toxicity. Notwithstanding, Shanker *et al*., (2005) reported a characteristic of *Albizia amara* as a potential chromium accumulator and recommended the plant for phytoremediation. Recently, *Eichhornia crassipes* is used for removal of Cr (VI) ions from polluted waste water and hence *Eichhornia crassipes* is considered as a means of phytoremediation (Kumar and Chauhan, 2019; Zelekew *et al*., 2022)

Mercury

As per ATSDR (Agency Toxic Substance and Disease registry) the four elements arsenic, lead, mercury and cadmium rank first, second, third and seventh respectively. Mercury has always been reported to be more toxic to plants compared to other heavy metals like cadmium(Fergusson,1990; Kneer and Zenk,1992; Gadallah,1994; Shaw,1995), chromium (Chandra and Garg,1992; Garg *et al.*,1994) and lead (Huang *et al*.,1987; De Grado *et al*.,1990; Xiong,1999; Orcutt and Nilsen,2000).

Mercury is an industrial heavy metal toxicant that causes phytotoxic effects. Even though angiosperms have not yet been reported to tolerant to mercury, some plants absorb and accumulate considerable quantity of mercury when grown in contaminated soil due to geogenic, anthropogenic and industrial activities (Lepp,1981; Ross,1994; Orcutt and Nilson,2000; Cseh,2002). Studies on phytotoxic effect of mercury have been mainly centered on tolerance mechanism, accumulation pattern and detoxification methods (Valasco-Alinsug *et al*., 2005). According to Hendry (2000) and Raskin and Ensley, (2000) most

plants are sensitive to Hg^{2+} toxicity, but no plants have been identified as mercury accumulators. Notwithstanding, earlier studies by Lenka *et al*., (1993) for biomonitoring purpose, two grasses *Chloris barbata* and *Cyperus rotundus* were identified from a mercury polluted area near chlor-alkali plant where mercury contamination was as high as 557 mg/kg soil and according to those authors these plant exhibited moderate tolerance towards mercury *Chloris barbata* being more tolerant.

Toxicity of mercury has been reported in many plants and in very low concentrations mercury causes hazards to plant growth (Vallee and Ulmer., 1972; Sandmann and Boger, 1983; Kagi and Hapke,1984; Baker *et al*., 1985; De *et al*.,1985). Various forms of growth retardation and physiological changes has been reported due to mercury toxicity (Nag *et al.*, 1980). In *Cyperus rotundus* and *Chloris barbata* root growth inhibition has been reported due to mercury treatment and the rate of inhibition increased with the increase in concentration of mercury (Lenka *et al*., 1993). Maitani *et al*., (1996) observed in reduction in the relative root elongation of *Rubia tinctorium* in root cultures when treated with 10µM mercury.

Mercury enters plants as inorganic forms from the soil or water or from the process of methylation that occurs in plants. According to Woolhouse (1983) mercury after being absorbed from the soil remains deposited mostly in root tissues. 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), one of the mechanisms by which mercury exerts its toxic effects is by interaction with essential –SH group of enzymes and structural proteins.

Mercury is an inhibitor of enzymes in biological system and all mercurial compounds are toxic to plants (Baker and Walker, 1989; Reed and Gatt,1990). Brzgska *et al*., (1991), reported that mercury inhibit both the soluble and

immobilized enzyme activity at low concentration (0.2mM) by 20 to 50% of the activity.

Boening., (2000) reviewed ecological effects, transport and fate of mercury. Suszcynsky and Shann (1995) conducted an experiment on the absorption, phytotoxicity and internal distribution of mercury in tobacco plants. Root exposed plants showed accumulation of mercury in the roots with movement to the shoots by day 10. Inhibition of root and shoot growth also occurred. In higher plants exposure to mercury reduced photosynthesis and transpiration, water uptake and chlorophyll synthesis (Godbold and Huttermann,1986). In Spruce (*Picea abies*) inorganic mercury affects the plasma membrane, methyl mercury may primarily affect organelle metabolism in the cytoplasm which subsequently affects membrane integrity. Mercury induced root damage may have serious consequences for nutrient and water supply to above ground plant parts (Godbold and Huttermann,1986).

Shaw (1995) reported that *Phaseolus aureus* when exposed to mercuric chloride showed enhanced lipid peroxidation and activities of antioxidative enzymes. Mishra and Choudhuri (1999) investigated the membrane damage caused by mercury in two rice cultivars and observed an increase in the activity of lipogenase and MDA due to treatment with both the heavy metals. Mercury increased the activity of peroxidase and the level of hydrogen peroxide while decreased the activity of SOD and CAT. An increase in leakage of electrolytes was also observed. The authors concluded that mercury caused membrane damage in *Oryza sativa* which was mediated by reactive oxygen species and hydrogen peroxide induced by this metal.

Studies on effect of mercury on growth of *Phaseolus vulgaris* seedlings revealed that mercury inhibited root and hypocotyl elongation and decreased chlorophyll content (Parmar *et al*.,2002). Peroxidase activity was higher in

mercury treated seedlings compared to control. According to Valsco-Alinsug (2005) *Chromolena odorata* is one of the most dominant species accumulating highly toxic level of mercury. Rooted cuttings of *Chromolena odorata* were subjected to mercury treatment and among the three vegetative organs, roots accumulated the highest level of mercury compared to the stem and leaves. Mercury binding peptides from roots, stem, and leaves of mercury treated *Chromolaena odorata* were isolated and partially characterized using RP-HPLC and ESI-MS (Valasco-Alinsug *et al.*,2005). The plant's ability to accumulate and sequester Hg ions was primarily attributed to the production of Hg- binding peptides.

Toxicity of mercury induce oxidative stress was studied in growing cucumber seedlings Cargnelutti *et al*., (2006). In this study, the effects of exogenous mercury on time dependent changes in the activities of antioxidant enzymes, lipid peroxidation, chlorophyll content and protein oxidation in cucumber seedlings were investigated. An important feature of Mercury toxicity is the generation of free radicals. The generation of Reactive Oxygen Species such as the superoxide anion, singlet oxygen, hydrogen peroxide and hydroxyl radical have been proven to be one of the underlying agents in the origin of tissue injury after the exposure of plants to a wide variety of stressful conditions such as drought, heat, chilling, high light intensity, UV radiation, heavy metals, various organic chemicals and air pollutants (Cho and Park,2000)

Memon *et al*., (2001) excellently reviewed heavy metal accumulation and detoxification mechanisms in plants. Mercury is among the most hazardous of the heavy metals and its pollution is regarded as one of the most serious environmental problems (Rugh *et al*.,1998: Bizly *et al*.,1997). Elemental mercury and mercury ions are released into the environment as a result of gold mining, industry, burning fossil fuels and medical waste. Once in the environment, these

forms of mercury are converted by sulphate reducing bacteria to the extremely toxic compound methylmercury, which bioaccumulates in the food chain. According to Rugh *et al*., (1998) organomercurials are 1-2 orders of magnitude more toxic in some eukaryotes and are more likely to bio magnify across tropic levels than ionic mercury. The biophysical behavior of organic mercury is thought to be due to its hydrophobicity and efficient membrane permeability.

Zhang and Tyeman (1999) reported mercury inhibited water uptake through aquaporins in plasma membrane of wheat. Higher concentration of mercury causes impaired photosynthesis and transpiration resulting in decreased growth (Chou and Park,2000; Basak *et al*.,2001). Consequences of mercury on enzymes in general and that of nitrogen metabolism in particular have been studied by several authors such as nitrate reductase (Vyas and Puran,1999), Glutamate dehydrogenase (Basak, 2001, Auvert *et al*.,2001; Aswathy *et al*.,2005). Toxic effect of mercury induces growth retardation due to impaired mineral nutrition uptake resulting in decreased chlorophyll content, enzyme activity and transpiration rate (Zhou *et al*.,2009).

Phytochelatins are most abundant class III metallothionines produced in higher plants due to Hg^{2+} exposure (Maitani *et al*.,1996). Those authors suggested that, since mercury II has a linear configuration in co-ordination compounds, phytochelatins can effectively protect plants against the Hg^{2+} toxicity. Reduced glutathione (GSH) in the predominant free thiol present in plants and the concentration of GSH in plant cells is modified by developmental and environmental factors such as heavy metals. Cell culture studies have indicated that GSH is the precursor for the synthesis of heavy metal binding phytochelatins (Grill *et al*.,1987; Rauser,1987; Scheller *et al*., 1987; Obata *et al*.,1994). According to Tukendorf and Rauser (1990) and De Vos *et al*., (1992) accumulation of phytochelatins is associated with decline in GSH. Jain and

Puranik, (1993) reported that supply of 0.01 to 0.1mM reduced glutathione to excised green maize leaf segments prevented the inhibitory effect of mercury on chlorophyll biosynthesis and the supply of other thiols such as dithiothretol, cysteine and mercaptoethanol also reduced this inhibition of chlorophyll formation by mercury. Increased content of malondialdehyde in plants treated with mercury was reported due to the inhibitor of the enzymes of photosynthetic carbon reduction (PCR) cycle (Sheoran *et al.*,1990; Van Assehe and Chiysters,1990). According to Prasad *et al*., (1991) mercury has got direct effect on photosynthetic electron transport causing generation of singlet of oxygen and superoxide radicals. Shaw (1995) reported that *Phaseolus arvens* when exposed to mercuric chloride showed enhanced lipid per oxidate and activities of antioxidant enzymes- Catalase, guaiacol peroxidase and ascorbate peroxidase.

Mercury inactivates GSH enzyme by binding to thiol (-SH) groups of protein and since GSH serves as a precursor of phytochelatins and mercury forms an inhibitor of key enzyme-y glutamyl cysteine synthase pathway (Jott and Subadera, 2010).

Zinc

As per the classification of plant mineral nutrients according to biochemical functions zinc comes under group III in which the mineral nutrients remain ionic form (Taiz *et al*.,2015). Zinc is a constituent of enzymes such as alcohol dehydrogenase, glutamic dehydrogenase, carbonic anhydrase etc. Many enzymes require zinc ions for their activity. Zinc is reported to be essential for chlorophyll biosynthesis in some plants. Zinc is typically the second most abundant transition metal in organism after iron and the only metal represented in all the six enzyme classes (Webb,1922). Zinc is the most common crop micronutrient and the role of excess and deficient zinc content in crop plants have been elaborately investigated and reported (Cakmak,2002; Alloway,2004). Zinc

is an essential component of thousands of proteins in plants, although it is toxic to plants in excess (Taiz *et al*.,2015). Zinc is an essential element for plant metabolism and growth. It is constituent of metalloenzymes or a cofactor for several enzymes such as anhydrases, dehydrogenases, oxidases and peroxidases and plays an important role in regulating nitrogen metabolism, cell multiplication, photosynthesis and auxin synthesis in plants (Palmer *et al*.,2009). Zinc also plays an important role in the synthesis of nucleic acids and proteins and help in the utilization of phosphorus and nitrogen during seed formation. While zinc is crucial for the above-mentioned processes, high levels of uncomplexed zinc are toxic to plants. Zinc is associated with the blockage of xylem elements and the inhibition of photosynthesis through alteration of electron transport and the capacity of Rubisco to fix CO_2 (Mateos-Naranjo *et al*.,2008).

Many plants inclusive of crops are zinc tolerant (Mathys,1977) according to whom in zinc tolerant plants *Silena vulgaris* the activity of metabolic enzymes is very higher level of Zn^{2+} is essential for the activity. Nutrient solutions with increased Zinc concentration inhibit the entry of other essential metals. Increased zinc concentration interferes the uptake and translocation of Fe by soyabean in nutrient solution explained by Ambler *et al*., (1971). According to Van Steveninck *et al*.,(1987) the zinc tolerant grass *Deschampsia caespitosa* formed specialized vacuoles in root cortex cells which accumulated high levels of zinc and appear to explain zinc tolerance by this ecotype. Malate a carboxylic group ligand with high binding affinity for zinc was proposed as a cytosolic Zn chelator in zinc tolerant plants (Mathys.,1977).

In plants, heavy metals induce the synthesis of metal binding peptides or phytochelatins which are considered to play an important role in cellular metal homeostasis (Rauser,1990; Steffens,1990; Grill *et al*.,1985). Among the metals, zinc appears to be a weak inducer requiring high external levels for induction of

phytochelatin synthesis (Steffens,1990). According to Harmens *et al*., (1993) in a study to elucidate the role of thiols in the synthesis of phytochelatins in *Silena vulgaris* plants both sensitive and tolerant to zinc in different concentration of zinc revealed increased concentration of –SH, -GSH and also non identified thiols. Those authors stated the cysteine concentration was increased equally in the roots of sensitive and tolerant plants, but accumulation of phytochelatin was same in both and it was concluded that increased zinc tolerance in *Silena vulgaris* is not due to increased production of phytochelatins.

Rosolem *et al*., (2005) stated that zinc in the growth medium resulted in the reduction of total area of the root, diameter of vascular bundles, number of xylem strands and the area they occupied in the stele in coffee plant compared to the control.

Reichman., (2001) explained the tolerance of Australian native plants- *Acacia holosericea*, *Eucalyptus camaldulensis* and *Melaleuca leucadendra* to high concentration of Zinc. *Acacia* showed growth reductions in the higher zinc treatments. In *Eucalyptus* chlorophyll found to be reduced and some reddening of the main vein of some younger leaves occurred. In *Melaleuca* the most conspicuous and wide spread symptom of toxicity was bronzing of affected leaves accompanied by tip necrosis and inward curling starting with the younger leaves but progressing down the plant with time.

Phytochelatins do not have much important role in binding Zn^{2+} ions in hyper accumulator plants (Kupper *et al*.,2000,2004). Studies employing GSH biosynthetic inhibitor buthionine sulfoximine suggested an increase in the level of phytochelatins and maintenance of GSH homeostasis in transgenic plants during exposure to excess zinc as the possible mechanism behind the tolerance potential (Singla Pareek,2006). Even though phytochelatins are reported as an essential component for the detoxification of non-essential metals and metalloids

such as cadmium and arsenic in plants, no direct evidence for a role of phytochelatins in essential metal homeostasis has been reported to date (Tennstedt *et al*.,2009). According to those authors phytochelatins formation is essential for zinc tolerance and provides driving force for the accumulation of zinc. Those authors further attributed that the function of phytochelatins may be due to the mysterious occurrence of phytochelatins synthase gene throughout the plant kingdom.

Zinc metal transporters have been identified in many plants such as *Arabidopsis thaliana, Oryza sativa, Arabidopsis halleri, Nicotiana tabacum* etc. (Memon and Schroder,2009). An excellent review on uptake, translocation and accumulation of zinc (Broadley *et al*.,2007) interprets the genetic strategies to address the wide spread problem of zinc-limited crop growth, genetic species variation and insights into the evolutionary potential of plants to respond to elevated levels of Zn^{2+} in terms of anatomical, physiological, chemical, genetic and molecular characterization of zinc hyperaccumulators such as *Thalapsi caerulesens* and *Thalapsi arvense* and *Arabidopsis halleri* (Broadley, *et al*.,2007).

A comparative study on root anatomy of zinc accumulator *Thlaspi caerulescens* and non-hyperaccumulator *Thlaspi arvense* registered remarkable difference between the structure of root in these two species (Zelko *et al*.,2008). In *Thlaspi caerulescens* the endodermal layer of cell exhibited thickened Casperian bands in a region from less than 1mm from the root tip and suberin lamellar are formed in all endodermal cells of a region 5-6 mm from the apex. In *T.arvense* Casparian bands developed from a region 2mm from the root tip and suberin lamella are formed in all endodermal cells greater than 10mm from the root tip. In *T.caerulescens* the endodermal cells with thickened inner tangential walls which is composed of secondary walls impregnated with suberin-lignin,

forming a compact cylinder surrounding the endodermis from the outer side whereas this layer is not seen in *T.arvense* . Based on the study on *T.caerulescens* and *T.arvense*, Zelko, *et al*., (2008) opined that zinc accumulation potential of plants are species specific and diverse root anatomy is integrally correlated with zinc accumulation pattern.

Mishra *et al*., (2020) focused on the concentration and speciation of zinc in different edible plants grown in soils contaminated with smelter wastes containing high levels of metals zinc, copper, lead, cadmium and their accumulation was different in plant parts such as root, stem and leaves. According to those authors although the accumulation of metals varied significantly with plant species, the average metal concentrations were Zn>Pb>Cu>Cd. The speciation and distribution of zinc in these plants showed a dynamic interplay between the histine and malate complexation of zinc in all plant species.

According to White and Broadley, (2009) zinc is transported within the plant as Zn^{2+} or complexed with proteins, amino acids and organic acids. In the xylem translocation of zinc occurs mainly as Zn^{2+} or complexed with histidine or nicotianamine (NA) while in the phloem zinc is mostly complexed with small proteins and NA. Zinc reaches storage tissues via xylem and phloem.

Common zinc toxicity symptoms in plants are stunted growth of shoots, curling and rolling of young leaves, death of leaf tips and chlorosis. To deal with toxic levels of zinc, plants possess a range of potential detoxification mechanisms for zinc homeostasis that allow uptake and distribution of zinc to tissues while maintaining zinc within cells or subcellular compartments below toxic levels (Clemens.,2002: Hall *et al*.,2002: Haydon and Cobbett.,2007). Tolerance mechanisms for zinc include restricted uptake, chelation by organic acids and polypeptides and isolation in vacuoles (Orcutt and Nilsen.,2000: Broadley *et al*.,2007). In addition to accumulation in vacuoles complexation of metals by

carboxylic and amino acids has been suggested to play an important role in tolerance and detoxification of heavy metals (Rauser.,1999; Clemens.,2001). According to Rauser, (1999), because of the high affinity of zinc with S, N and O carboxylic and amino acids are potential ligands for zinc complexation and their availability can aid detoxification.

An exhaustive review on "Implications of metal accumulation mechanisms to phytoremediation" was done by Memon and Schroder, (2009) interprets heavy metal accumulation pattern, characteristics of phytoremediation technique, transport/tolerance mechanism and analysis of genetic aspects of bioaccumulation. Plants exhibit different strategies to cope with fluctuations in the environment in order to minimize the adverse effects of metal toxicity

Mechanism of zinc absorption in plants: uptake, transport, translocation and accumulation were integrally narrated by Gupta *at al*., (2016). Roots absorb zinc as divalent zinc ionic form. Like all the nutrients the need for zinc by plant is different. As per the genetic makeup and physiological need for zinc, plants increase the solubility and absorption of zinc ions from soil, by changing the rhizosphere. In plant's exudate, organic acids like citric acid, malic acid, oxalic acid, actively absorb zinc by hyperpolarizing root cell plasma membrane with the aid of ATP's (Gupta *et al*.,2016). According to those authors, zinc absorption is a complex physiological trait which is mainly governed by zinc transporters and metal chelators of plant system. Plant growth stage, edaphic factors, season etc. also influence zinc efficiency of particular species. Molecular studies in zinc hyperaccumulators have already demonstrated the participation of specific zinc transporters, vacuolar sequestration and detoxification mechanisms in maintenance of zinc homeostasis. Within the plant, transporter proteins are necessary for translocation of zinc. These proteins play essential role in translocation of zinc ions in and out of the cell microcompartments, sequestering

these ions in vacuoles to act as a reservoir to reduce toxicity. Mainly three transporters' systems like ZIP, ZRT, IRT like proteins are seen on plasma membrane. The CDF's (Cation diffusion facilitator) like MTP (Metal tolerance proteins) seen on tonoplast and as heavy metal ATP ase, P-type ATP ase localized on the plasma membrane, tonoplast and endomembrane system are elucidated in plants (Caroli *et al*.,2020). According to Caroli *et al*., (2020), zinc induces endomembrane reorganization for compartmentalization and secretory functions to maintain homeostasis in plants.

Exposure to heavy metals-one of the important components of pollutants causes increased synthesis of secondary metabolite accumulation in plants in general and medicinal plants in particular (Rai *et al*.,2004).

Zinc is one of the essential micronutrients that is required in a specific amount for the proper function of physiological processes. Nevertheless, as per the phytotoxicity studies on metals, it is known that zinc is a major environmental pollutant (Orcutt and Nilsen, 2000: Alloway, 2008; Azzi *et al*., 2015; Saha *et al*., 2015). According to Nasiri *et al.*, (2010) zinc treatment enhanced secondary metabolites especially essential oils in *Matricaria chamomilla*. In *Lepidium sativum* and *Beta vulgaris*, the synthesis of secondary metabolites (Zepidine and Betaline) was enhanced as a result of zinc treatment (Savitha *et al*.,2006).Stimulation of secondary metabolites, synthesis and accumulation in medicinal plants is strongly influenced by factors like plant growth stage, concentration and duration of treatment and composition of growth medium (Nasim and Dhir, 2010).

Increased content of alkaloids in the roots of *Catharanthus roseus* subjected to cadmium, manganese and nickel treatments was reported by Srivastava and Srivasthava (2010). According to those authors cadmium and

nickel treatments resulted in two fold increase in serpentine content in the roots whereas lead treatment resulted in three fold increase of serpentine.

Mukta *et al*., (2019) stated that in order to mitigate and reduce negative impact of chromium stress in rice plants, effect of exogenous supplementary calcium can be used. Those authors further suggested that calcium function as signaling molecules at cellular level and growth inhibition. Protein content and membrane stability were found to be restored due to Ca^{2+} ions. Sequestration of chromium was reported in *Sulam hytoperia* subjected to treatments with calcium and sulfur (Singh *et al.*, 2020). According to those authors, calcium and sulfur stimulate the vascular sequestration of Cr (VI) and reduce its absorption to the cell walls.

Bioactive secondary metabolites of plants in general and medicinal plant in particular analyzed by GCMS study have reported to possess different types of phytochemical potential. *Strobilanthes alternata* control plants contained 15 secondary metabolites namely were Neophytadine, 3,7,11,15 Tetramethyl 2 hexadecen 1ol, Methyl palmitate, Methyl octadeca 9,12 dienoate, Methyl laurate, Methyl myristate, Hexahydrofarnesyl acetone, 9-octadecenoic acid, Isophytol, 2,5-Dimethyl 4 hexen 3 ol, Linolenic acid, Phytol, Methyl stearate, 1,2-Benzenedicarboxylic acid, and Squalene.

Secondary metabolites have been analyzed and screened in several medicinal plants such as *Erythrina sandwicensis* (Saidu *et al*.,2000), *Helichrysum* (Aiyegoro and Okoh,2010), (Yadav *et al*., 2014), *Clerodendrum inerma* (Thilagavathi *et al.,* 2015), *Ocimum tenuiflorum* (Mousavi *et al*., 2018), Premna (Gomathipriyadharshini *et al*., 2019). Majority of the identified secondary metabolites are found to be of medicinal properties. GC-MS analysis of *Baccopa monerrii* revealed qualitative and quantitative distribution of saponins (Phrompittayarat *et al*.,2011). Singh and Aeri (2013) analyzed secondary

metabolites of *Acanthes ilicifolius* and reported components like saponins, tannins, alkaloids, flavonoids and triterpenoids. *Crotton bonplanbianum* plants revealed the occurrence of many secondary components possessing antibacterial, antitubercular and anticytotoxic activity by GC-MS studies. According to Abid and Touquer (2015) phytochemicals of *Breynia disticha* include antimicrobial and antioxidant activities in methanolic extracts. Tamboli *et al*., (2018) compared the presence of phytoconstituents like alkaloids, phenolics, saponins and pyrenoids in natural and micro propagated from *Bacopa* plants. Almost all studies using GC-MS to analyze bioactive components are centered around the identification and estimation of bioactive secondary metabolites of plants in general and medicinal plants in particular. GC-MS analyses of phytochemicals and potentially bioactive components of many medicinal plants have been reported in plants like *Moringa oliffera* (Igwe *et al*.,2015), *Euphorbia kansuii* (Yu *et al*.,2005), *Cassia italica* (Sermakram and *thangapandian*,(2011), *Artimesia princeps* (Ryu *et al*.,2011), *Moringa peregram* (Al-owasn *et al*.,2014), *Vernonia elangifolia* and *Bregaria disticha* (Abid and Touquer (2015), *Huru crepitans* (Igwe *et al*.,2016), *Ipomea violaeae* (Mereuro *et al*.,2017), Equisetum arvanse (Balogun et al.,2017), *Bacopa monnerri* (Tamboli *et al*.,2018), *Homolomena aromatic* (Roy *et al*.,2019).

Terpenoid compounds identified in *Strobilanthes alternata* include neophytadine, hexahydrofarnesyl acetone, squalene, isophytol and phytol. Terpenoids play vital role in anti-inflammatory effects as well as aid defense against environmental stresses (Prakash, 2017; Yazaki *et al*., 2017).

According to Ichihara and Fukubayashi (2010), fatty acids are the major component of lipids and the physical, chemical and physiological properties of lipids primarily depends on its fatty acid composition. The fatty acid composition is determined as the fatty acid methyl esters by Gas-liquid chromatography.

Occurrence of Fatty acids methyl esters have been reported from vegetable oils of soyabean, corn and sunflower (Pinto *et al*.,2017). Their study was focused on evaluating antifungal activity as well as testing the interaction of these compounds with commercial antifungal drugs and also their antioxidant potential. GC-MS analysis revealed the occurrence of 83-88% of unsaturated fatty acids whereas saturated fatty acids are found to be less. Methyl palmitate was abundant among the saturated fatty acids. Antioxidant potential is measured using DPPH assay. Pinto *et al*., (2017) elaborately investigated the effect of antifungal activity in number of fungal species and the antifungal activity showed significant variation among the fatty acid methyl esters of sunflower oil, corn oil and soyabean oil and varies according to fungal species. Antioxidant property of squalene was studied in epithelial tissues. Squalene molecule is highly abundant in skin which is the target tissue exposed to different environmental stresses leading to oxidative stress such as pollutants, photooxidation and UV light (Mishra *et al*.,2020). Aioin *et al*., (1995) showed the scavenging activity of squalene on super oxides (ROS) formation in the Kerathionocytes exposed to oxidative stressors and suggested a protective role of squalene that acts in combination with SOD. The potential of squalene as a topical skin lubricant and protectant and also can be correlated to wound healing in accordance with the view of Mishra *et al*., (2020).

A review by Anjitha *et al*., (2021) analysed and interpreted the role of secondary metabolites as metal precipitators, antioxidants and/or metal chelators in plants growing under environmental conditions contaminated with toxic metals. An important aspect of toxicity/influence of heavy metals is induction of secondary metabolites. In *Oryza sativa* toxicity of chromium is found to produce Procatechuic, p-hydroxybenzoic, vanillic, p-coumaric, caffeic and gallic acids (Dubey *et al*., 2018).

MATERIALS AND METHODS

CHOICE OF PLANT

Strobilanthes alternata (Burm.F.) Moylan Ex. J.R.I.Wood plants growing in the botanical garden, SNGS College, Pattambi. The twigs consisting of 7-8 unfolded leaves and approximately 15 cm length were collected.

CULTIVATION

Earthen pots half filled with potting mixture were used for cultivation. Cuttings were planted and irrigated with water and maintained under greenhouse condition. Most profusely growing plants were selected for nutrient culture studies. Healthy plants were maintained for the availability throughout the period of experimentation. Healthy cuttings of length 15-18 cm consisting of 3-4 nodes and approximately 8 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 Zn ($ZnSO_4$).

Composition and preparation of nutrient solution (Hoagland solution.)

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

Table 1. Composition of nutrient solution employed in the present study

Compounds	Molecular weight	Concentration of stock solution	Concentration of stock solution	Volume of stock solution per liter final solution
	(g mol^{-1})	(mM)	(g L^{-1})	(mL)
KNO3	101.10	1,000	101.10	6.0
$Ca(NO_3)_2$ $4H_2O$	236.16	1,000	236.16	4.0
$NH_4H_2PO_4$	115.08	1,000	115.08	2.0
$MgSO_4.H_2O$	246.48	1,000	246.49	1.0
KCl	74.55	25	1.864	2.0
H_3BO_3	61.83	12.5	0.773	2.0
$MnSO_4.H_2O$	169.01	1.0	0.169	2.0
$ZnSO_4.7H_2O$	287.54	1.0	0.288	2.0
$CuSO_4.5H_2O$	249.68	0.25	0.062	2.0
H_2MoO_4	161.97	0.25	0.040	2.0
NaFeEDTA	558.50	53.7	30.0	0.3

Treatment with heavy metals

One Molar stock solution of heavy metal salts- aluminium, chromium, mercury and zinc were prepared and required dilutions were made. The optimal concentrations were determined by trial and error method. Rooted propagules were grown in different concentrations. Concentrations of these metals to impart about 50% growth retardation symptoms maintaining the survival of the plant was determined and following concentrations were selected for further experiments

Aluminium chloride ($AlCl_3$) - 400µM Al

Potassium dichromate ($K_2Cr_2O_7$) - 70µM Cr.

Mercuric chloride ($HgCl_2$) - 20µM Hg.

Zinc sulphate ($ZnSO_4$) -250µM Zn

Experimental setup

Fifty milliliter of each of the solution was taken in glass culture tube consisting of 25×150 mm size. Rooted cuttings (1 number) were planted in one culture tube containing 50 ml of Hoagland solution to which the heavy metal solutions were added to obtain the final standardized concentrations. The

hydroponic system was maintained under greenhouse conditions. Plants cultivated in Hoagland solution without any heavy metal salt served as the control.

Sampling

Cultured plants of treatments and control were collected at comparable interval of 4 days up to 20 days of growth. At each interval on 0, 4,8,12,16 and 20 days, five plants were sampled at each interval. Plants harvested from each treatment and control were washed thoroughly in distilled water and blotted to dryness. A minimum of 5 plants of each treatments were cut to separate root, stem, leaf and each were cut into pieces, randomized and sampled in duplicates for each analysis.

MORPHOLOGICAL PARAMETERS

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

ROOT LENGTH, STEM LENGTH AND LEAF AREA

The sampled propagules were washed in distilled water, blotted and length of root and stem were measured manually using a graduated scale. Leaf area was measured using graph paper.

TOLERANCE INDEX PERCENTAGE

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

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

STOMATAL INDEX

Stomatal density on abaxial and adaxial sides of the leaf was counted under a light microscope, by using nail polish impressions of leaf surface.

Stomatal index was calculated according to the method of Meidner and Mansfield (1968)

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

ANATOMICAL STUDIES

For anatomical studies, uniformly cut pieces of root, stem and leaf of control and treatments were collected on 20th day and fixed in FAA. Free hand sections were taken and sections were stained with Toluidine blue according to the procedure of Khasim (2002) 0.5% of Toluidine blue prepared with distilled water for the staining purpose. Stained sections were observed using Leica DM 1000 microscope and photographs were taken using DFC 295 Camera.

SEM-EDX ANALYSIS

For scanning electron microscopic study leaf segments as well as their cross sections of root, stem and leaves were fixed in 2.5% glutaraldehyde, made on 0.1M phosphate buffer (pH 6.9) for overnight. Fixed specimens were rinsed twice in distilled water and dehydrated in steps by passing through ascending alcohol series. Ten minutes incubation time was provided for incubation in each alcohol series. Dried specimens were mounted onto grooves cut on aluminium stubs using double side adhesive conducting carbon tapes to expose the sections. Then the specimens were gold coated and further photomicrographs were taken using the photographic attachment of the Scanning electron microscope. (Make; JEOL Model JSM-6390LVEDS Resolution: 3nm (Acc V 30KV, WD 8mm, SEI): 8nm (Acc V 3.0 KV, WD 6mm, SEI):15 nm (Acc V 1.0 KV, WD 6mm, SEI) Magnification:5X to 300,000X). Energy dispersive X-Ray

Analysis (EDX) technique used to identify the elemental composition of materials.

PHYSIOLOGICAL AND BIOCHEMICAL STUDIES

DRY WEIGHT

Suitable quantities of root, stem and leaves collected and processed as described earlier were weighed in pre weighed containers using electronic balance. Fresh weight obtained were recorded and the weighed samples were then placed in hot air oven at 100^0C for one hour followed by at 60^0C for overnight. Dry weight of each samples was taken on the next day and drying and weighing were repeated until values become constant.

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

ESTIMATION OF PIGMENT COMPOSITION OF THE LEAF

Estimation of chlorophyll and carotenoids pigments were done according to Arnon (1949). Fresh leaves of control as well as experimental were washed with water and blotted between sheets of filter paper. To estimate chlorophyll and carotenoids,80% acetone was used as the extracting medium.

One hundred milligram fresh leaf sample was weighed in an electronic balance and crushed using mortar and pestle in 20 ml of 80%acetone (v/v). Then the homogenate was centrifuged at 5000 rpm for 10 minutes and the supernatant was collected. The residue was re extracted with 80%acetone and centrifuged. The process was repeated till the pellet become colorless. The final volume of the pooled supernatant was noted. The absorbance of extract was read at 663 nm,646 nm,750 nm and 470 nm against the solvent blank (80% acetone). Then quantity of chlorophyll and carotenoids present in the extract

was calculated as µg chlorophyll and carotenoids per gram dry weight using the following formula.

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

Chlorophyll b µg/g fresh weight=22.9(A663)-4.68(A663) V/1000×W

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

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

Where, A663 – Absorbance at 663 nm

A645- Absorbance at 645 nm

V – Volume of extract

W- Weight of tissue

ESTIMATION OF TOTAL PROTEIN

Total protein content of *Strobilanthes alternata* samples was estimated according to Lowry *et al*., (1951). Bovine Serum Albumin fraction V protein (66KDa) was used as a standard.

Extraction

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

The precipitated homogenate was centrifuged for 10 minutes, supernatant was decanted off and 2%TCA was added to the residue and again centrifuged and supernatant was decanted off. The precipitate was washed with 80%

acetone to remove the pigments. Two washes were carried out in 80% acetone and final washing in anhydrous acetone. Five ml of 0.1N NaOH was added to the pellet in each centrifuge tube and boiled for 5 minutes in water bath, cooled and centrifuged. The supernatant was then transferred to test tubes and used to protein estimation.

Estimation: Reagents

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

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

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

D- Folin-Ciocalteau reagent.

Suitable aliquots were taken in duplicates from each preparation. Volume was made up to 1ml with double distilled water. Then 5ml of reagent C was added to each tube mixed well and kept at room temperature for 10 minutes and 0.5ml 1n Folin ciocalteau reagent was added with immediate mixing. The tubes were kept for 30 minutes for colour development. Absorbance was read at 700nm using UV-Visible 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 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 minutes at 10,000rpm 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, 2ml was taken in test tubes in triplicate and equal volume of glacial acetic acid and ninhydrin were adds to it. The tubes were then heated in a boiling water bath for one hour and then the reaction was terminated by placing the tubes in ice bath. For colour development,4ml of toluene was added to the reaction mixture and stirred well for 20-30 seconds. Then the chromophoric toluene layer was separated carefully and brought to room temperature. The colour intensity of the solution was measured at a wave length of 520nm using spectrophotometer. L-proline was used as the standard.

PHENOLICS

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

Extraction

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

Estimation

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

ESTIMATION OF MALONDIALDEHYDE (MDA) CONTENT

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

Extraction

Two hundred milligram of plant tissue was weighed in duplicate and homogenized in 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 milliliter of the supernatant was mixed with an equal aliquot of 0.5% of Thiobarbituric acid (TBA) in 20% trichloroacetic acid (TCA). The solution was heated at 95^0 C for 24 minutes, cooled and then centrifuged at 3000 rpm for 2 minutes. The absorbance of the supernatant was measured at 532 nm and 600nm against reagent blank using UV-visible spectrophotometer. The absorbance value at 532nm was corrected for nonspecific turbidity by subtracting absorbance value at 600 nm and then the MDA content was calculated using an extinction coefficient of 155.

SUPEROXIDE DISMUTASE ASSAY

For the estimation of Superoxide dismutase activity, the protocol of Giannopolits and Ries (1977) with minor modifications was adopted.

Extraction: Five hundred milligram of plant tissue was weighed and homogenized gently with 50mM phosphate buffer of pH 7.8 in ice-cold mortar and pestle. It was centrifuged at 16,000 rpm for 15 min in refrigerated centrifuge (Thermo scientific XIR, Osterode am Harz, Germany) at 4ºC. The supernatant was collected and it was used for enzyme assay.

Enzyme assay: The activity of SOD was measured by monitoring the ability of SOD to inhibit the photochemical reduction of nitro blue tetrazolium chloride (NBT). The reaction mixture contained 0.1 mL of 1.5 M sodium carbonate, 0.3 mL of 0.13 M methionine, 0.3mL of 10µM EDTA, 0.3mL of 13µM riboflavin and 0.3mL of 0.63mM NBT and 0.1 mL enzyme extract. By using phosphate buffer (50mM, pH 7.8) the reaction mixture was made up to 3mL. For the assay of SOD activity, different assay systems were set, such as dark -control, light-control and test samples. Test tubes consisting of only assay mixture without enzyme extract were illuminated under fluorescent lamp for 30 min (light-control). Test samples (test tubes containing assay mixtures with enzyme extract and assay mixtures in dark (dark-control). The accumulation of formazan in different tubes is measured using UV-VIS spectrophotometer by reading the absorbance of the developed blue color at 560nm against the blank (reaction mixture without NBT). Result was expressed in units SOD mg^{-1} $protein^{-1}$. one unit of SOD was the enzyme activity inhibited the photo reduction of NBT to the blue formazan by 50%.

CATALASE ASSAY

CAT activity in the fresh samples was measured according to the protocol of Kar and Mishra (1976).

Extraction: Five hundred milligram of root, stem and leaf tissue of *Strobilanthes alternata* were homogenized using a chilled glass mortar and pestle in a medium consisting of 50mM phosphate buffer (pH 7.0) and 200 mg of polyvinyl polypyrolidone as phenolic binder. The homogenate was filtered through two layers of muslin cloth and was made up to 10ml using phosphate buffer. The filtrate was then centrifuged at 16000xg for 15 minutes in refrigerated centrifuged at 4ºC. The collected supernatant was used for the enzyme assay.

Enzyme assay: Enzyme assay mixture contained 1 mL of 50mM phosphate buffer (pH 7.0), 2mL enzyme extract and 1mL of 30 mM hydrogen peroxide. The enzyme assay mixture contained 2.4mL of 50mM phosphate buffer, 0.3 mL enzyme extract and 0.3mL of 30mM hydrogen peroxide. The enzyme extract and phosphate buffer was pipetted out and mixed well in a test tube. Hydrogen peroxide was added into this mixture which initiated the enzyme activity. After the addition of hydrogen peroxide immediately the enzyme activity was measured at 240nm for 90 seconds. Readings were recorded at 15 seconds interval. The activity of CAT was determined in terms of μmol hydrogen peroxide oxidized per min per gram fresh weight. The CAT activity was expressed in terms of decrease in absorbance at 240 nm for 1 min following the decomposition of hydrogen peroxide. One unit of the enzyme was defined as μmols hydrogen peroxide decomposed per min per mg protein.

QUANTITATIVE ESTIMATION OF VARIOUS HEAVY METALS

Various heavy metals namely Hg, Cr, Al and Zn contents of root, stem and leaf tissues were analyzed using Inductively Coupled Plasma- Optical Emission Spectrometry (ICP-OES). ICP-OES instrument PERKIN ELMER-AVIO 200 at Kerala Forest Research Institute (KFRI), Peechi, Thrissur was used for the estimation of heavy metals present in the digested samples. Dried plants (root, stem and leaves) are digested in an acid solution with a PerkinElmer Titan MPS. Weigh 400 mg of the sample into the digestion vessel. Add 5 ml of nitric acid and 3 ml of H_2O_2. Gently swirl the mixture and wait approximately 10 min before closing the vessel. Temperature is adjusted as per requirement. To avoid splashing wait until the vessels have cooled to room temperature. Carefully open the digestion vessel. A clear solution of digested sample is got as a result of digestion.

GC-MS ANALYSIS

Fresh leaves of treatment and control of sixth interval (20^{th} day) were collected and shade dried. Dried leaves were ground into fine powder and 5g of which was subjected to extraction using 200ml of Methanol in Soxhlet apparatus. After running several cycles, the extract obtained was concentrated and was used for GC-MS analysis.

Gas Chromatography Mass Spectrometry analysis of leaf extracts was performed using Shimadzu GC-MS, with model number QP2010S available at Kerala Forest Research Institute, Thrissur, Kerala. Helium gas was used as the carrier gas at constant flow rate of 1ml/min and an injection volume of 1µL was employed. The injection port temperature was set at 260ºC and ion source temperature at 200ºC. The oven temperature was programmed from 80ºC for 4 minutes with an increase of 5ºC/min to 280ºC with a hold time of 6 minutes. The total GC running time was 43 minutes. The components were identified

based on the comparison of their relative retention time and Mass spectra with those of Wiley NIST 7N Library data.

STATISTICAL ANALYSES

All experiments were carried out for a minimum of five times and the mean values are given in tables and figures. Standard deviation and standard error were calculated. The values in tables are mean ± standard error. Test of significance was done following Fisher's 't' test.

RESULTS

Root length

Strobilanthes alternata showed visible growth retardation in root length due to the toxicity of aluminium, chromium, mercury and zinc compared to control (Table-2; Fig- 2). Growth retardation rate was more or less similar in the case of all heavy metals. Visible retardation were evident from 8th day onwards of all metals, and the trend was continued throughout the growth period.

Stem length

During growth, gradual increase in stem length was observed in *Strobilanthes alternata* plants treated with aluminium, chromium, mercury and zinc during growth upto 20 days. More or less similar stem length was observed in *Strobilanthes alternata* treated with aluminium and control. But inhibition of stem growth was evident in the case of chromium, mercury and zinc during all intervals (Table-3: Fig-3).

Leaf area

Growth retardation in terms of leaf area was evidently shown in *Strobilanthes alternata* due to the toxicity of aluminium, chromium, mercury and zinc (Table-5:Fig-5). Leaf area measurements were made on newly opened leaves because no significant differences were found due to the treatments with heavy metals in the already matured leaves. Aluminium exhibited only negligible difference in leaf area compared to control Leaf area of plants treated with chromium was reduced. Leaf growth retardation was more or less similar in plants treated with Cr and Hg. More leaf growth retardation was shown by mercury than plants treated with chromium. Zinc treatment showed maximum retardation of leaf growth compared to control and other treatments.

Fig.1 Standardization of treatment concentration of heavy metals in *Strobilanthes alternata*

A: Control, B: Aluminum, C: Chromium ,D: Mercury, E.Zinc

Table- 2

Effect of aluminum, chromium, mercury and zinc on root length in *Strobilanthes alternata*.

Root length in Cm

Metals	Interval- Days					
	0	**4**	**8**	**12**	**16**	**20**
Control	7.58±0.11	10.04±0.42	12.34±0.35	14.4±0.23	16.58±0.19	18.5±0.31
Aluminium (400µM)	7.84±0.17	8.56±0.16	9.38±0.27	10.56±0.18	12.02±0.32	12.98±0.28
Chromium (70µM)	7.86±0.20	8.86±0.10	9.76±0.23	10.70±0.31	11.93±0.22	12.52±0.23
Mercury (20µM)	7.46±0.27	9.8±0.14	10.44±0.15	11.14±0.23	12.18±0.18	12.94±0.08
Zinc (250µM)	7.56±0.11	8.42±0.17	9.41±0.11	10.32±0.21	11.24±0.12	12.34±0.10

Values given are mean of 5 replicates ± S.E

Figure-2

Effect of aluminium, chromium, mercury and zinc on root length in *Strobilanthes alternata*

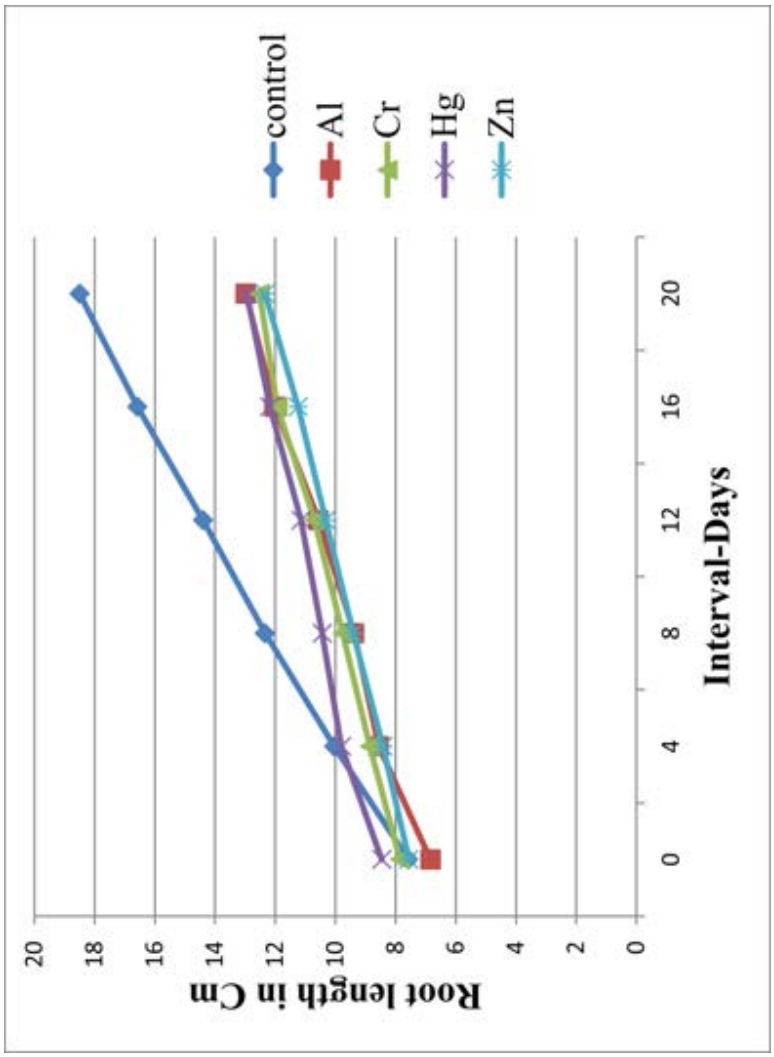


Table: 3

Effect of aluminium, chromium, mercury and zinc on stem length in *Strobilanthes alternata*

Stem length in Cm

Metals	Interval- Days					
	0	4	8	12	16	20
Control	17.14±0.19	20.82±0.27	23.56±0.22	26.34±0.21	28.7±0.11	31.26±0.13
Aluminium (400µM)	17.82±0.16	21.6±0.17	26.54±0.18	27.84±0.11	29.28±0.17	30.96±0.21
Chromium (70µM)	17.88±0.14	19.14±0.11	20.08±0.24	21.04±0.31	22.42±0.37	23.16±0.29
Mercury (20µM)	17.76±0.23	19.24±0.29	20.76±0.34	22.68±0.21	23.6±0.28	24.66±0.15
Zinc (250µM)	17.06±0.21	19.04±0.22	20.9±0.16	22.1±0.12	23.1±0.34	24.52±0.27

Values given are mean of 5 replicates ± SE

Figure-3

Effect of aluminium, chromium, mercury and zinc on stem length in *Strobilantes alternata*

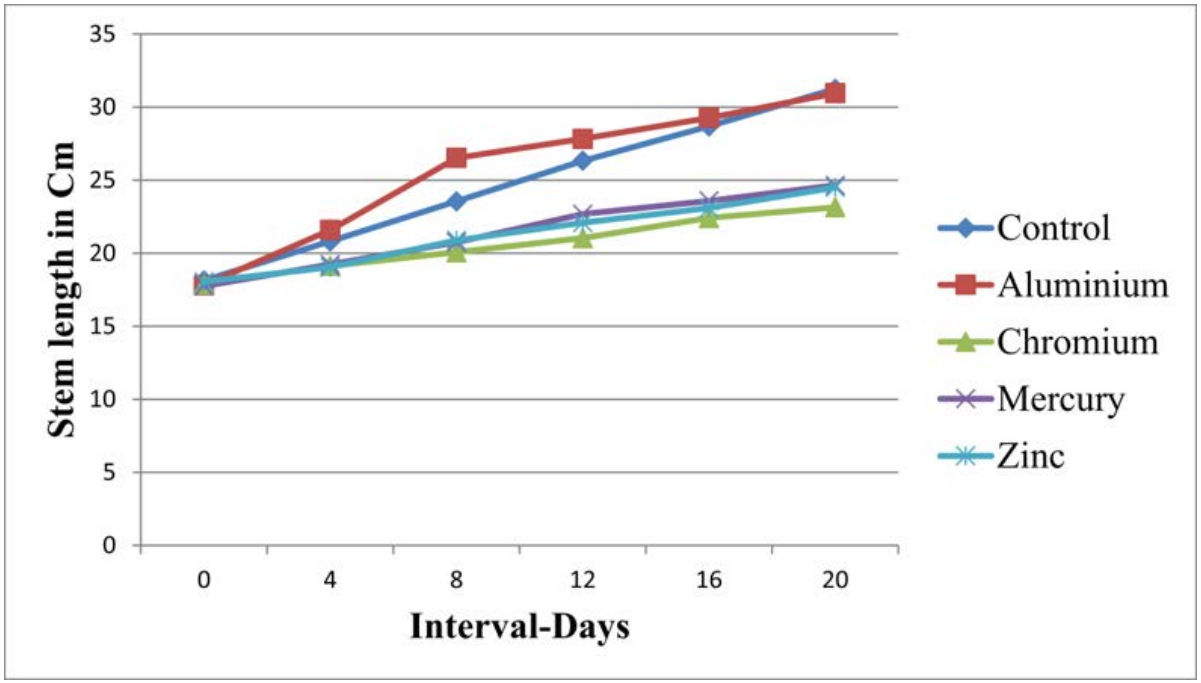


Table-4
Effect of aluminium, chromium, mercury and zinc on leaf area in *Strobilanthes alternata*

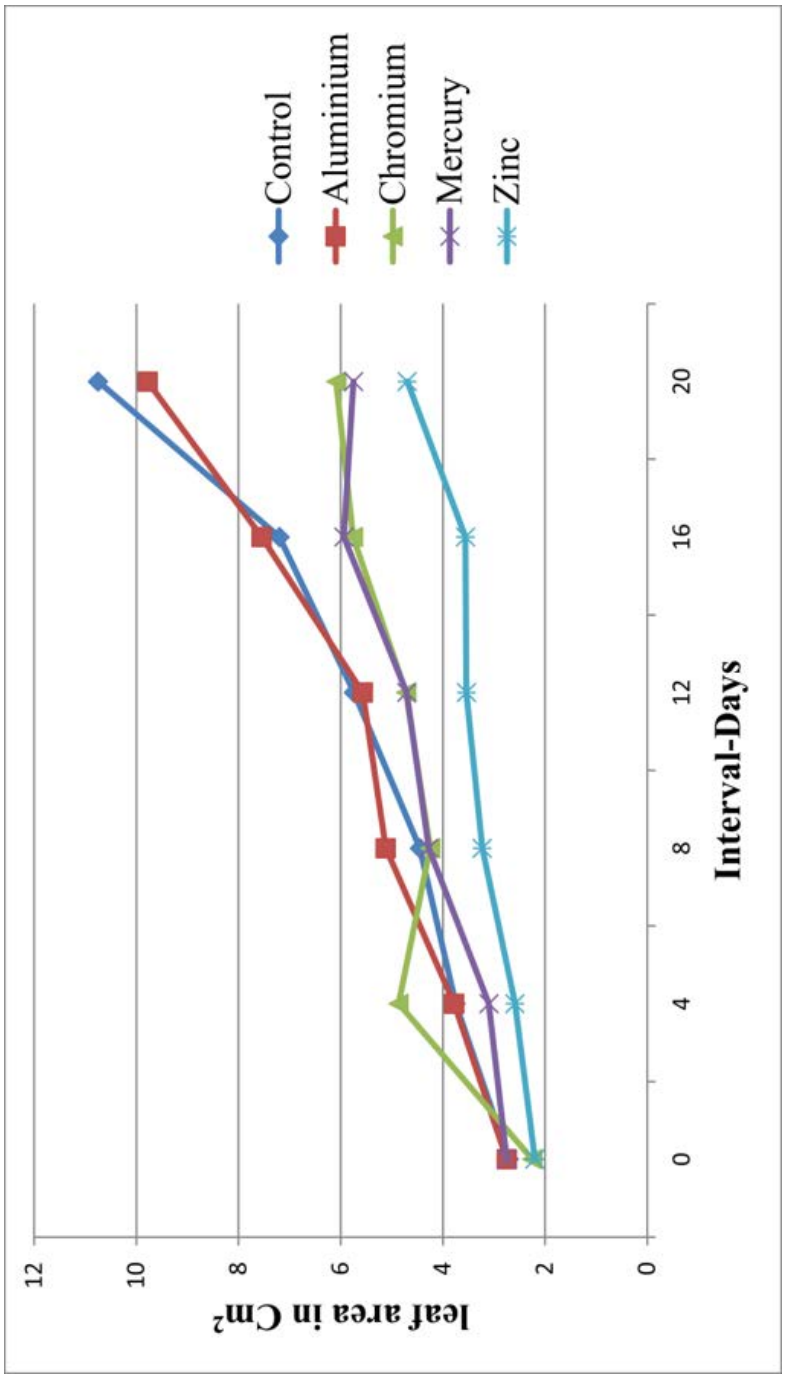
Leaf area in cm^2

Treatments	Intervals-Days					
	0	4	8	12	16	20
Control	2.71±0.09	3.75±0.11	4.45±0.07	5.74±0.14	7.20±0.15	10.75±0.20
Aluminium (400µM)	2.75±0.29	3.78±0.25	5.12±0.23	5.57±0.13	7.55±0.09	9.78±0.13
Chromium (70µM)	2.25±0.16	4.87±0.18	4.25±0.15	4.72±0.25	5.76±0.14	6.09±0.17
Mercury (20µM)	2.75±0.18	3.10±0.24	4.28±0.26	4.71±0.17	5.94±0.21	5.75±0.18
Zinc (250 µM)	2.20±0.11	2.59±0.24	3.23±0.29	3.54±0.16	3.56±0.20	4.70±0.19

Values given are mean of 5 replicates ±S.E.

Figure-4

Effect of aluminium, chromium,mercury and zinc on leaf area in *Strobilanthes alternata*



Stomatal index

Stomatal index of *Strobilanthes alternata* plants treated wilth all heavy metals showed significant changes (Table-5 ; Fig-5). Changes in the stomatal index of plants treated with aluminium remained almost unchanged compared to control. Chromium treatment resulted in significant increase of stomatal index in the lower epidermis in comparison with the control, whereas stomatal index of upper epidermis showed negligible increase. Plants treated with mercury showed maximum value of stomatal index of both upper and lower epidermis($P < 0.01$) compared to control as well as other treatments. Plants treated with zinc showed only negligible difference in stomatal index in both upper and lower epidermis compared to the control.

Tolerance index

Tolerance index was calculated as percentage difference in the ratio of root length due to heavy metal treatments in comparsion with the root length of the control (Table-6: Fig-6) . When compared to the control, plants treated with aluminium, chromium, mercury and zinc shows decrease in tolerance index. In plants treated with metals, tolerance index percentage showed negligible differences in each interval. In the case of mercury treatment on 20^{th} day there was a significant decrease in tolerance index ($P < 0.01$) When compared with each treatment, difference in tolerance index was also insignificant.

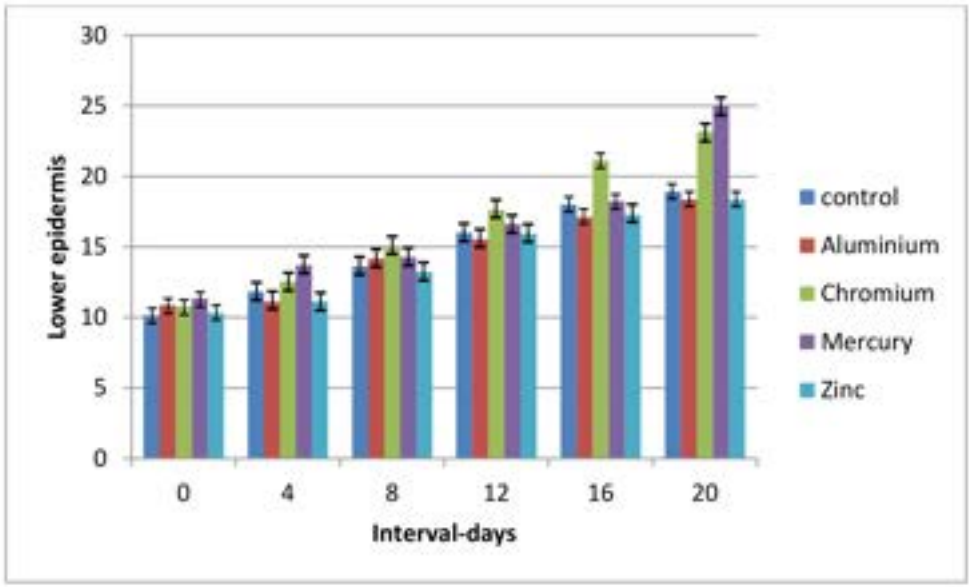
Table-5
Effect of aluminium, chromium, mercury and zinc on stomatal index in *Strobilanthes alternata*

Treatments		Interval-Days					
		0	4	8	12	16	20
Control	Lower Epidermis	10.14 ± 0.19	11.83 ± 0.13	13.61 ± 0.41	16.01 ± 0.15	18 ± 0.22	18.92 ± 0.08
	Upper Epidermis	6.41 ± 0.12	8.32 ± 0.10	9.17 ± 0.36	10.21 ± 0.13	11 ± 0.31	12.63 ± 1.2
Aluminum (400 µm)	Lower Epidermis	10.86± 0.11	11.12 ± 0.14	14.16 ± 0.25	15.54 ± 0.62	17.12 ± 0.17	18.36± 0.23
	Upper Epidermis	7.12 ± 0.38	8.15 ± 0.08	9.47 ± 0.07	10.63 ± 0.22	11.21 ± 0.17	12.40 ± 0.53
Chromium (70 µm)	Lower Epidermis	10.77 ± 0.27	12.54 ± 0.03	14.98 ± 0.17	17.64 ± 0.21	21.13 ± 0.13	23.16 ± 0.16
	Upper Epidermis	6.50 ± 0.20	8.39 ± 0.16	9.75 ± 0.13	11.05 ± 0.43	12.23 ± 0.30	12.46 ± 0.39
Mercury (20µm)	Lower Epidermis	11.34 ± 0.17	13.71 ± 0.75	14.28 ± 0.25	16.61 ± 0.36	18.25 ± 0.53	24.98 ± 0.76
	Upper Epidermis	7.49± 0.09	8.30 ± 0.62	10.88 ± 0.23	12.87 ± 0.12	13.24 ± 0.24	15.64 ± 0.13
Zinc (250 µm)	Lower Epidermis	10.30 ± 0.12	11.11± 0.14	13.24 ± 0.28	15.94 ± 0.02	17.26 ± 0.16	18.33 ± 0.83
	Upper Epidermis	6.21 ± 0.11	7.56 ± 0.54	9.75 ± 0.01	10.37 ± 0.35	12.87 ± 0.19	12.23 ± 0.17

Values given are mean of 5 replicates ±S.E

Figure-5

Effect of aluminium, chromium, mercury and zinc on stomatal index in *Strobilanthes alternata*



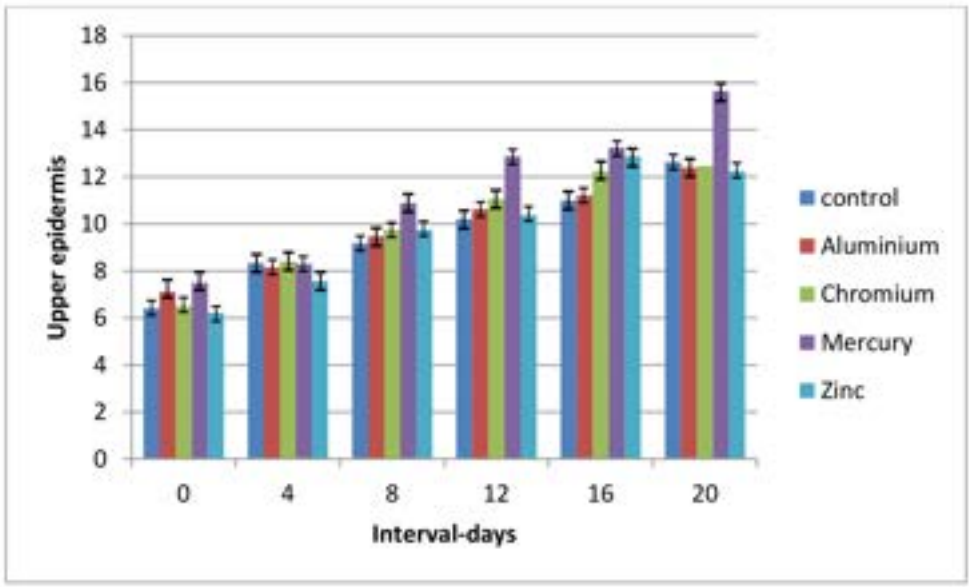


Table-6

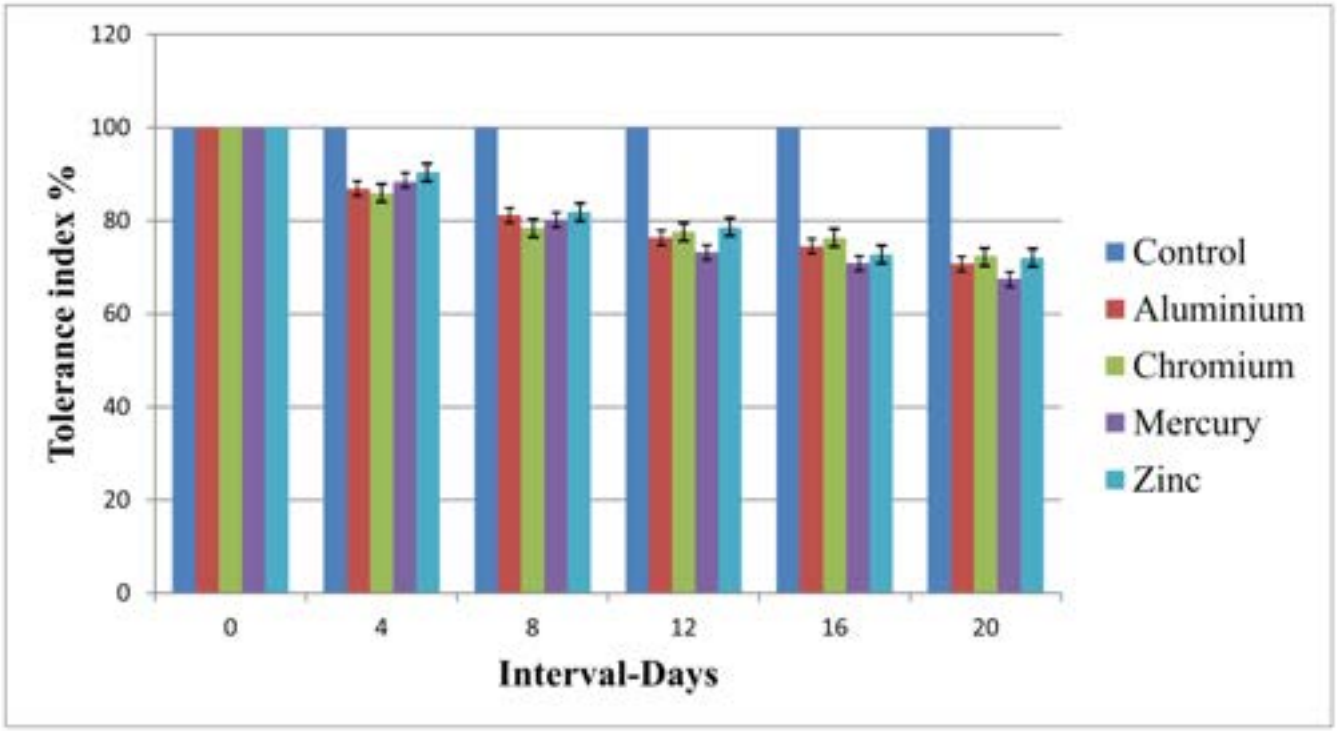
Effect of aluminium, chromium, mercury and zinc on tolerance index percentage pertaining to root length in *Strobilanthes alternata*

Treatments	Intervals-Days					
	0	4	8	12	16	20
Control	100	100	100	100	100	100
Aluminium (400µM)	100	86.86±1.35	81.26±1.80	76.39±2.90	74.49±2.53	70.77±1.69
Chromium (70µM)	100	85.9±2.83	78.5±1.76	77.65±1.99	76.29±2.72	72.44±3.01
Mercury (20µM)	100	88.37±2.42	80.25±1.94	73.26±1.55	70.90±1.90	67.56±3.15
Zinc (250µM)	100	90.47±1.76	81.94±1.59	78.57±2.74	72.78±2.22	71.99±2.93

Values given are mean of 5 replicates ±S.E

Figure-6

Effect of aluminium, chromium, mercury and zinc on tolerance index percentage pertaining to root length in *Strobilanthes alternata* during growth



Anatomical studies/Scanning Electron Microscopic analysis

Cross section of roots of *Strobilathes alternata* cultivated in hoagland solution consisting of distinct piliferous (rhizodermal) layer, cortex and vasculature (Fig- 7).Outermost piliferous layer has undulated margin. Root hairs occurred in plants. Cellular details of the cortical cells were distinctly seen. Phloem consists of small and thin walled lightly stained cells whereas xylem cells were thick walled and densely stained. Pith is narrow and consists of parenchyma cells. Scanning electron microscopic images, study on root section showed that roots of the control are composed of three concentric rings of tissues- Vascular, ground and epidermis (Fig-12). Cells of the ground tissues are compactily arranged and the cells showed well defined configuration. There is no deposit masses or localized bodies found in both vascular and ground tissues. Cortex cells are wavy like appearance

Anatomy of stem exhibited a typical pattern of dicot stem consisting of distinct epidermis layer with thick walled cells and epidermal hairs (Fig-7). Multilayered epidermal cells consisting of small, slightly thick walled cells and cortex consist of broad thin walled cortex. Vascular shows small sized phloem cells and xylem cells are thick walled and deeply stained. Vast pith consisting of thin walled parenchyma cells is also present. Medullary cells are thick walled and angular in shape in which large thick walled xylem vessels were present. The stem is composed of three tissue systems including the epidermis, vascular and ground tissues all of which are made from simple cell types in SEM images(Fig-12). Epidermis and epidermal hairs cannot be captured in detail during SEM analysis. Even though there causes a damage in epidermal cell due to pretreatment, epidermal cells without any localization were seen in the ground tissues. Cortex cells are unique and arranged in well organized manner. Vascular

tissues consists of protoxylem and metaxylem with opened pores like cells found in stem .Wide pith with compactly arranged cells were found in stem

Leaf midrib consists of outer epidermis with thick walled cells, mesophyll cells and densely stained palisade cells (Fig-7). Vasculature consists of thin walled small phloem cells and angular thick walled xylem cells. In the lower epidermis, vast mesophyll cells which are thin walled and plenty intercellular spaces were present. Lower epidermis were almost similar to upper epidermis consisting of thick walled cells. SEM images of leaf consists of three distinct type of tissues- epidermis, vasculature and mesophyll tissues (Fig-12). Epidermal cells were thin and cuticle was surrounded over epidermis

Structure of piliferous/ rhizodermal layer was not distinctly seen due to dark stained cells (Fig-8) and root hairs were low in number in roots of *Strobilanthes alternata* treated with aluminium compared to control and were densely stained also. Cortex and endodermis is almost similar to control. Structural details were similar to control but, dark spots with different sizes were seen distributed unevenly in different region of the sections. In comparison with the control, root tissues become more prominent and cells are cospicous and well arranged in SEM images of *Strobilanthes alternata* treated with aluminium (Fig-13).Treatment with aluminium, *Strobilanthes alternata* did not exhibit any structural damage to root tissues compared to control. Due to the presence of aluminium, complete stellar region become more distinct. Noticeable character of aluminium treated root is the presence of shapeless, solid deposits unevenly distributed at cortex region.

Stem treated with aluminium showed almost similar structure with control, epidermis, cortex and vasculature (Fig-8).Whereas stealer region particularly xylem region become more distinct consisting of large number of vessels. In the stem section also dark stained spots with different sizes were seen unevenly

distributed. SEM images of the stem treated with aluminium showed more or less similar configuration compared to control (Fig-13). All cell types including cortex, vasculature and pith is distinctly seen. Vasculature of stem treated with aluminium is conspecous and cells were well arranged. Cell wall thickening is characteristic in aluminium treated stem compared to control stem. Different types of deposits were seen scattered all over the sections.

Aluminium treated plant leaf shows typical structure of dicot leaf and the structure is almost similar to control (Fig-8). Structural variartions is meager in the leaves of aluminium treated plants compared to control (Fig-13) in SEM images. Presence of aluminium is not occurred in leaves of treated plants. Cells are distinct than the control.

In the root of plants treated with chromium, piliferous layer are not at all clear, and are comparatively thicker outer layer which are darkly stained (Fig-9). Root hairs were unevenly distributed and found broken. Vasculature consist of thick walled phloem cells and increased number of vascular bundles compared to control in chromium treated plants. The structure of root is almost similar to control. The entire region of cortex having a light yellow hue. Eventhough the xylem vessels were almost similar to control and uniformly stained in blue color, the entire pericycle region appeared light yellowish in color. Cross sections of the root of plant treated with chromium showed difference in its shape compared to control in SEM analysis (Fig-14). Cell walls of root treated with chromium becomes undulated. Cell walls of xylem and phloem appeared as thickened. Cell details were not distinct in chromium root. Scattered masses as deposits were seen all over the sections

In stem tissues, structure of epidermis, exodermis, cortex and vasculature is almost similar to control plants but epidermal hairs are almost absent (Fig-9). In general vascular region become enlarged resulted in broad pith

parenchymatous cells with unevenly thickened walls. Wider medullary rays were present. Most noticeable change in stem anatomy of chromium treated *Strobilanthes alternata* was damage of inner and outer cortex cells compared to control (Fig-14). Difference in cell wall striations are distinct in the treatments. Dense masses of deposits were found scattered all along the section.

Unlike the control, midrib region consist of epidermis showing number of epidermal hairs in the leaves of *Strobilanthes alternata* treated with chromium (Fig-9). Other structural details of leaf anatomy is almost similar to control. In SEM analysis *Strobilanthes alternata* treated with chromium imparted only low structural damage to leaf tissue cells compared to root and stem (Fig-14). Cell structures was appeared somewhat similar to that of control. Noticable change due to chromium treatment was shrinkage of vasculature. Accumulated chromium was seen as dense bodies in the vasculature and other tissues

Section of root treated with mercury shows thick walled rhizodermal cells which are densely stained(Fig-10). Root hairs were totally absent. Vascular region became small and cellular details are not at all distinct due to dark staining. Dark stained patches are seen over the rhizodermal cells and dark stained spots were seen distributed in the cortical region. Treatment with mercury, *Strobilanthes alternata* root resulted changes in the entire structure of the root in SEM analysis (Fig-15). Due to treatment with mercury stealar area was not distinct. Most of the cells found damaged. Cellular details were obscure. Many embedded masses of different sizes were seen irregularly distributed all over sections , mainly in stealar region.Carbon, oxygen, sodium, magnesium, aluminium, silicon, phosphorous, sulfur, chlorine, potassium, calcium, mercury, boron, iron, copper, and zinc were present in the root treated with mercury.

Structure of stem tissue of plant treated with mercury is found to be almost similar to control but the protoxylem vessels were found filled with stained

masses (Fig-10). Stem size is comparatively small in SEM analysis of Strobilanthes alternata treated with mercury, compared to the control (Fig-15) . Damage of cells of stem due to mercury treatment was not much conspicious. There occured a slight loss of compactness in cortex and epidermal cells. Small and large colourless deposits were found unevenly distributed all over the stem section.

Epidermis layer of midrib region of leaf treated with mercury showed the presence of large number of epidermal hairs (Fig-10). Other structures are same as control. SEM analysis, structure leaf of *Strobilanthes alternata* treated with mercury was almost uniform or similar to the control (Fig-15). All cell types including cortex, vasculature were distinctly seen

In *Strobilanthes alternata* treated with zinc rhizodermal cells are not clearly seen due to over staining resulting in a dark layer (Fig-11). Root hairs are completely spoiled. Typical character of root of plants treated with zinc, shows dull light brown tinch all over the section especially in stealar region but pith cells were seen without any light brown coloration. Hence the masking of blue colour of the xylem vessels. In contrast to the other treatments and control were xylem vessels were thick walled and brightly blue colour. Due to zinc treatment roots of *Strobilanthes alternata* in SEM showed that the cell wall become more distinct than the control, compared to control (Fig-16). All type of cells were clearly seen. Cell wall thickening was prominent in vascular tissues. White deposits were seen scattered all over the sections.

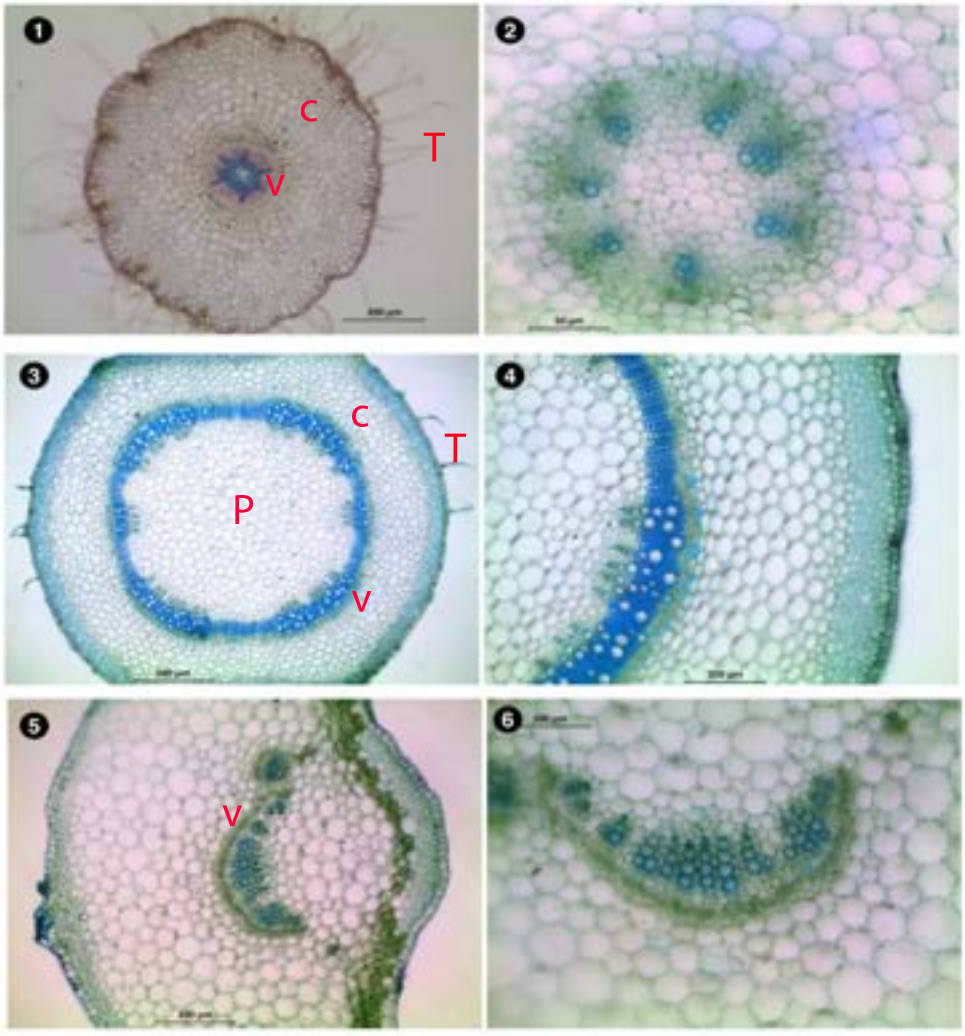
All anatomical features are almost similar to the control in stem (Fig-11). In the stem cell types including cortex, vasculature and pith were distinctly seen in the stem of zinc treated plants (Fig-16). Pith cells became more wider. Deposits were seen scattered all over the sections which were more concentrated in phloem region.

Leaf tissues treated with zinc showed a reduction in size of vasculature. Epidermal hairs are absent in the leaf tissues(Fig-11). Due to zinc treatment leaf tissues structure was distinct than that of control in SEM analysis (Fig-16). Deposits were found as shapeless spots unevenly distributed with more concentrated in vascular regions.

Figure 7

Effect of aluminium, chromium, mercury and zinc on the anatomy of *Strobilanthes alternata* (Figure7-11)

Control

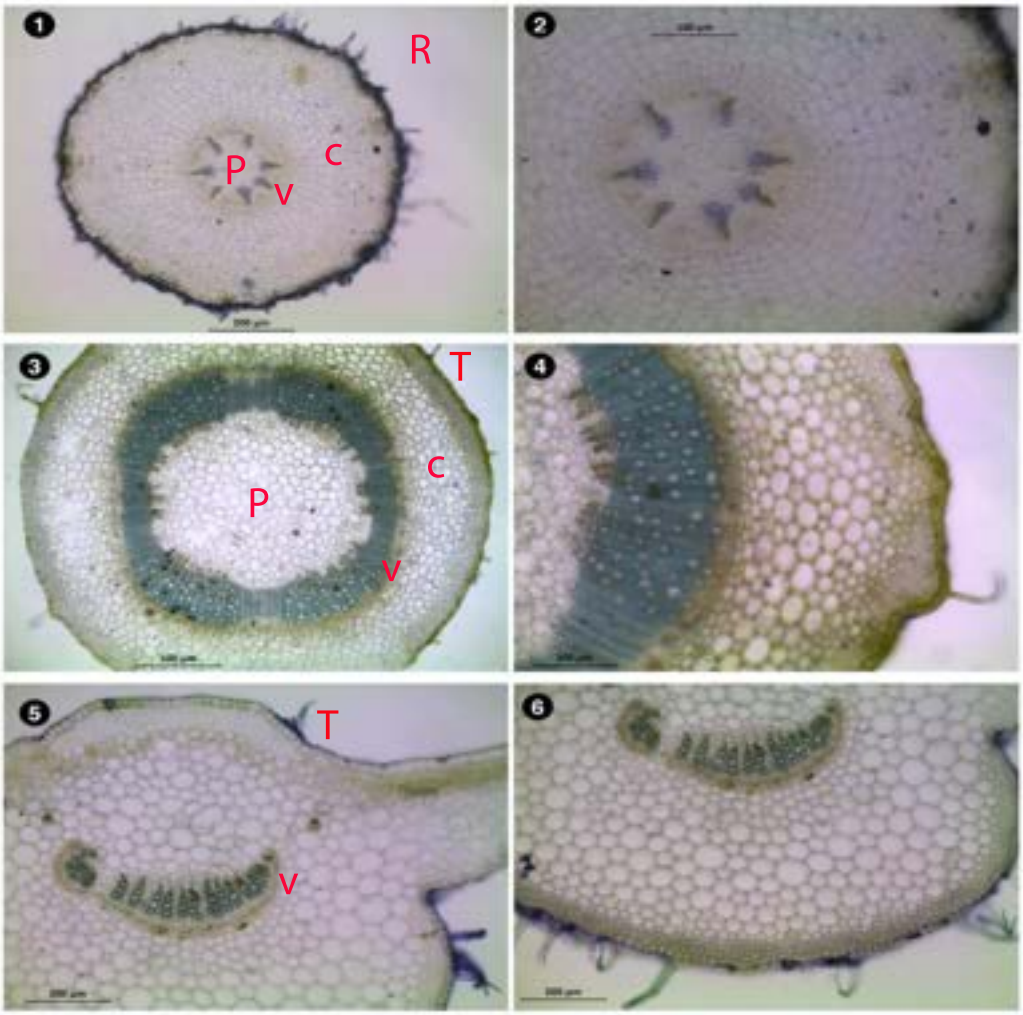


1-Root T.S
2- Portion Enlarged (Root)
3- Stem T.S
4- Portion Enlarged (Stem)
5- Leaf T.S
6- Portion Enlarged (Leaf)

C-Cortex; P-Pith; V-Vascular bundle; T-trichome; R-Root hairs

Figure 8

Effect of aluminium on the anatomy of *Strobilanthes alternata*

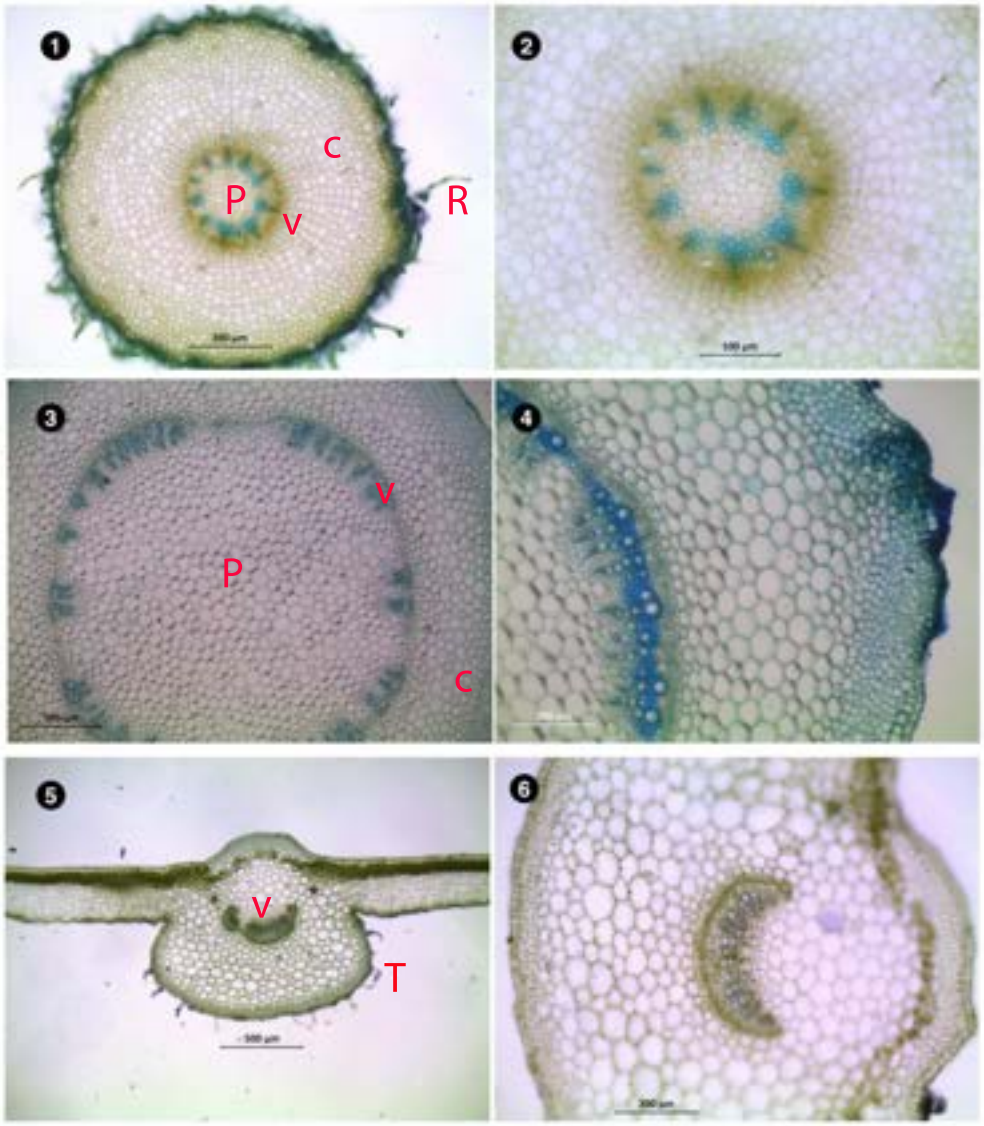


1-Root T.S
2- Portion Enlarged (Root)
3- Stem T.S
4- Portion Enlarged (Stem)
5- Leaf T.S
6- Portion Enlarged (Leaf)

C-Cortex; P-Pith; V-Vascular bundle; T-trichome; R-Root hairs

Figure 9

Effect of chromium on the anatomy of *Strobilanthes alternata*

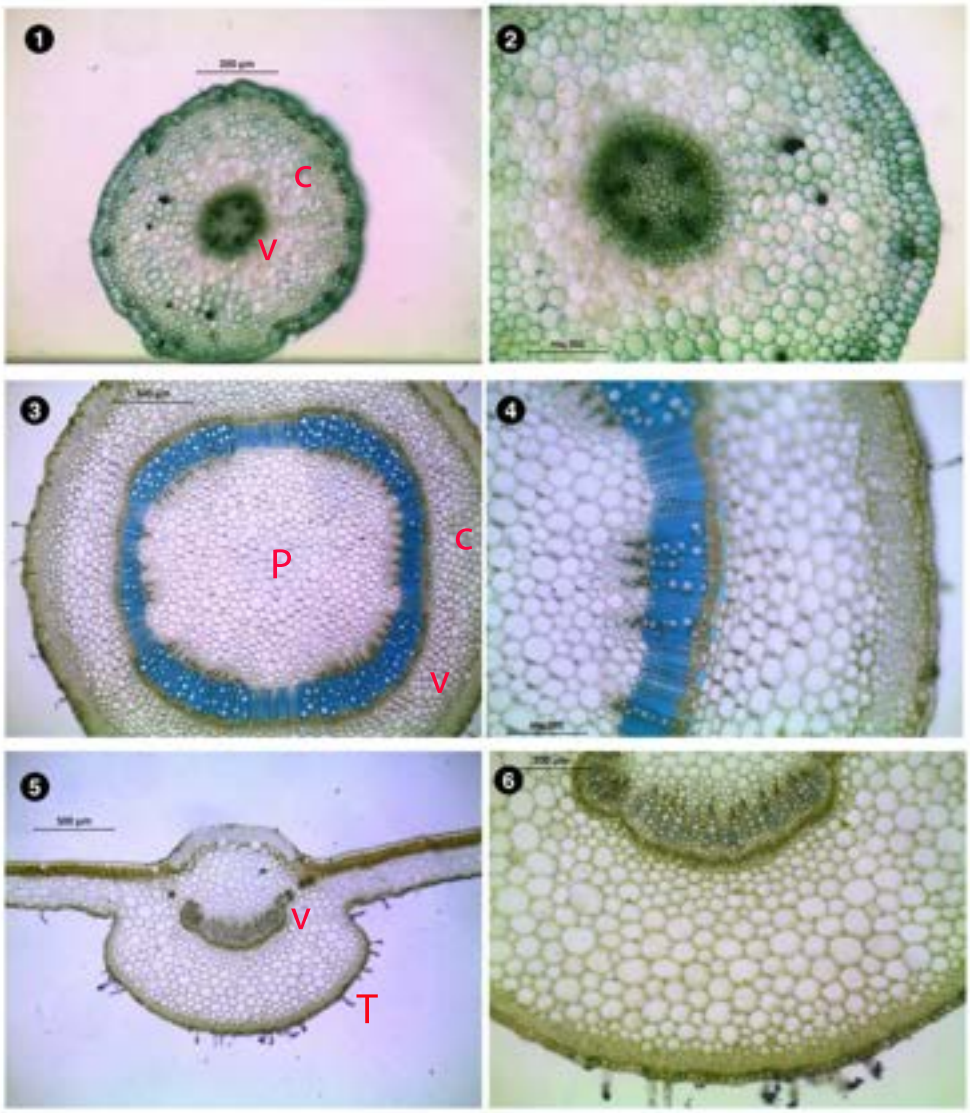


1-Root T.S
2- Portion Enlarged (Root)
3- Stem T.S
4- Portion Enlarged (Stem)
5- Leaf T.S
6- Portion Enlarged (Leaf)

C-Cortex; P-Pith; V-Vascular bundle; T-trichome; R-Root hairs

Figure 10

Effect of mercury on the anatomy of *Strobilanthes alternata*

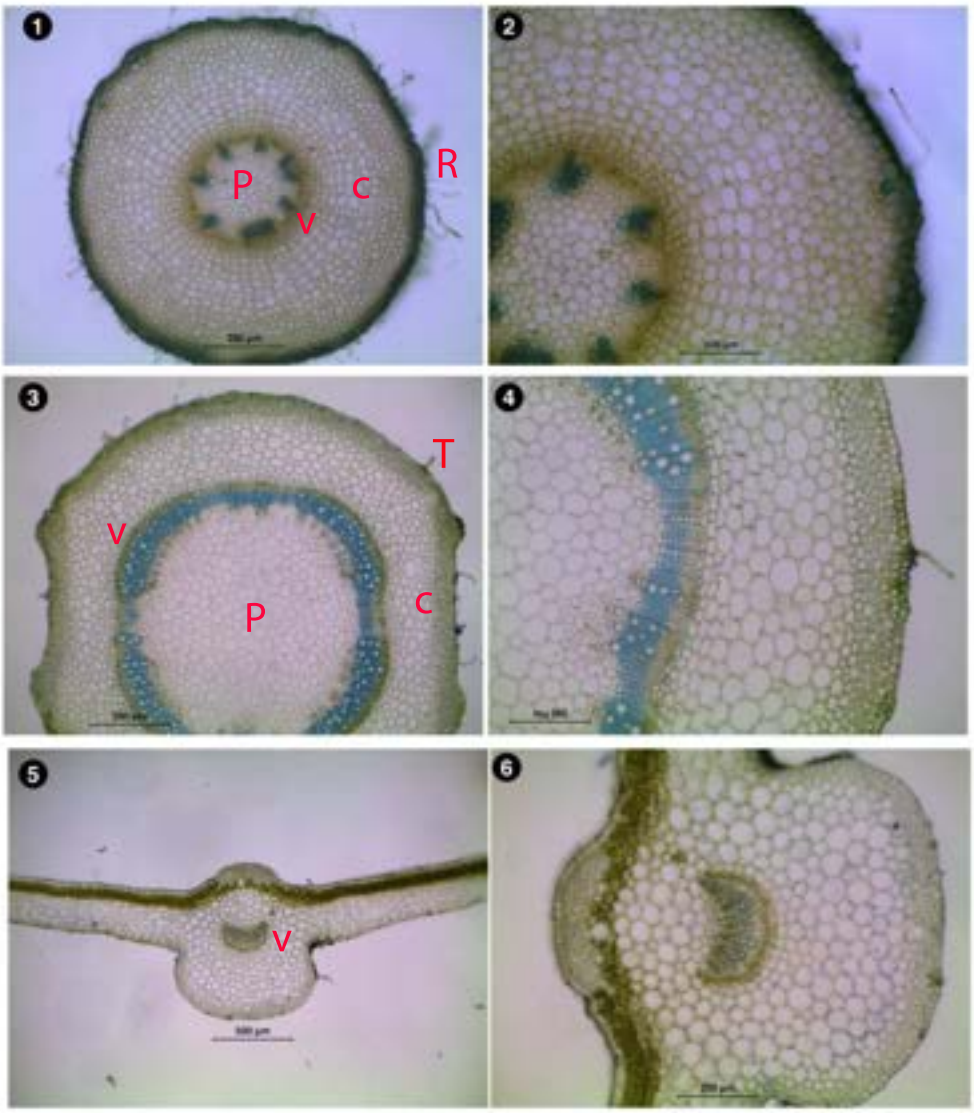


1-Root T.S
2- Portion Enlarged (Root)
3- Stem T.S
4- Portion Enlarged (Stem)
5- Leaf T.S
6- Portion Enlarged (Leaf)

C-Cortex; P-Pith; V-Vascular bundle; T-trichome; R-Root hairs

Figure 11

Effect of zinc on the anatomy of *Strobilanthes alternata*



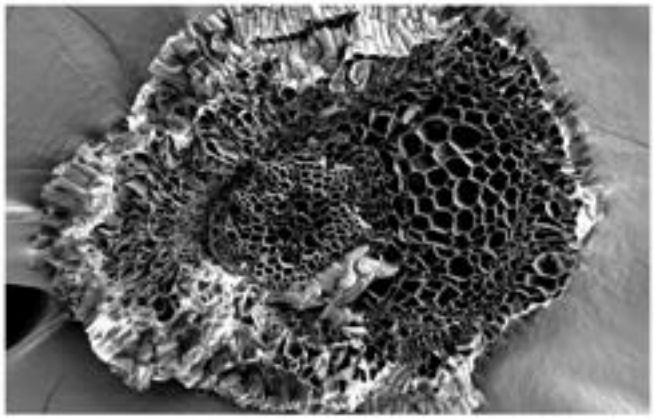
1-Root T.S
2- Portion Enlarged (Root)
3- Stem T.S
4- Portion Enlarged (Stem)
5- Leaf T.S
6- Portion Enlarged (Leaf)

C-Cortex; P-Pith; V-Vascular bundle; T-trichome; R-Root hairs

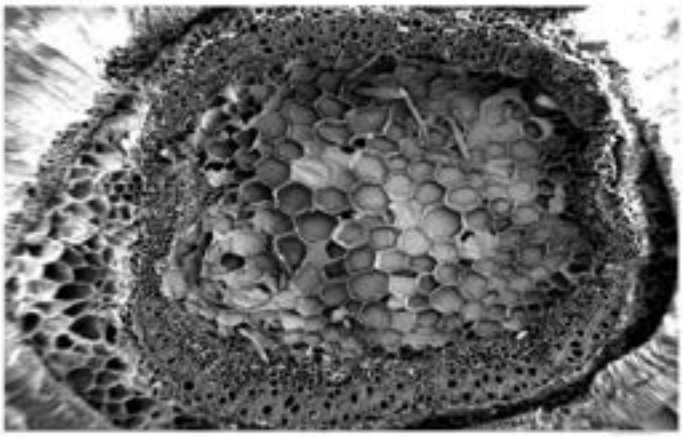
Figure 12

Scanning Electron Micrographs of *Strobilanthes alternata*

A-ROOT



B-STEM

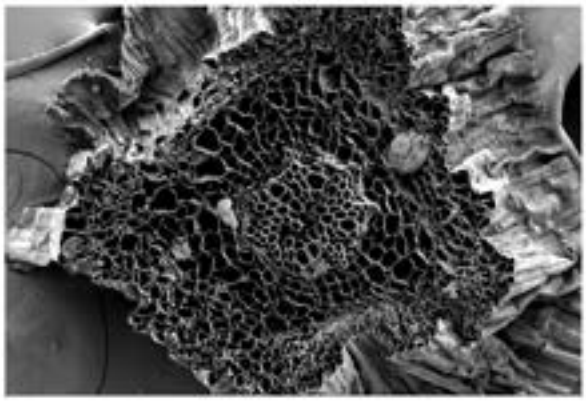


C-LEAF

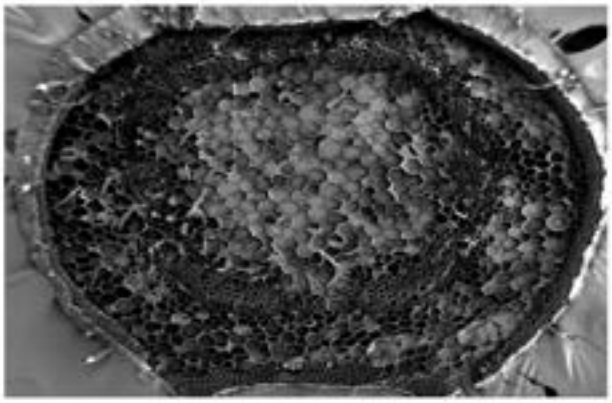
Figure 13

Scanning Electron Micrographs of *Strobilanthes alternata subjected to aluminium treatment*

A-ROOT



B-STEM



C- LEAF

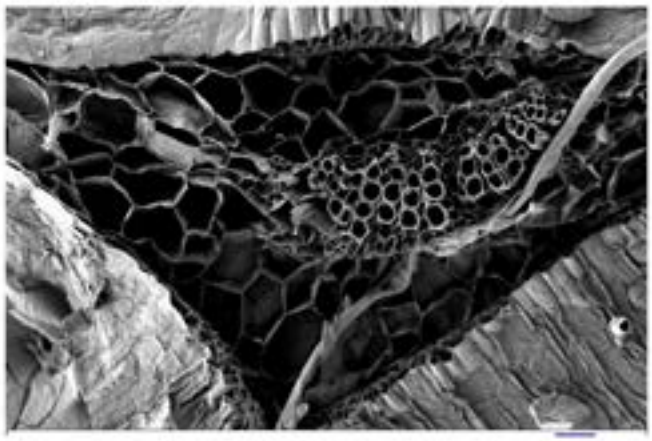
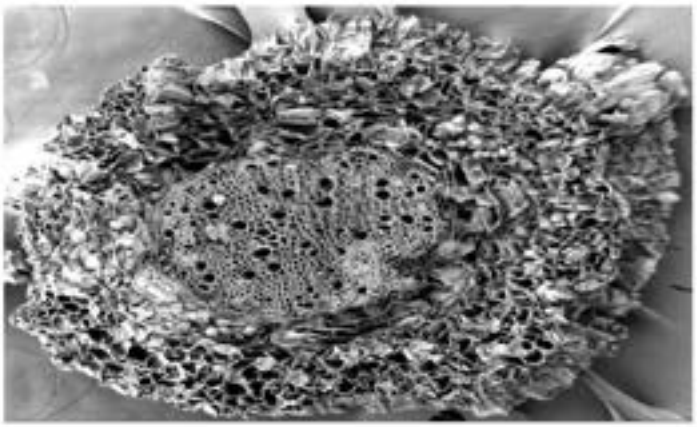


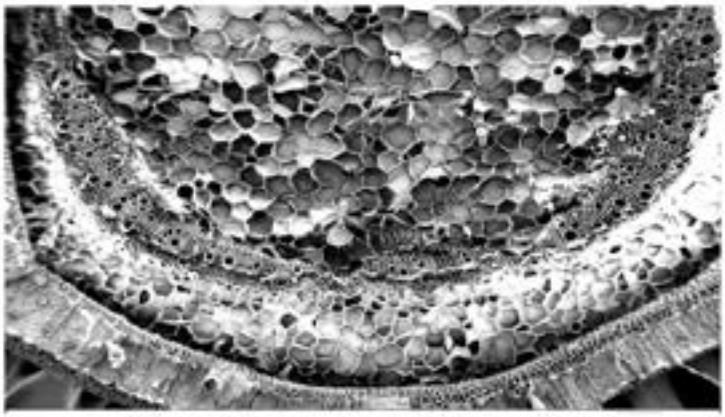
Figure 14

Scanning Electron Micrographs of *Strobilanthes alternata subjected to chromium treatment*

A-ROOT



B-STEM



C-LEAF

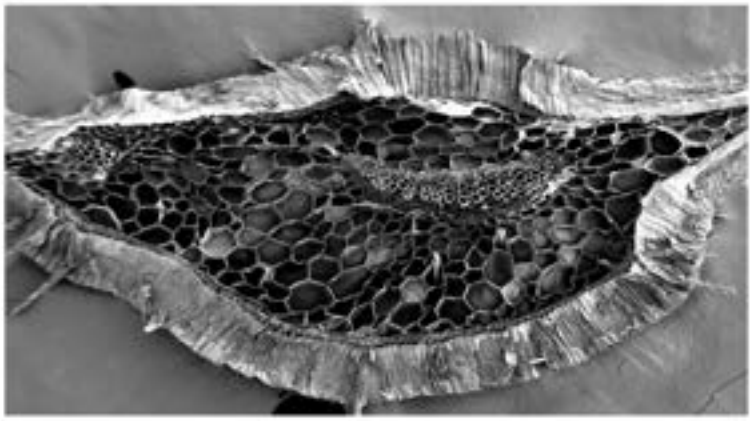
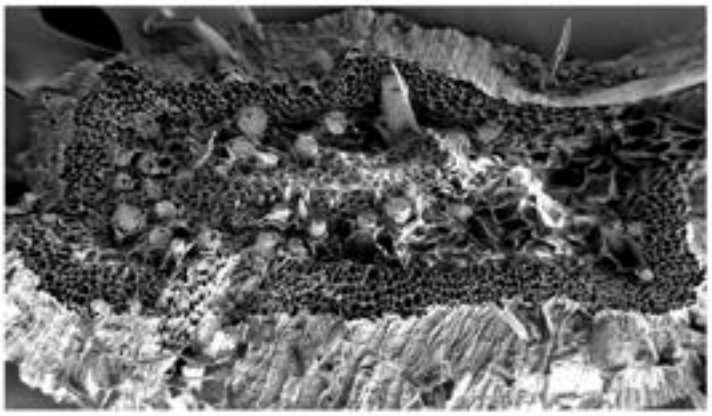


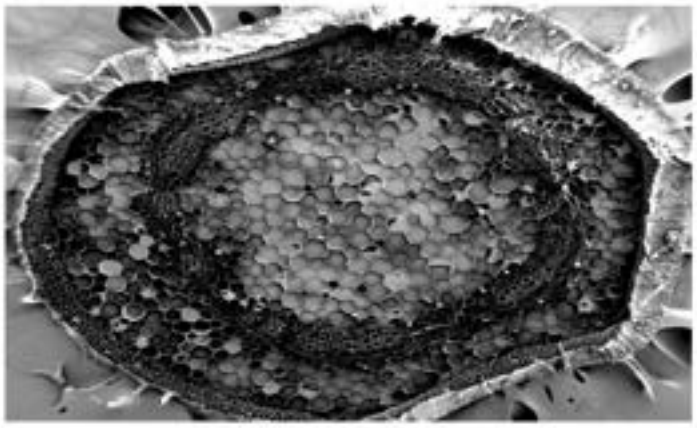
Figure 15

Scanning Electron Micrographs of *Strobilanthes alternata subjected to mercury treatment*

A-ROOT



B-STEM



C-LEAF

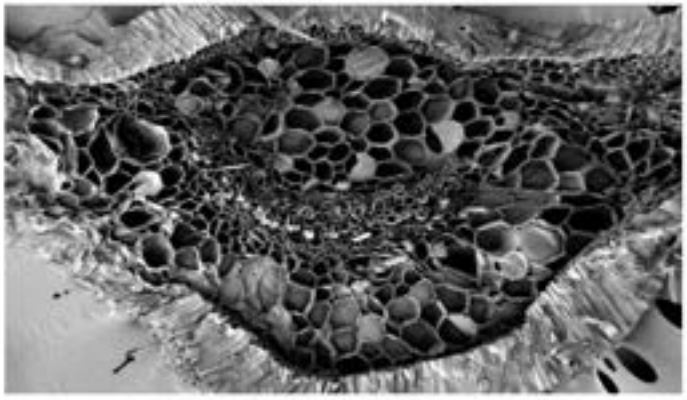
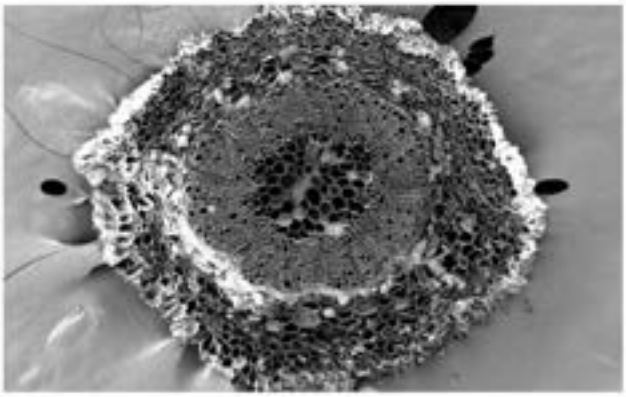


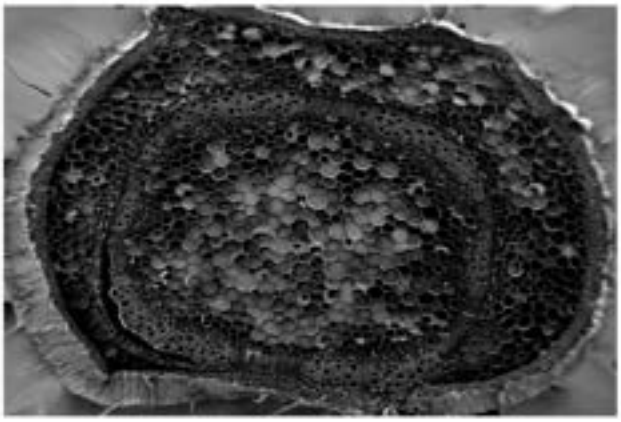
Figure 16

Scanning Electron Micrographs of *Strobilanthes alternata subjected to zinc treatment*

A-ROOT



B-STEM



C-LEAF

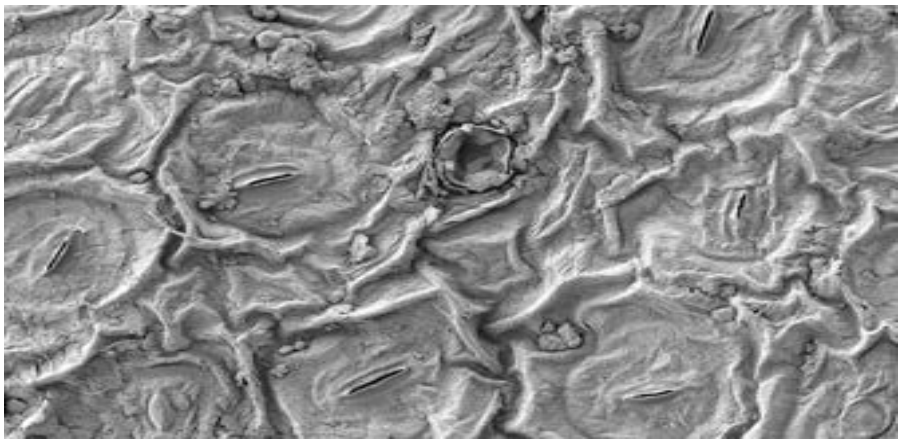
Scanning electron micrographs showing effect of heavy metals on the abaxial leaf surfaces of *Strobilanthes alternata*

Scanning electron microscopic study of lower epidermis of leaf showed evenly arranged epidermal cells in which stomatal cells are clearly seen (Fig-17). In the control, stomata appeared almost closed leaving a small opening in all cells. In plants treated with aluminium stomata of lower epidermis was found to be widely opened (Fig -17). Stomatal aperture was clearly seen. Plants treated with chromium also, all stomata are found open with slight difference in aperture size compared to aluminium.. Distribution of stomatal number was slightly increased in the leaves of plants treated with mercury than the control and stomatal aperture was widely openend (Fig-18). Zinc treatment resulted in uneven distribution of stomata in the epidermal cells and stomatal aperture was fully opened in some other cell aperture was particularly closed(Fig-18).

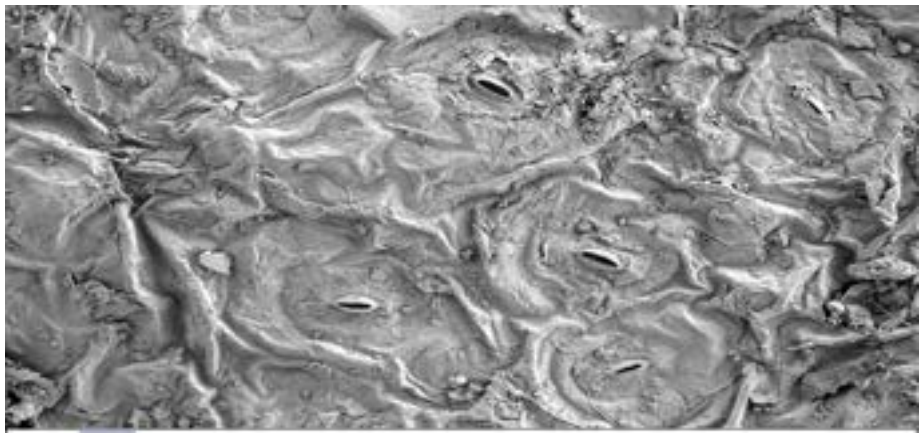
Figure 17

Scanning electron micrographs of leaf (adaxial side) in *Strobilanthes alternata* treated with aluminium and chromium

A- CONTROL



B- EFFECT OF ALUMINIUM



C-EFFECT OF CHROMIUM

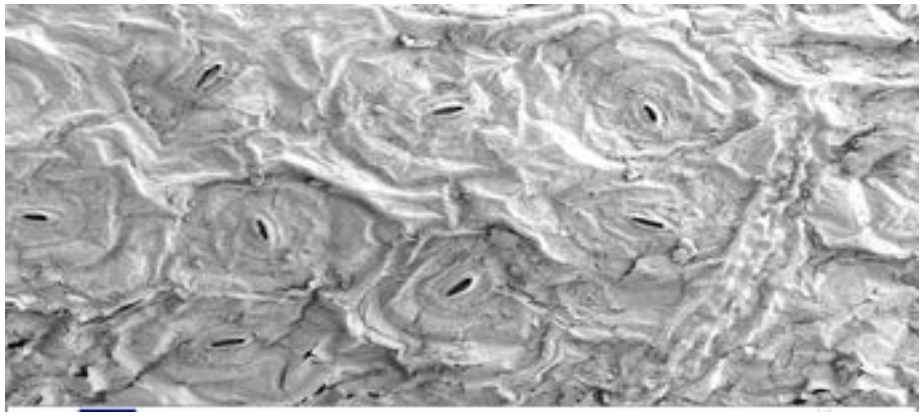
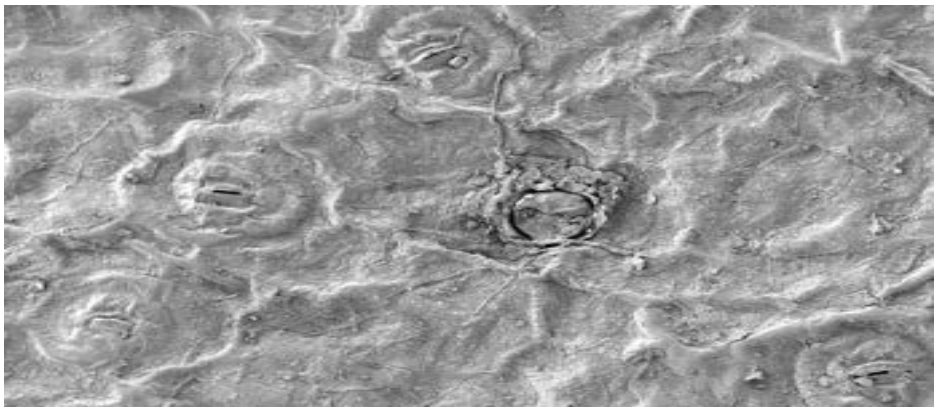


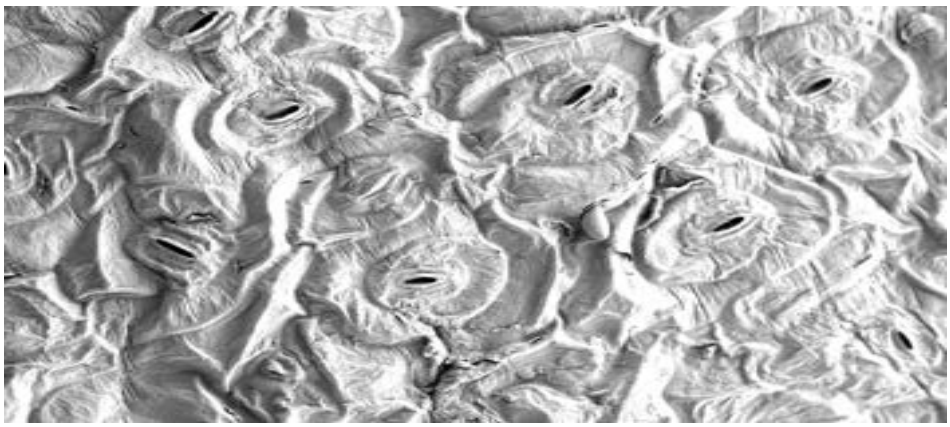
Figure 18

Scanning electron micrographs of leaf (adaxial side) in *Strobilanthes alternata* treated with mercury and zinc

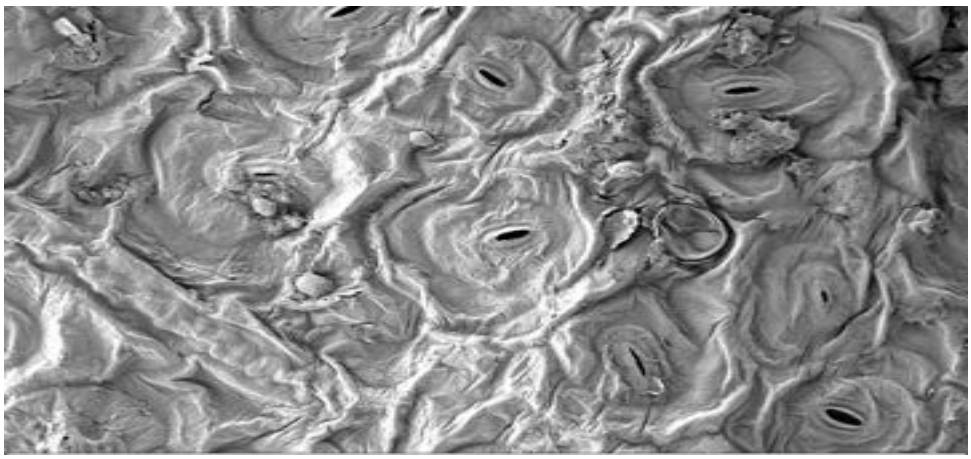
A-CONTROL



B-EFFECT OF MERCURY



C-EFFECT OF ZINC



Scanning electron micrographs –Energy dispersive X-ray analysis of *Strobilanthes alternata*

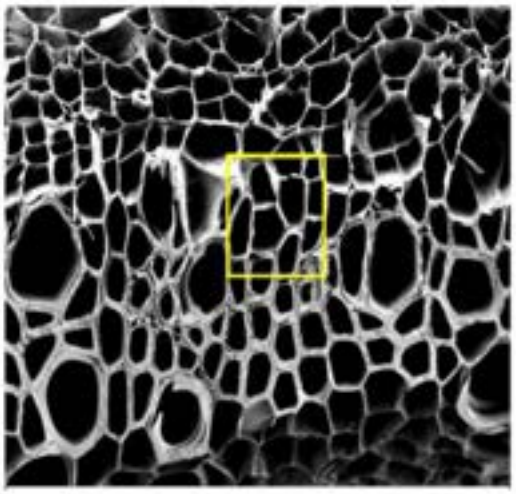
CONTROL

EDX data revealed the presence and quantity of elements in the localized masses . Energy dispersive X-ray (EDX) analysis data of zoomed region of root section of *Strobilanthes alternata* showed essential macro and micro elements such as carbon, oxygen, sodium, magnesium, aluminium, silicon, phosphorous, sulfur, chlorine, potassium, calcium, boron, iron, copper and zinc present in the tissues (Fig-19). In the stem tissues and leaf also showed the same essential macro and microelements as that of root (Fig-20,21)..

Figure- 19

Scanning Electron Micrographs and EDX spectrum of *Strobilanthes alternata*

ROOT



Distribution of individual elements present in selected area of SEM

Elements	Weight %	Atomic %
CK	46.95	55.14
OK	47.39	41.78
NaK	0	0
MgK	0.06	0.03
AlK	0	0
SiK	0.02	0.01
PK	0.14	0.06
SK	0.04	0.02
ClK	0.98	0.08
KK	0.65	0.23
CaK	1.82	0.64
BK	1.43	1.86
FeK	0.23	0.05
CuK	0.29	0.06
ZnK	0.1	0.02

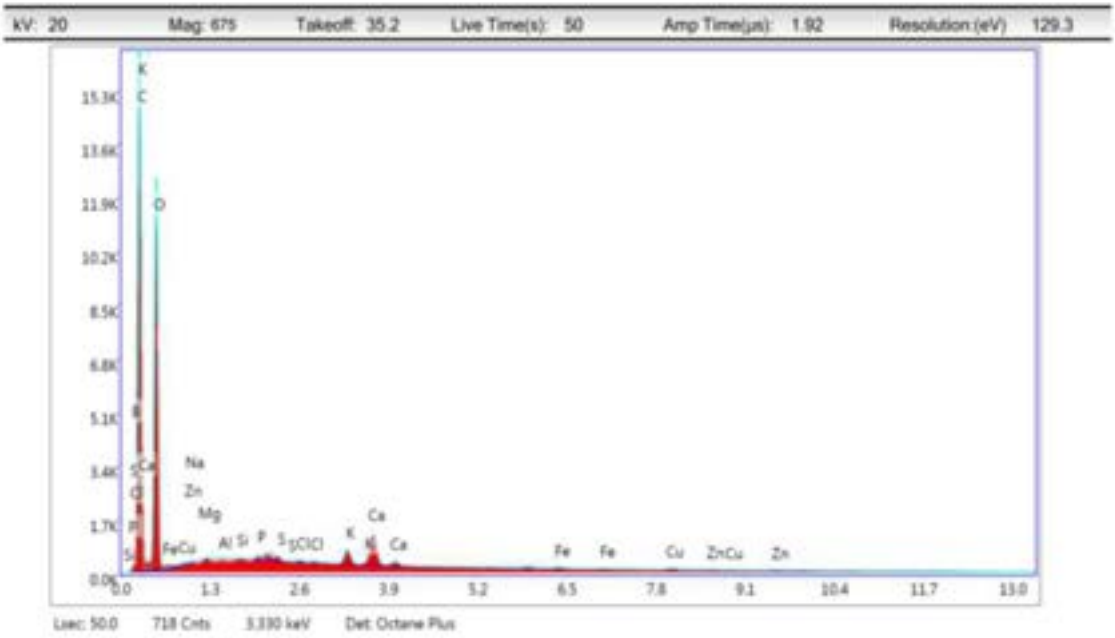
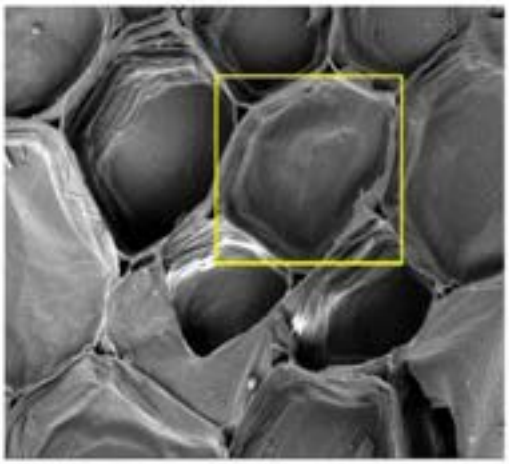


Figure-20

Scanning Electron Micrographs and EDX spectrum of *Strobilanthes alternata*

STEM



Distribution of individual elements present in selected area of SEM

Elements	Weight %	Atomic %
CK	50.46	58.7
OK	43.73	38.19
NaK	0.09	0.06
MgK	0.01	0.01
AlK	0.03	0.01
SiK	0.04	0.02
PK	0.34	0.15
SK	0.44	0.07
ClK	0.19	0.08
KK	2.39	0.86
CaK	0.6	0.21
BK	1.21	1.56
FeK	0.17	0.05
CuK	0.23	0.05
ZnK	0.05	0.01

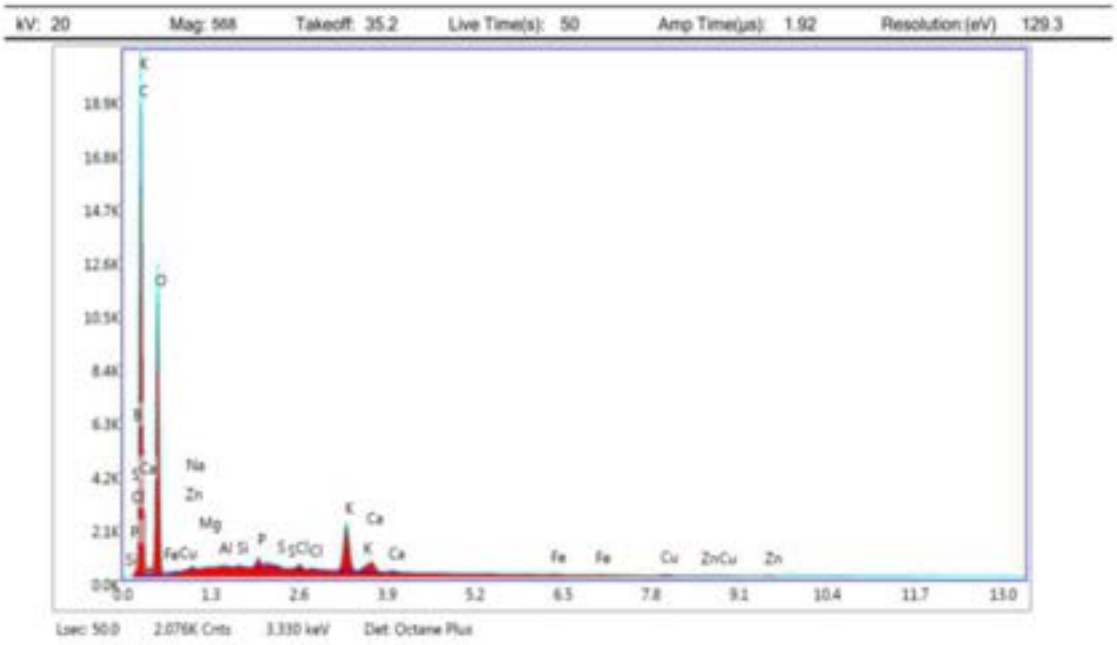
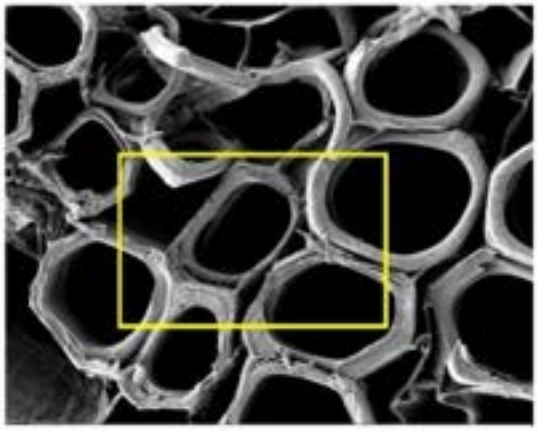


Figure-21

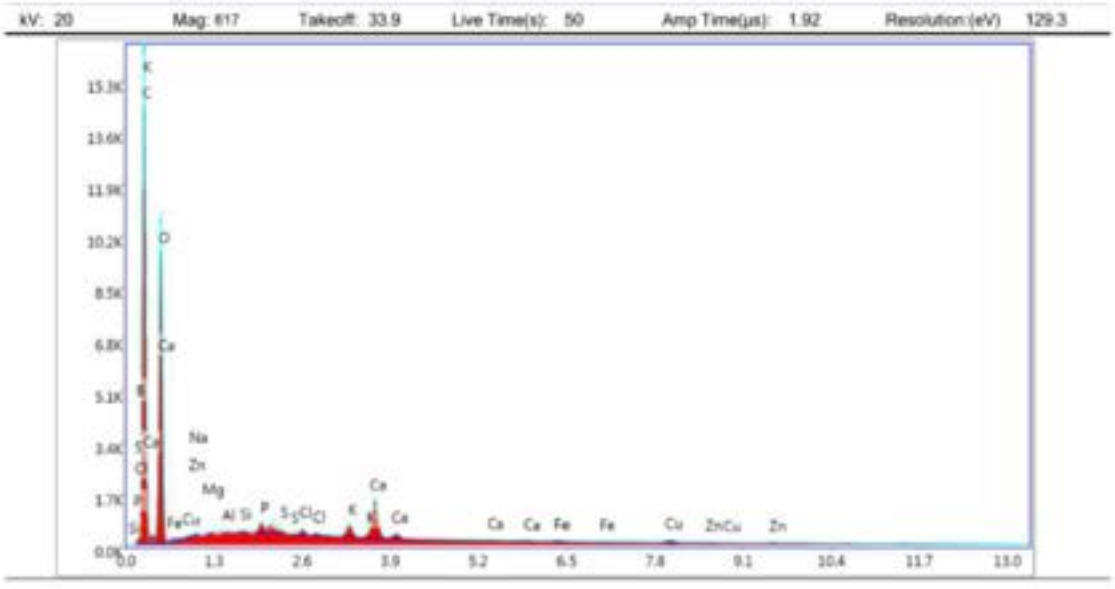
Scanning Electron Micrographs and EDX spectrum of *Strobilanthes alternata*

LEAF



Distribution of individual elements present in selected area of SEM

Elements	Weight %	Atomic %
CK	47.9	56.34
OK	46.03	40.65
NaK	0.09	0.06
MgK	0.06	0.03
AlK	0.08	0.04
SiK	0.18	0.09
PK	0.25	0.11
SK	0.08	0.04
ClK	0.26	0.11
KK	0.78	0.28
CaK	1.76	0.62
BK	1.11	1.45
FeK	0.28	0.11
CuK	0.26	0.06
ZnK	0.89	0.08



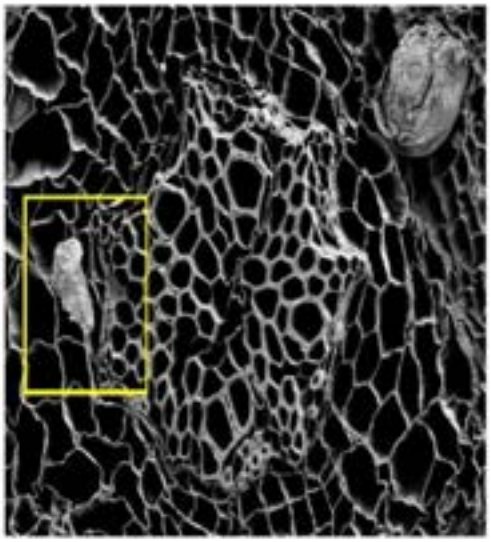
EFFECT OF ALUMINIUM

Comparative study using energy dispersive X-ray analysis on the effect of aluminium treatment on *Strobilanthes alternata* showed maximum occurrence of aluminium compared to control (Fig- 22, 23, 24). The root tissue of plant treated with aluminium showed an increase of magnesium by three fold and a marginal increase of potassium, silicon, sulfur and reduction of calcium and zinc (Fig- 22). All other elements remained unchanged in the root tissues. Increase of silicon and reduction of potassium, phosphorous, sulfur, chlorine and iron was observed in the stem tissues(Fig-23). Magnesium, calcium, copper and zinc remained unchanged. Leaves of plants treated with aluminium showed maximum aluminium content compared to other tissues(Fig-24). Sodium content also was increased in the leaf tissues due to aluminium treatment. About five times decrease in the distribution of potassium, iron and zinc in leaf tissues. Other elements such as magnesium, silicon, phosphorous, sulfur, chlorine, iron, and copper remained same as control.

Figure-22

Scanning Electron Micrographs and EDX spectrum of *Strobilanthes alternata* subjected to aluminium treatment

ROOT



Distribution of individual elements present in selected area of SEM

Elements	Weight %	Atomic %
CK	46.83	57.62
OK	43.72	38.73
NaK	0.07	0.04
MgK	0.17	0.1
AlK	1.43	0.26
SiK	0.48	0.24
PK	0.4	0.18
SK	0.11	0.05
ClK	0.1	0.04
KK	1.95	0.71
CaK	0.79	0.28
BK	1.19	1.56
FeK	0.46	0.12
CuK	0.22	0.05
ZnK	0.07	0.02

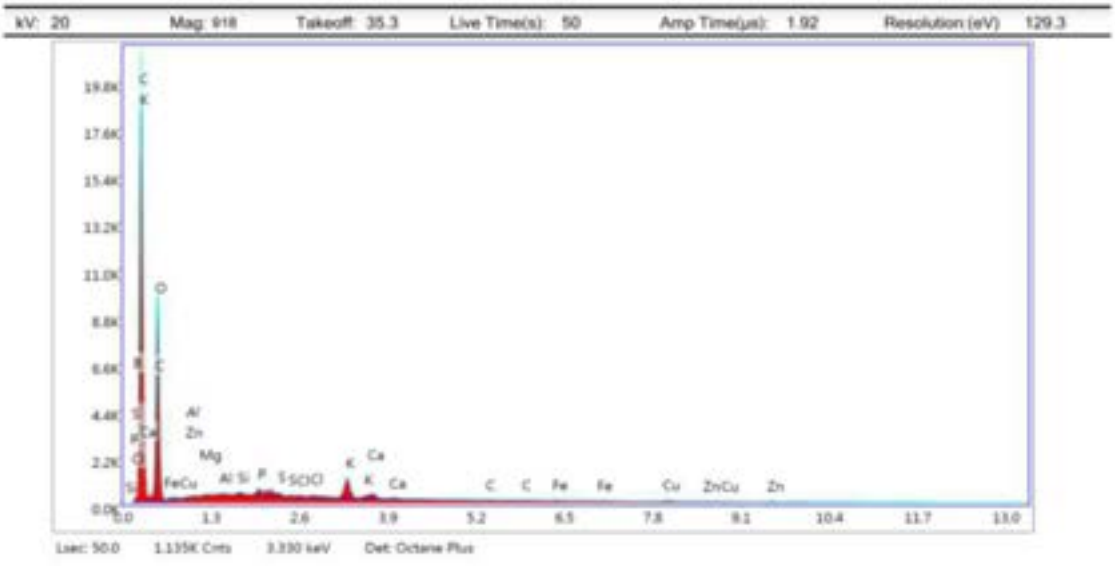
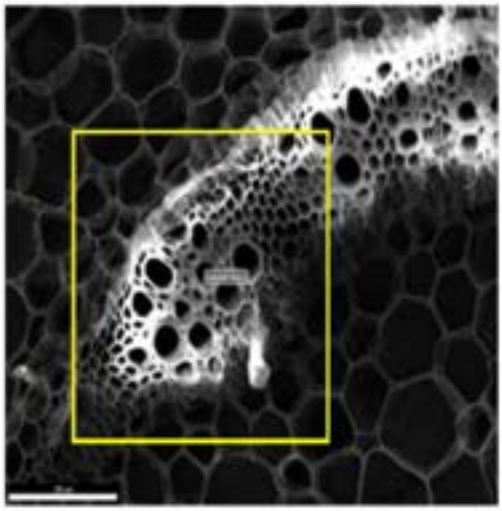


Figure-23

Scanning Electron Micrographs and EDX spectrum of *Strobilanthes alternata* subjected to aluminium treatment

STEM



Distribution of individual elements present in selected area of SEM

Elements	Weight %	Atomic %
CK	54.23	62.2
OK	41.27	35.5
NaK	0.04	0.02
MgK	0.02	0.01
AlK	0.9	0.08
SiK	0.08	0.04
PK	0.28	0.13
SK	0.03	0.01
ClK	0.06	0.02
KK	1.28	0.45
CaK	0.37	0.13
BK	1.05	1.34
FeK	0.09	0.02
CuK	0.22	0.05
ZnK	0.05	0.01

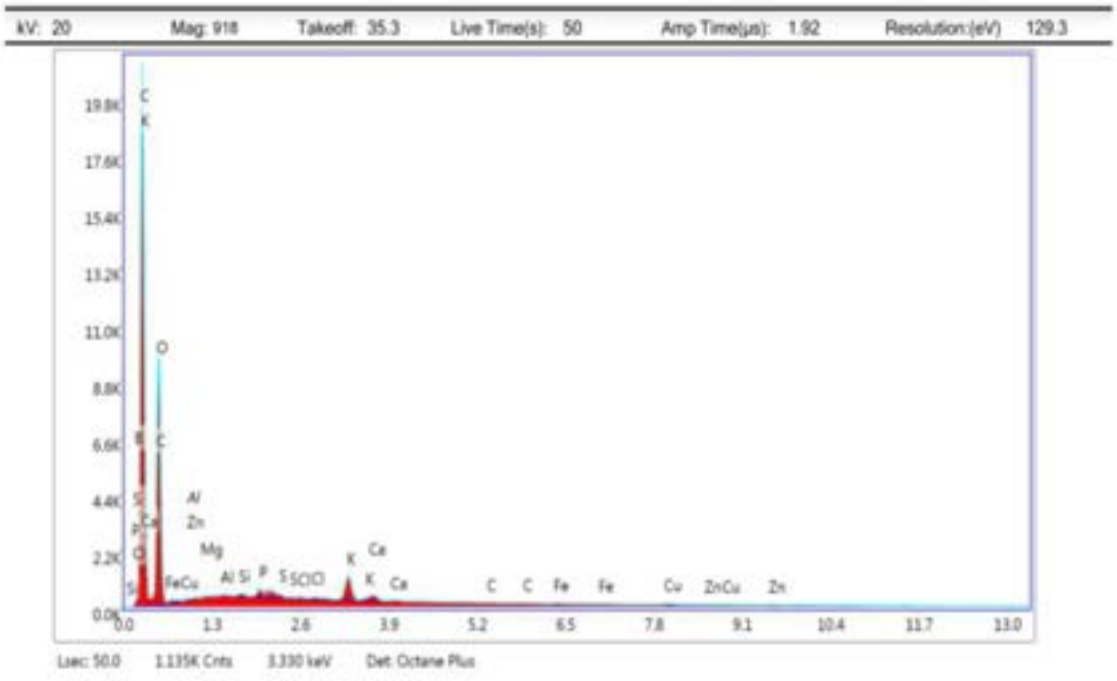
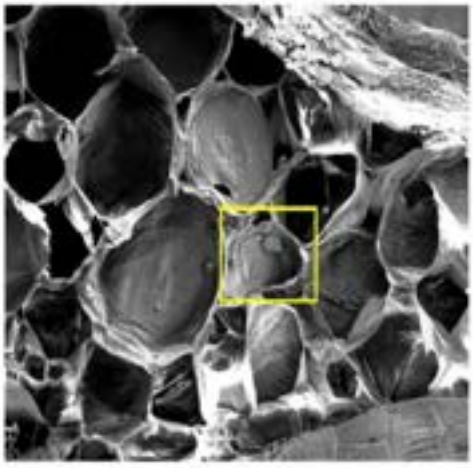


Figure-24

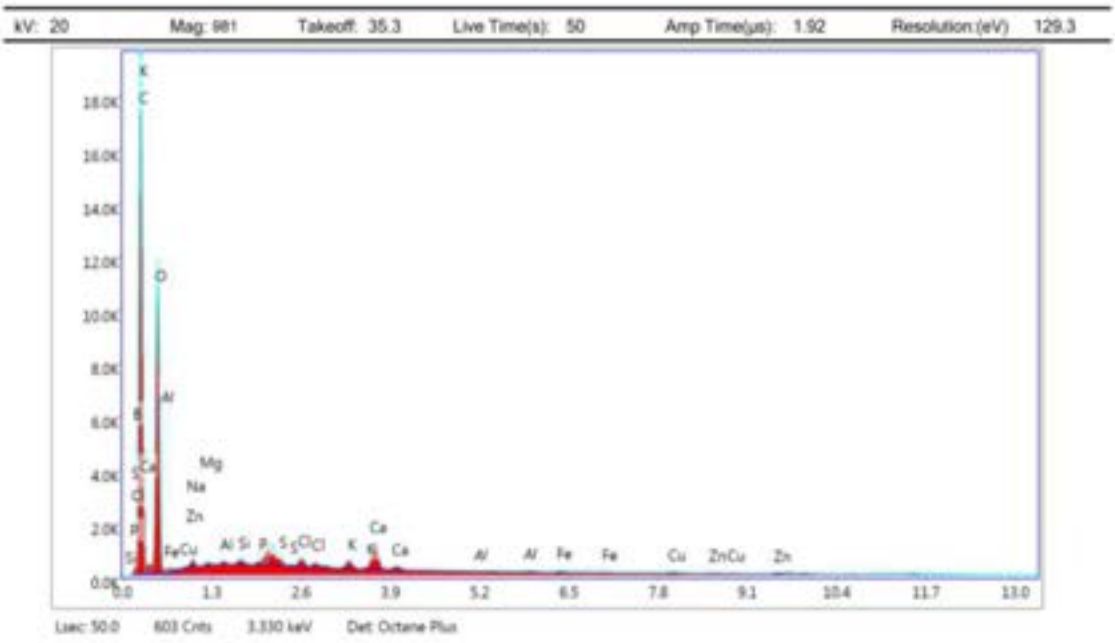
Scanning Electron Micrographs and EDX spectrum of *Strobilanthes alternata* subjected to aluminium treatment

LEAF



Distribution of individual elements present in selected area of SEM

Elements	Weight %	Atomic %
CK	50.44	59.11
OK	43.3	38.09
NaK	0.3	0.18
MgK	0.08	0.05
AlK	1.67	0.15
SiK	0.11	0.06
PK	0.19	0.08
SK	0.04	0.02
ClK	0.32	0.13
KK	0.4	0.15
CaK	1.58	0.55
BK	0.99	1.29
FeK	0.16	0.04
CuK	0.26	0.06
ZnK	0.16	0.03



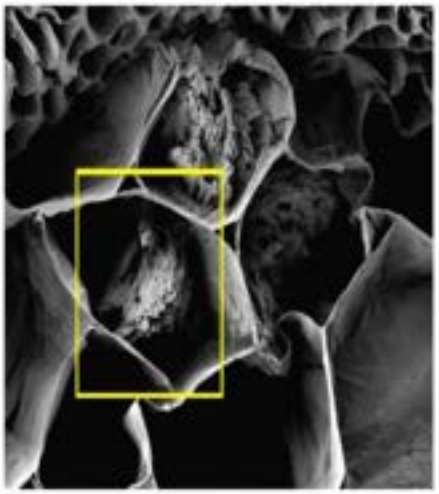
EFFECT OF CHROMIUM

Root tissues of plants treated with chromium showed maximum chromium atoms compared to other tissues (Fig-25). Chromium treatment resulted in remarkable variation in the distribution of atoms in the root tissues of *Strobilanthes alternata* . In the root tissues, due to chromium treatment there is no increase in the distribution of elements whereas chlorine and iron decreased significantly. The other elements sodium, magnesium, silicon, potassium, phosphorous, sulfur, calcium, copper and zinc remained the same as control. In stem tissues, chromium treatment showed increase of magnesium, phosphorous and calcium compared to control(Fig-26). Distribution of phosphorous atoms has doubled and calcium increased upto five times in the stem tissues. Leaf tissues treated with chromium showed high chromium content (Fig-27). Magnesium and aluminium increased in leaf tissues whereas sodium, chlorine, potassium, calcium, iron, copper and zinc was decreased.

Figure-25

Scanning Electron Micrographs and EDX spectrum of *Strobilanthes alternata* subjected to chromium treatment

ROOT



Distribution of individual elements present in selected area of SEM

Elements	Weight %	Atomic %
CK	46.95	55.14
OK	47.39	41.78
NaK	0	0
MgK	0.06	0.03
AlK	0	0
SiK	0.02	0.01
PK	0.14	0.06
SK	0.04	0.02
ClK	0.06	0.02
KK	0.65	0.23
CaK	1.82	0.64
CrK	0.75	0.05
BK	1.43	1.86
FeK	0.18	0.04
CuK	0.29	0.06
ZnK	0.1	0.02

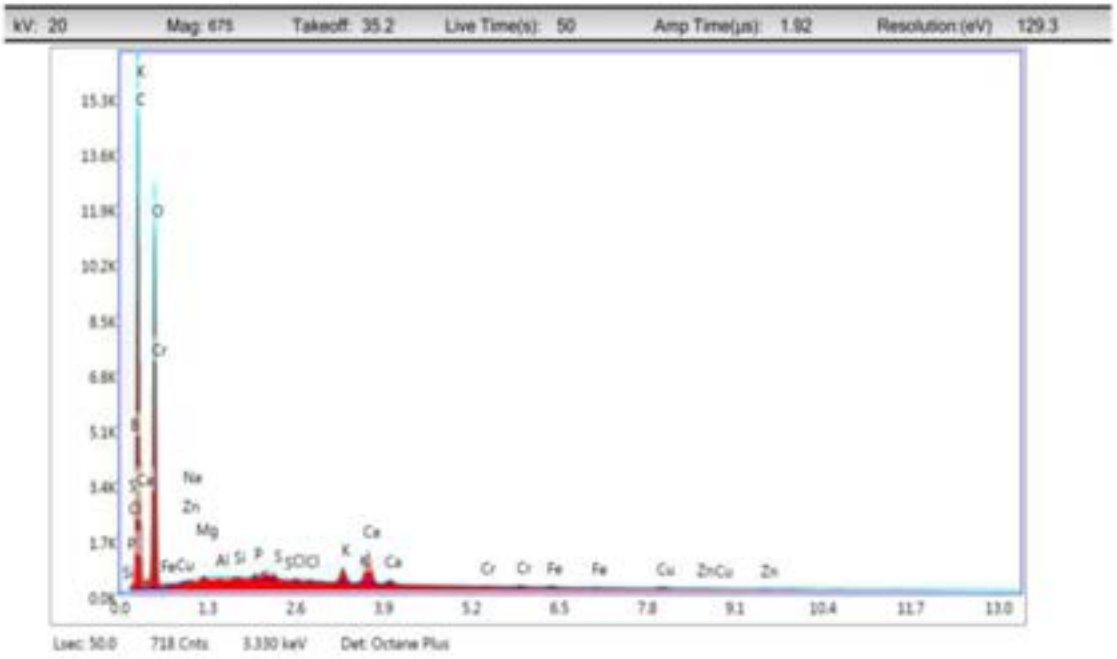
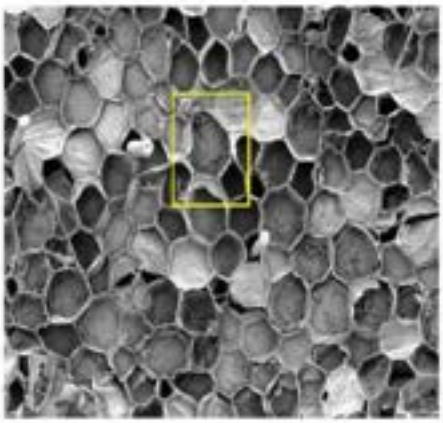


Figure-26

Scanning Electron Micrographs and EDX spectrum of *Strobilanthes alternata* subjected to chromium treatment

STEM



Distribution of individual elements present in selected area of SEM

Elements	Weight %	Atomic %
CK	46.05	54.86
OK	46.03	41.16
NaK	0.12	0.08
MgK	0.04	0.02
AlK	0.05	0.03
SiK	0.03	0.02
PK	0.93	0.43
SK	0.04	0.02
ClK	0.12	0.05
KK	1.84	0.67
CaK	2.69	0.56
CrK	0.55	0.45
BK	1.2	1.58
FeK	0.07	0.02
CuK	0.22	0.05
ZnK	0.02	0.01

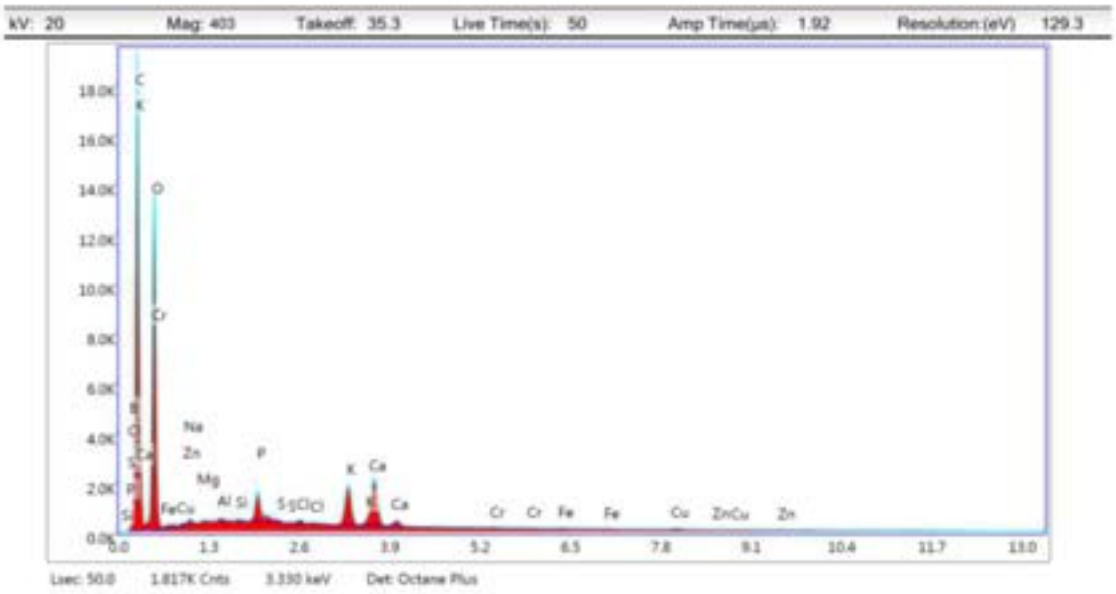
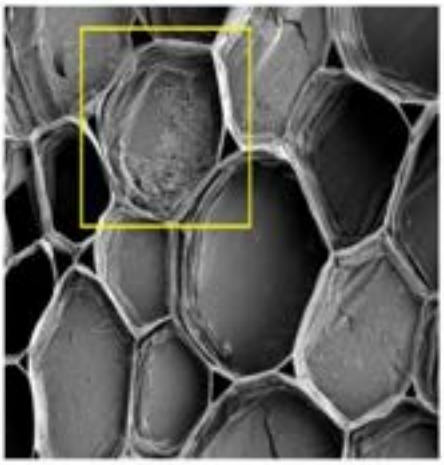


Figure-27

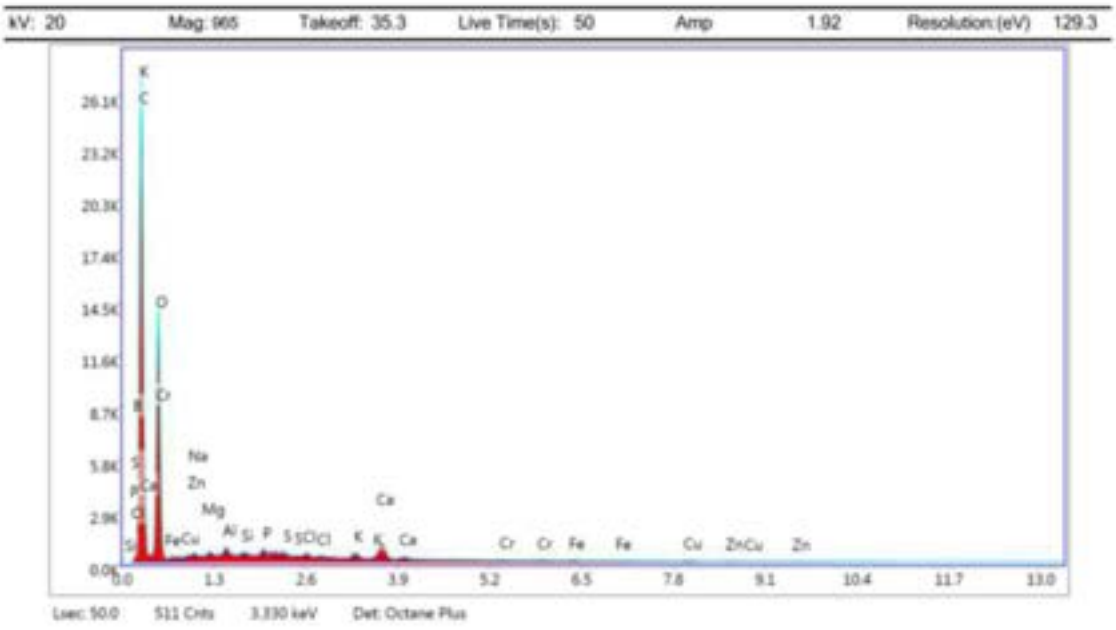
Scanning Electron Micrographs and EDX spectrum of *Strobilanthes alternata* subjected to chromium treatment

LEAF



Distribution of individual elements present in selected area of SEM

Elements	Weight %	Atomic %
CK	53.27	61.1
OK	42.35	36.47
NaK	0	0
MgK	0.16	0.09
AlK	0.28	0.14
SiK	0.11	0.06
PK	0.28	0.13
SK	0.12	0.05
ClK	0.16	0.06
KK	0.2	0.1
CaK	1.12	0.38
CrK	0.49	0.04
BK	1.03	1.31
FeK	0.1	0.02
CuK	0.16	0.03
ZnK	0.09	0.02



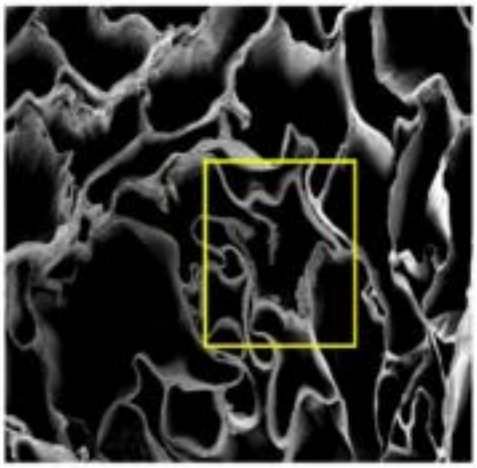
EFFECT OF MERCURY

In *Strobilanthes alternata* treated with mercury, maximum mercury atoms were present in the root tissues (Fig-28). No significant increase or decrease were observed in any atoms present. Distribution of mercury atoms were seen in the stem tissues of mercury treated plant(Fig-29). There is no increase in the distribution of other atoms but sulfur and iron get reduced. Sodium, magnesium, aluminium, silicon, phosphorous, chlorine, potassium, calcium, boron, copper and zinc remained unchanged. Leaf tissues showed maximum mercury atoms and no change of distribution of other atoms (Fig-30). Distribution of mercury atoms were seen in the stem tissues of mercury treated plants. There is no increase in other atoms distribution, but sulfur and iron get reduced. Sodium, magnesium, aluminium, silicon, phosphorous, chlorine, potassium, calcium, boron, copper and zinc remained unchanged. Maximum mercury atoms were present in the leaf tissue treated with mercury and there is no atoms increased. Iron and zinc showed significant (10 fold) reduction. Sodium, magnesium, aluminium, silicon, phosphorous, sulphur, chlorine, potassium, calcium, boron and copper remained same as the control.

Figure-28

Scanning Electron Micrographs and EDX spectrum of *Strobilanthes alternata* subjected to mercury treatment

ROOT



Distribution of individual elements present in selected area of SEM

Elements	Weight %	Atomic %
CK	46.95	55.14
OK	47.39	41.78
NaK	0	0
MgK	0.06	0.03
AlK	0	0
SiK	0.02	0.01
PK	0.14	0.06
SK	0.04	0.02
ClK	0.06	0.02
KK	0.65	0.23
CaK	1.82	0.64
HgK	0.92	0.07
BK	1.43	1.86
FeK	0.18	0.04
CuK	0.29	0.06
ZnK	0.1	0.02

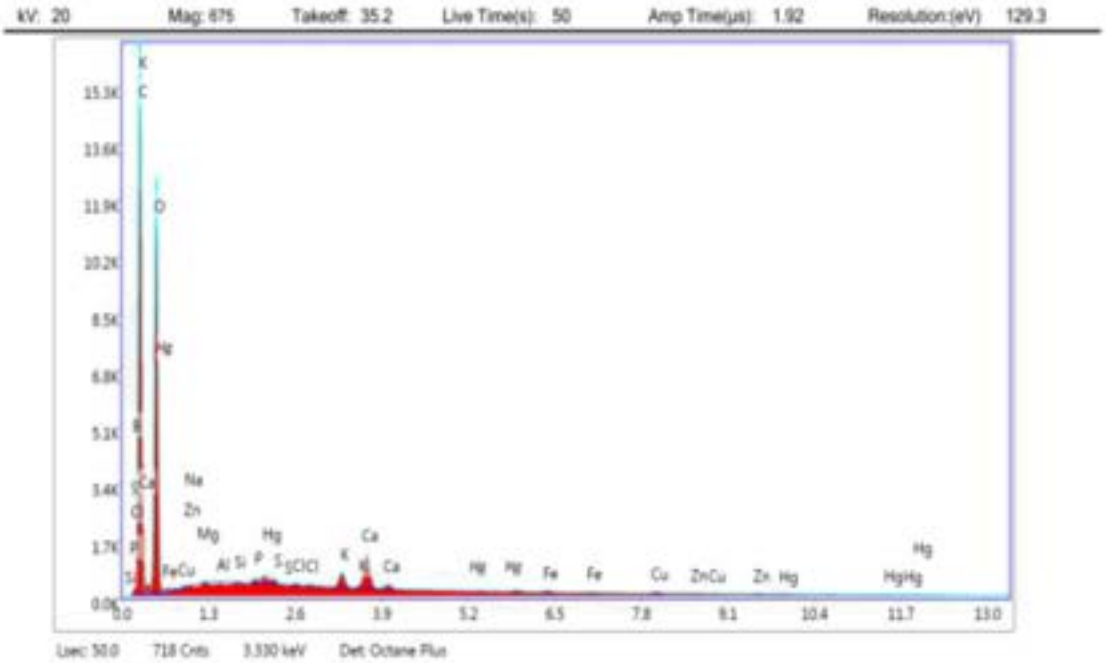
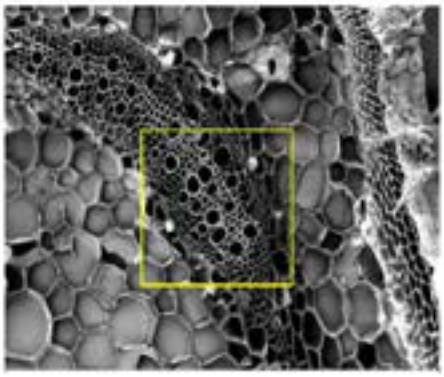


Figure-29

Scanning Electron Micrographs and EDX spectrum of *Strobilanthes alternata* subjected to mercury treatment

STEM



Distribution of individual elements present in selected area of SEM

Elements	Weight %	Atomic %
CK	50.46	58.7
OK	43.73	38.19
NaK	0.09	0.06
MgK	0.01	0.01
AlK	0.03	0.01
SiK	0.04	0.02
PK	0.34	0.15
SK	0.04	0.02
ClK	.19	0.08
KK	2.39	0.86
CaK	0.6	0.21
HgK	0.46	0.05
BK	1.21	1.56
FeK	0.11	0.03
CuK	0.23	0.05
ZnK	0.05	0.01

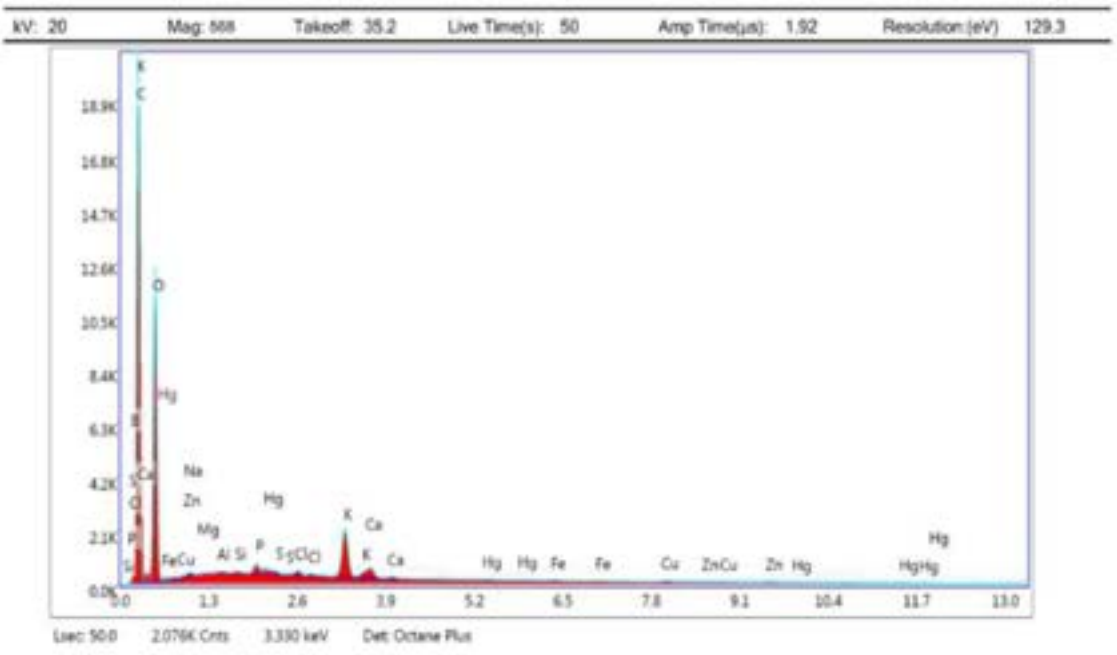
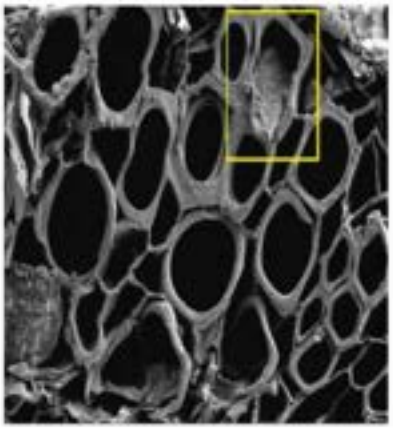


Figure-30

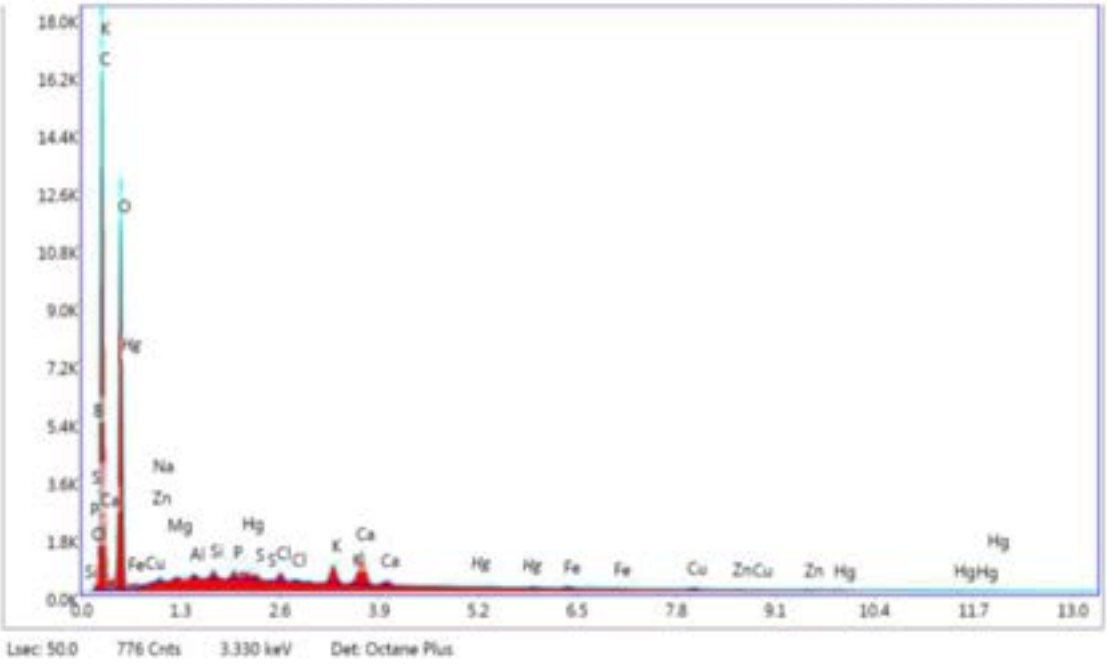
Scanning Electron Micrographs and EDX spectrum of *Strobilanthes alternata* subjected to mercury treatment

LEAF



Distribution of individual elements present in selected area of SEM

Elements	Weight %	Atomic %
CK	47.9	56.34
OK	46.03	40.65
NaK	0.09	0.06
MgK	0.06	0.03
AlK	0.08	0.04
SiK	0.18	0.09
PK	0.25	0.11
SK	0.08	0.04
ClK	0.26	0.11
KK	0.78	0.28
CaK	1.88	0.61
HgK	0.72	0.07
BK	1.11	1.45
FeK	0.2	0.05
CuK	0.26	0.06
ZnK	0.09	0.02



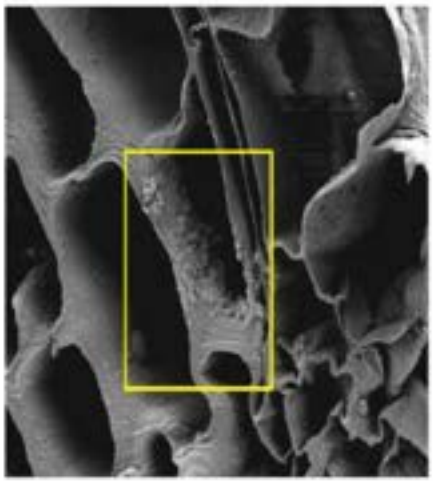
EFFECT OF ZINC

Zinc treatment resulted in maximum zinc atoms in the root compared to the control. Increase of magnesium, silicon, phosphorous and sulphur atoms was observed in the root tissue of *Strobilanthes alternata* due to zinc treatment (Fig-31). Potassium and copper atoms were reduced. Stem tissues also showed high zinc atoms compared to the control(Fig-32). In the stem tissues, magnesium, phosphorous and calcium were increased. Phosphorous and calcium increased about seven fold and six fold respectively. Sulphur, chlorine and potassium decreased significantly in stem tissues. Leaf tissues showed maximum zinc compared to control(Fig-33). Increase of calcium and copper was seen and the reduction occurred in the distribution of aluminium, silicon, sulphur, potassium and iron. The atoms of sodium, magnesium, phosphorous, chlorine, boron and zinc remained unaltered.

Figure-31

Scanning Electron Micrographs and EDX spectrum of *Strobilanthes alternata* subjected to zinc treatment

ROOT



Distribution of individual elements present in selected area of SEM

Elements	Weight %	Atomic %
CK	51.28	59.29
OK	43.89	38.09
NaK	0	0
MgK	0.15	0.09
AlK	0.08	0.04
SiK	0.21	0.11
PK	0.3	0.13
SK	0.16	0.07
ClK	0.1	0.04
KK	0.43	0.15
CaK	1.14	0.39
BK	1.14	1.46
FeK	0.14	0.04
CuK	0.11	0.02
ZnK	0.95	0.08

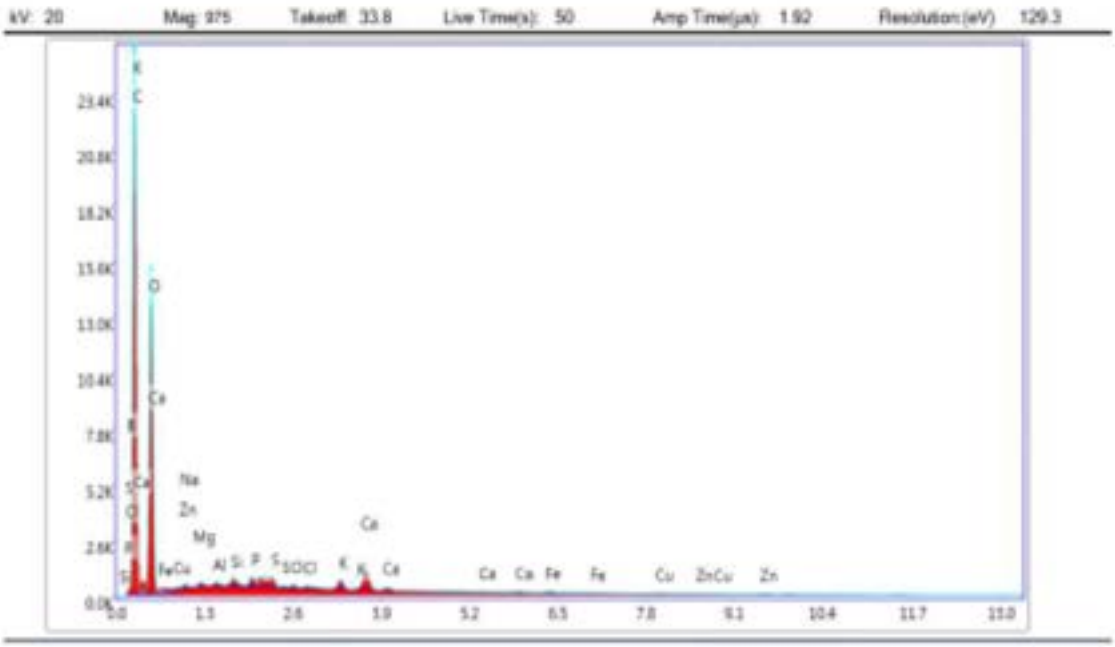
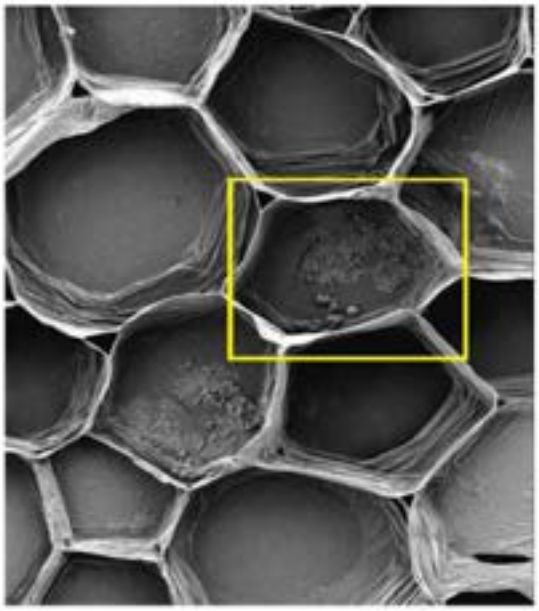


Figure-32

Scanning Electron Micrographs and EDX spectrum of *Strobilanthes alternata* subjected to zinc treatment

STEM



Distribution of individual elements present in selected area of SEM

Elements	Weight %	Atomic %
CK	44.09	53.43
OK	45.67	41.55
NaK	0	0
MgK	0.07	0.04
AlK	0.03	0.02
SiK	0.07	0.03
PK	2.06	0.97
SK	0.07	0.03
ClK	0.05	0.02
KK	0.4	0.15
CaK	4.54	1.65
BK	1.39	1.87
FeK	0.16	0.04
CuK	0.3	0.07
ZnK	1.1	0.12

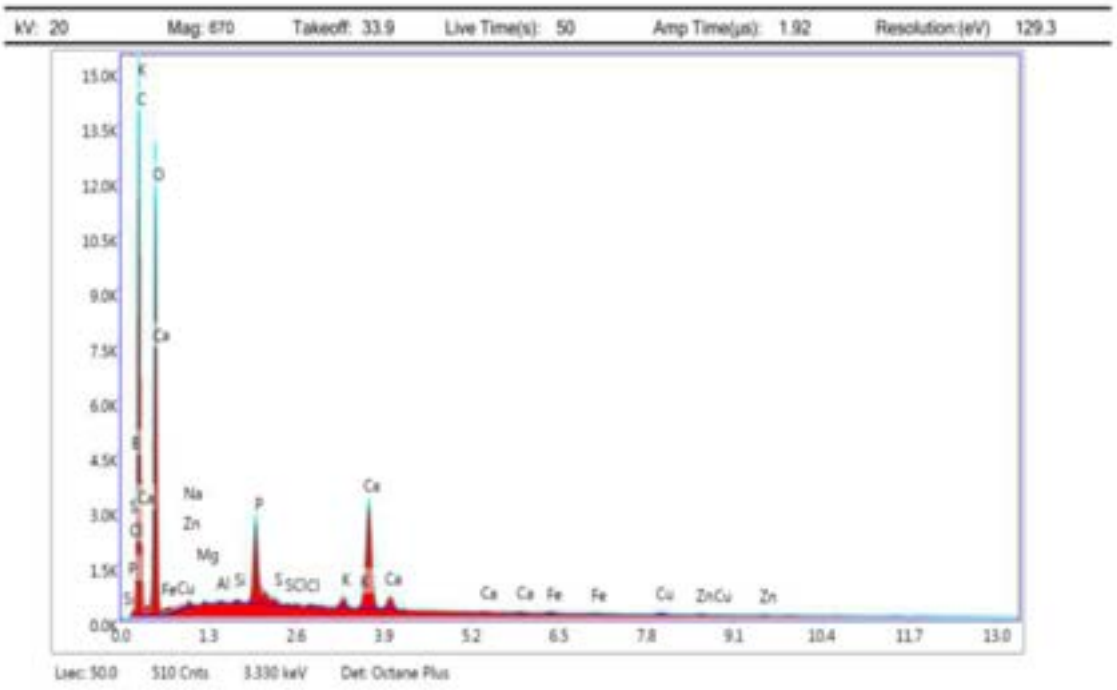
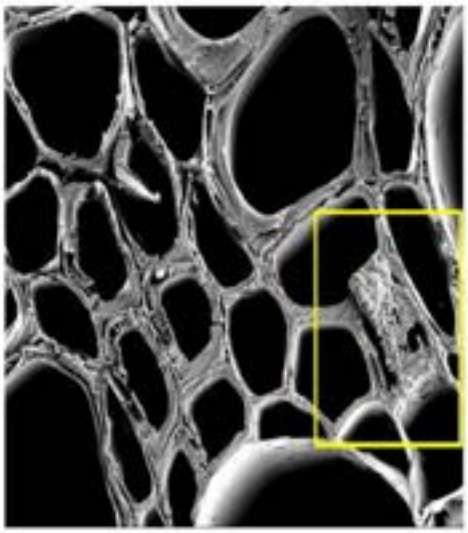


Figure-33

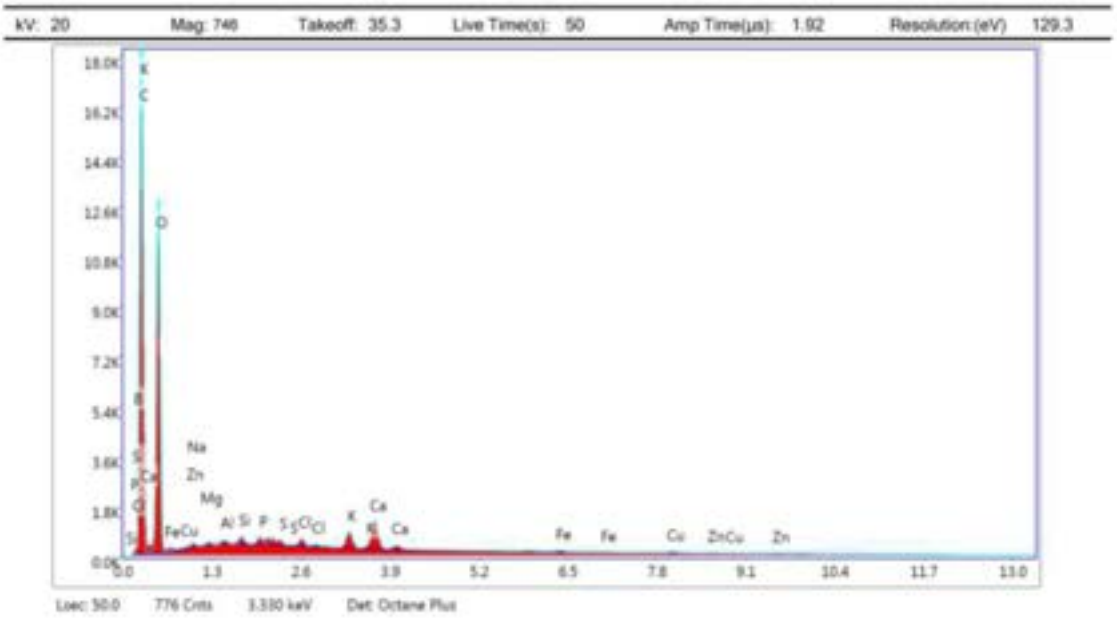
Scanning Electron Micrographs and EDX spectrum of *Strobilanthes alternata* subjected to zinc treatment

LEAF



Distribution of individual elements present in selected area of SEM

Elements	Weight %	Atomic %
CK	48.49	56.98
OK	45.18	39.86
NaK	0.12	0.07
MgK	0.02	0.01
AlK	0	0
SiK	0.03	0.02
PK	0.34	0.16
SK	0.04	0.02
ClK	0.2	0.08
KK	0.6	0.22
CaK	2.25	0.79
BK	1.2	1.57
FeK	0.15	0.04
CuK	0.5	0.11
ZnK	0.89	0.09



Dry weight percentage

Distribution of dry weight percentage of plant parts of *Strobilanthes alternata* of various treatments are shown in table 7 ;Fig-34. Dry weight percentage of root registered gradual but insignificant increase due to Al treatments. Stem and leaf tissues maintained somewhat similar trend same as control. Dry weight percentage of leaves of plant treated with Al showed slight increase during all developmental stages compared to the control. In the case of chromium, insignifant but slight increase of dry weight percentage were observed in root tissues compared with the control. During the initial stage in stem tissues increase in dry weight percentage can be observed, which then on resulted in negligible changes compared to control. Slight increase in dry weight percentage was observed in leaves of chromium treated plants compared to the control. In plants treated with mercury insignificant but gradual reduction in dry weight percentage were observed during all intervals. Same trend of gradual reduction were observed in stem tissues also due to mercury treatment. Leaf dry weight percentage of plants treated with mercury showed gradual increase compared to control. Compared to control and other heavy metal treatments zinc exhibited significant changes in dry weight percentage. Linear and significant increase of dry weight percentage was observed in the root tissues of the plant treated with zinc. Increase in dry weight percentage was maximum during last stage of growth. Slight increase of dry weight percentage was observed in the stem tissues of plant treated with Zn. Leaf tissues also exhibited significant increase ($P < 0.01$) of dry weight percentage compared to the control.

Table-7

Effect of aluminium, chromium, mercury and zinc on dry weight percentage in *Strobilanthes alternata*.

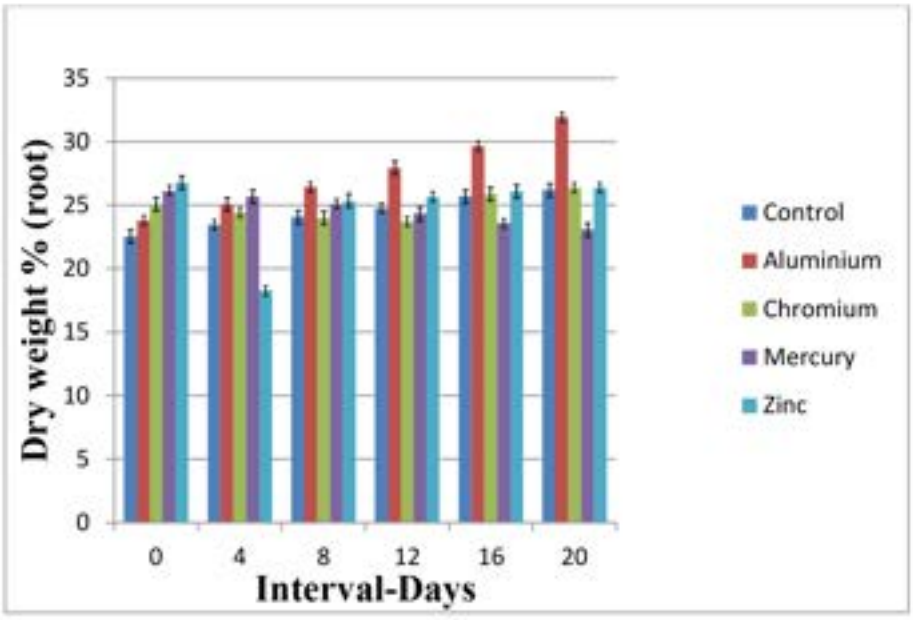
Dry weight percentage

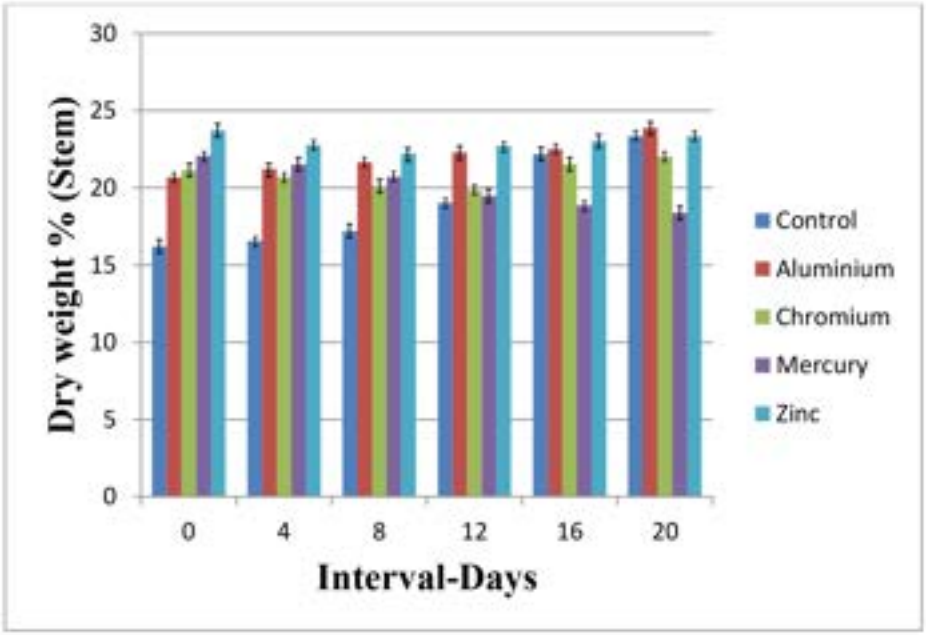
Treatments	Tissues	Interval -Days					
		0	4	8	12	16	20
Control	Root	22.53±0.16	23.47±0.21	24.10±0.36	24.77±0.33	25.70±0.31	26.21±0.11
	Stem	16.21±0.36	16.56±0.54	17.2±0.78	19.04±0.78	22.18±0.13	23.34±0.23
	Leaf	13.39±0.41	14.19±0.43	14.76±0.63	15.45±0.75	16.13±0.11	17.22±0.68
Aluminium (400µM)	Root	23.80±0.32	25.08±0.59	26.44±0.26	27.94±0.24	29.64±0.36	31.93±0.81
	Stem	20.70±0.49	21.23±0.40	21.67±0.31	22.31±0.54	22.49±0.52	23.89±0.50
	Leaf	16.01±0.21	16.52±0.32	16.91±0.71	17.56±0.53	18.01±0.49	18.45±0.73
Chromium (70µM)	Root	24.92±0.39	24.42±0.36	23.98±0.62	23.70±0.70	25.86±0.66	26.38±0.77
	Stem	21.14±0.25	20.72±0.49	20.12±0.51	19.86±0.88	21.54±0.53	22.05±0.82
	Leaf	17.96±0.21	17.38±0.37	18.90±0.33	19.28±0.19	19.50±0.39	21.18±0.24
Mercury (20µM)	Root	26.12±0.19	25.68±0.51	25.11±0.23	24.34±0.28	23.60±0.41	23.10±0.67
	Stem	22.08±0.36	21.52±0.54	20.72±0.19	19.50±0.75	18.86±0.34	18.40±0.92
	Leaf	18.40±0.43	17.74±0.39	17.58±0.22	18.46±0.63	18.32±0.27	19.12±0.64
Zinc (250µM)	Root	26.78±0.22	28.28±0.57	29.32±0.26	29.64±0.52	31.10±0.16	33.36±0.52
	Stem	21.72±0.40	22.78±0.66	22.22±0.47	22.72±0.23	23.02±0.87	23.34±0.39
	Leaf	18.26±0.52	18.28±0.42	19.62±0.33	23.02±0.88	25.32±0.21	29.84±0.44

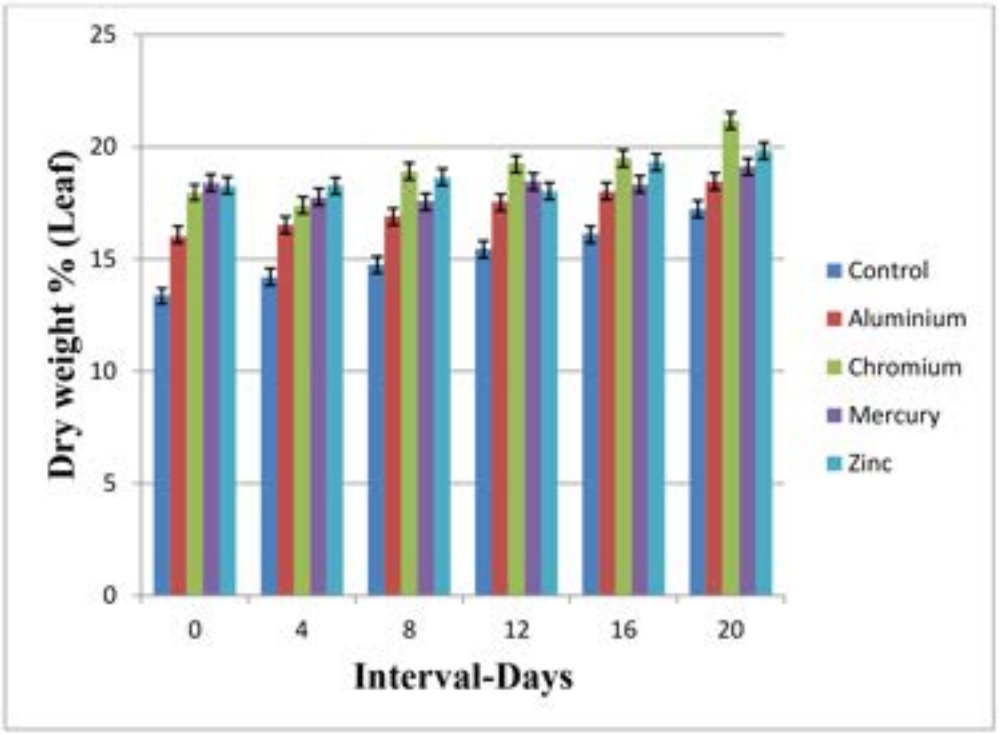
Values given are mean of 5 replicates ± SE

Figure-34

Effect of heavy metals on dry weight percentage in *Strobilanthes alternata*






25
20
15
10
5
0
Dry weight % (Leaf)
0
4
8
12
16
20
Interval-Days
Control
Aluminium
Chromium
Mercury
Zinc

Protein content

Protein content of tissues of *Strobilanthes alternata* showed significant variation among treatments with aluminium, chromium, mercury and zinc (Table-8,Fig-35). From the first day of treatment itself protein content of root tissues was increased significantly ($P < 0.01$) compared to control. During all stages of growth, protein of plants treated with aluminium increased continuously in all intervals.

Plants treated with aluminium showed increase in protein content of stem compared to the control. The increase of protein of the stem was not much significant like root. Protein content of leaf tissues of aluminium treated plants showed very high protein content at initial stages of growth compared to control.

Treatment with chromium exhibited high protein content of all parts of the plant during all stages of growth compared to control. Stage to stage increase of protein content was insignificant in root tissues. On 20^{th} day of treatment, root tissues showed maximum protein content due to chromium treatment. Stem tissues of plants treated with chromium showed more or less similar trend of increase in protein content compared to control. At initial stages of growth leaf tissues showed maximum increase of protein content but in chromium treated plants at final stages increase was not much significant.

Treatment with mercury showed maximum increase of protein content in root tissues compared to the control and other treatments. Increase of protein content was significant ($P < 0.01$) and maintained same trend during all intervals in root tissues. Protein content of stem of plants treated with mercury was significantly increased during the final stages of growth which is more or less similar to that of leaf tissues in which protein content was increased gradualy and significantly.

Zinc treatment resulted in significant increase of protein content during all stages of growth in root tissues in comparison with control. During the initial stages of growth stem tissues exhibited increase of protein content but a significant reduction was observed during the final stages of growth. Stem tissues of zinc treated plants exhibited only negligible changes in protein content. But the leaf tissues showed significant increase of protein content in all stages of growth compared to the control. During growth on 4^{th} day significant increase of protein content was observed in plants treated with zinc ($P < 0.01$) followed by meager but gradual reduction in protein content.

Table-8

Effect of heavy metals on total protein content of root, stem and leaf in *Strobilanthes alternata*

Protein mg/g dry weight

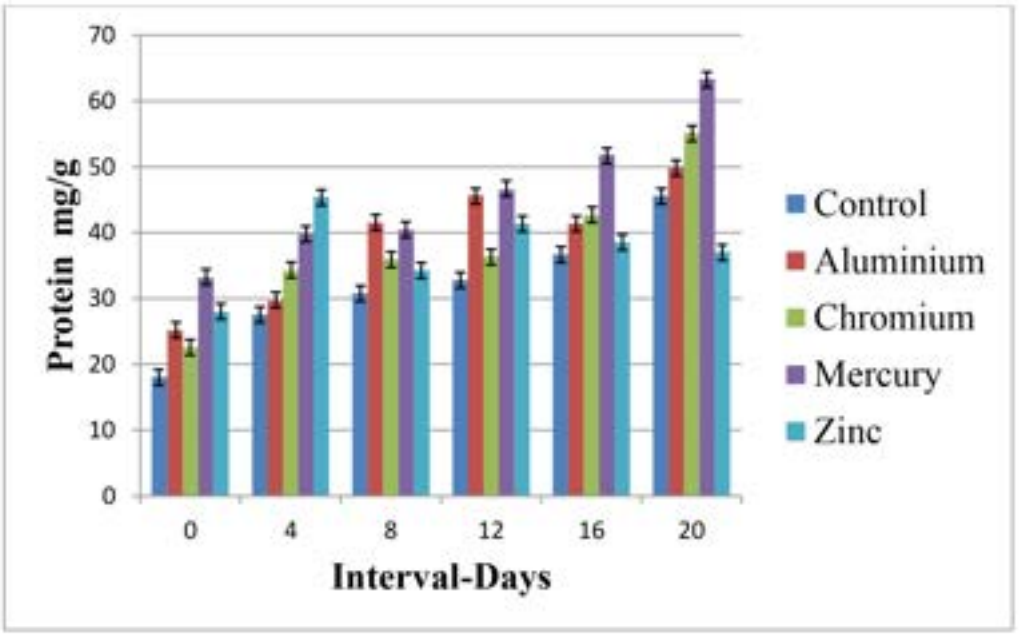
Treatments	Tissues	Intervals-Days					
		0	**4**	**8**	**12**	**16**	**20**
Control	Root	18.10±2.56	27.54±0.14	30.78±0.97	32.83±2.37	36.70±1.64	45.61±2.75
	Stem	11.01±0.24	13.14±3.4	19.90±1.07	20.10±3.1	30.56±1.53	30.26±1.63
	Leaf	10.19±0.22	12.23±1.6	15.12±2.3	15.77±2.02	18.25±2.66	20.24±3.8
Aluminium (400µM)	Root	25.20±1.91	29.64±1.82	41.54±3.7	45.71±3.6	41.35±2.7	49.83±4.7
	Stem	16.46±1.5	16.01±2.2	20.63±1.8	25.74±3.4	28.34±3.3	25.85±5.1
	Leaf	18.75±0.67	15.25±1.25	17.73±1.09	25.15±1.64	28.57±1.8	33.98±4.01
Chromium (70µM)	Root	22.58±2.1	34.22±3.9	35.98±2.2	36.40±3.8	42.70±4.6	55.13±2.6
	Stem	16.18±3.2	21.52±1.22	23.72±1.7	25.71±2.9	24.74±3.23	33.49±3.02
	Leaf	17.93±1.7	20.95±2.04	24.66±0.99	25.17±2.5	23.11±2.5	25.0±2.7
Mercury (20µM)	Root	33.16±0.95	39.79±1.3	40.44±3.86	46.54±4.7	51.83±5.73	63.34±7.1
	Stem	14.44±1.76	15.86±2.07	20.58±1.53	20.78±2.6	38.34±7.2	38.13±5.3
	Leaf	13.09±1.38	18.25±2.22	19.43±1.84	22.73±3.6	35.16±3.5	32.21±2.9
Zinc (250µM)	Root	27.94±2.23	45.41±3.96	34.29±2.24	41.35±4.6	38.57±4.92	37.10±5.9
	Stem	19.77±1.66	20.52±1.93	23.93±1.6	27.15±2.1	25.83±4.6	22.75±6.3
	Leaf	16.64±3.1	28.11±3.7	25.17±2.57	24.92±2.2	24.42±2.4	27.29±2.4

Values given are mean of 5 replicates ± SE

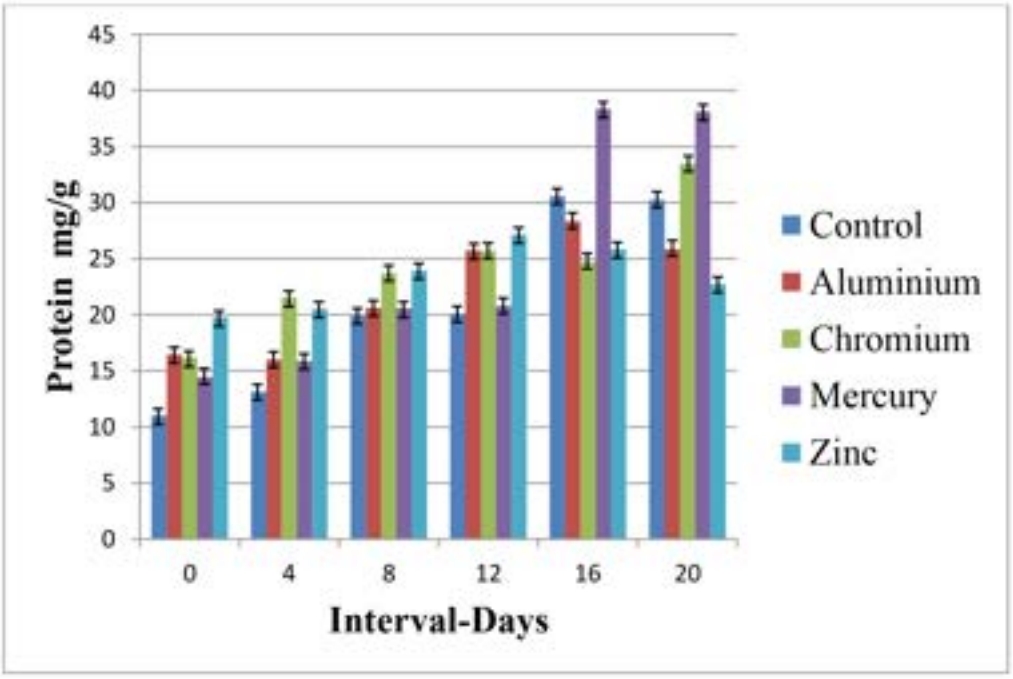
Figure-35

Effect of heavy metals on total protein content of root, stem and leaf in *Strobilanthes alternata*:A-Root,B-Stem,C-Leaf

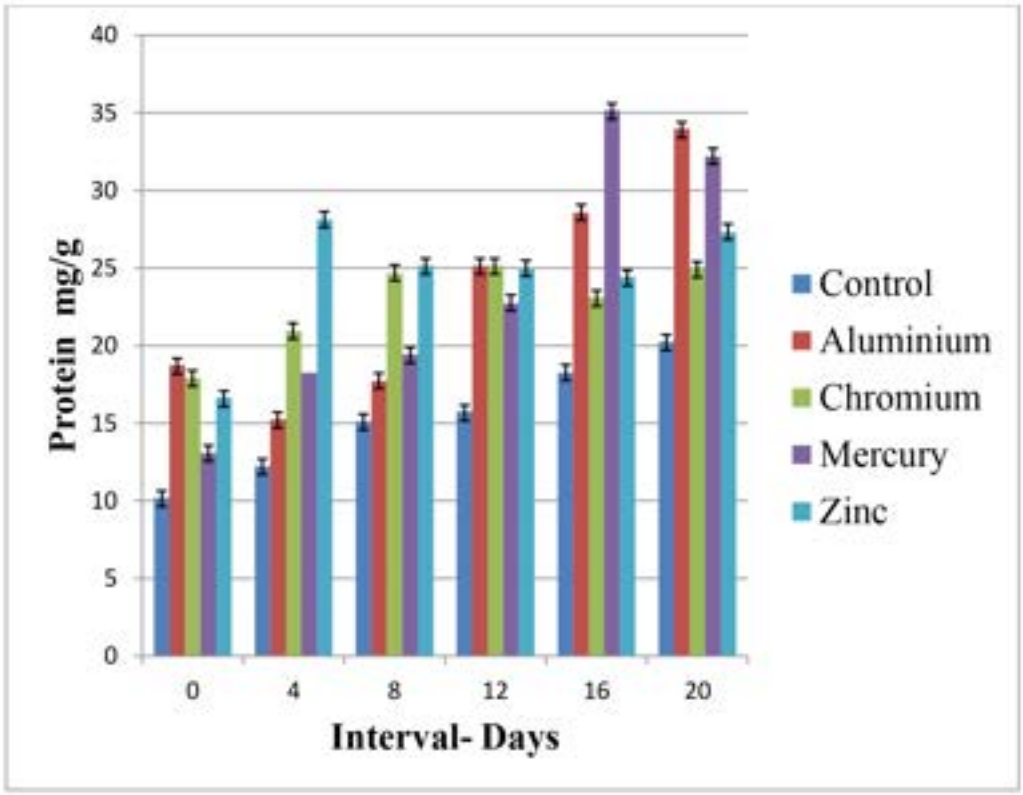
A



B



C


40
35
30
25
20
15
10
5
0
Protein mg/g
0
4
8
12
16
20
Interval- Days
Control
Aluminium
Chromium
Mercury
Zinc

PROLINE CONTENT

Increasing trend of proline content was observed in stem, root and leaf of *Strobilnthes alternata* treated with aluminium, chromium, mercury and zinc compared to control (Table-9,Fig-36). Plants treated with aluminium showed increase in proline content compared to control. Proline content of control plant roots showed only a slight increase. In the roots of plants treated with aluminium, increase was gradually higher upto 12th day, but a slight decline of proline content was observed after the 12th day of treatment. When compared to control stem of plants treated with aluminium showed increase in proline content upto 12th day of treatment and maintained the same trend on further days. Proline content of the leaf tissue of the control plants exhibited a decrease during all stages of growth. Increase in proline content was tremendous in leaf tissues upto 8th day and increase was significant at each intervals and maintained similar trend afterwards. Leaf of aluminium treated plants exhibited a continuos increase of proline content during the entire period of growth.

Chromium treatment resulted in exponential increase in the root in general and particularly during 16th and 20th day of treatment. Similar trend in proline content was shown by stem tissues also. But in comparison with root, stem contain more proline than the root. Proline content of leaf showed about doubling of proline content during growth. Stem, root and leaves of plants treated with chromium showed an increasing trend of proline content compared to the control. All these plant tissues showed same pattern of increase at all stages of growth. In the root increase of proline content was more than double during 8th day onwards and several fold increase of proline was observed afterwards. Stem tissues also showed similar trend of proline content.

Maximum proline content was present in the leaf tissues of plants treated with mercury where increase was linear during the entire period of growth. Plants exposed to mercury showed gradual increase in proline content compared to

control. Increase was evident during all stages of growth. Two fold increase in proline content was observed during the last stage of growth. The plants maintained this same trend during all intervals.

In the roots of plants treated with zinc, increase in proline content was gradual upto 8^{th} day of treatment. After the initial stage of growth, the plant showed about two fold increase in proline content compared to the control. Stem of zinc exposed plants showed a gradual but significant hike in proline content and maintained the same trend at all intervals. Leaf tissues exhibited about two fold increase of proline content compared to control.

Table-9
Effect of heavy metals on distribution of total proline content in *Strobilanthes alternata*

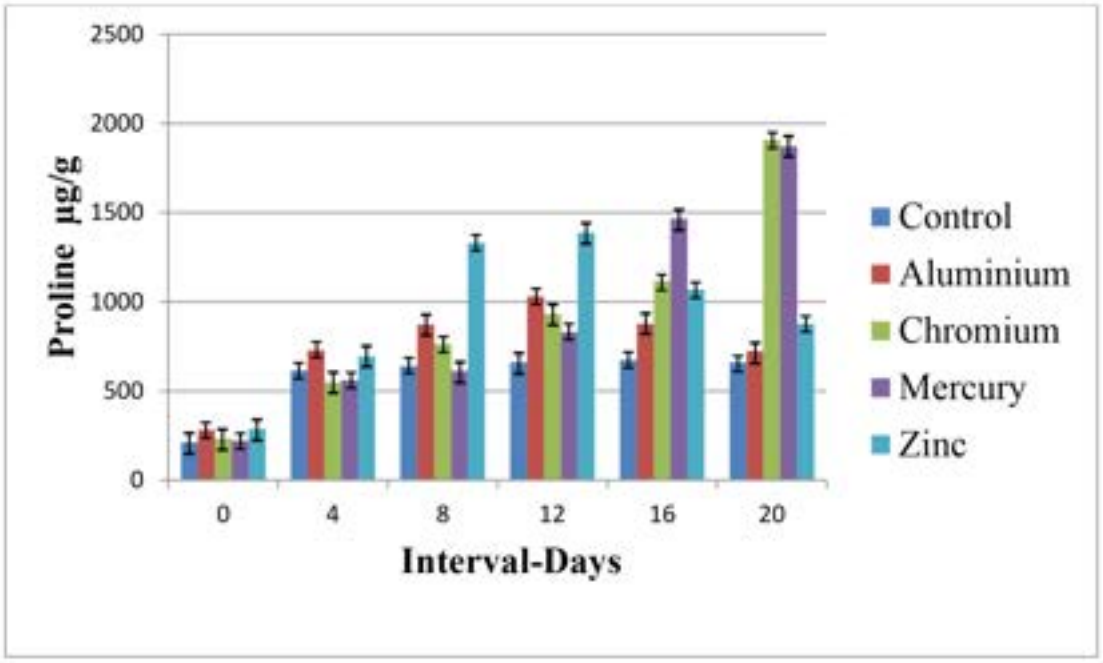
Proline µg/g gry weight

Treatments	Tissues	Intervals-Days					
		0	4	8	12	16	20
Control	Root	213±12	315±10.1	439±16.5	661±14.2	674±10.1	661±9.3
	Stem	118±23.1	174±13.4	223±2.8	455±5.58	564±12.4	616±6.8
	Leaf	431±19.3	441±7.95	463±11.02	623±5.32	319±21	228±4.9
Aluminium (400µM)	Root	283±10.6	729±16	873±5.3	1031±11.7	877±3.1	723±25.3
	Stem	139±11.4	678±12.04	932±16.8	1423±24.3	1027±26.5	1029±22.4
	Leaf	662±2.69	932±11.8	1537±12.3	1584±13.2	1592±12.7	1451±34.6
Chromium (70µM)	Root	232±5.44	548±7.92	763±11.9	932±8.6	1117±29.5	1909±20.1
	Stem	136±21.3	511±26.8	666±1.83	966±12.2	1207±8.5	870±19.2
	Leaf	482±7.44	1005±31.6	1067±24.1	1246±27.1	1676±18.4	1591±10.3
Mercury (20µM)	Root	221±3.78	560±17.8	613±8.32	829±7.41	1466±8.90	1872±22.3
	Stem	174±1.07	580±3.87	646±7.34	887±13.6	1428±23.04	1619±15.35
	Leaf	471±14.5	713±14.2	1313±3.6	1040±26.8	1681±4.68	2060±28.3
Zinc (250µM)	Root	287±8.33	694±17.13	1330±9.34	1388±22.1	1068±10.5	876±14.95
	Stem	162±12.8	759±12.07	1080±13.25	1100±18.4	1451±22.9	1358±3.23
	Leaf	629±4.71	1159±39.6	1044±17.7	1607±6.20	1392±12.32	1310±15.31

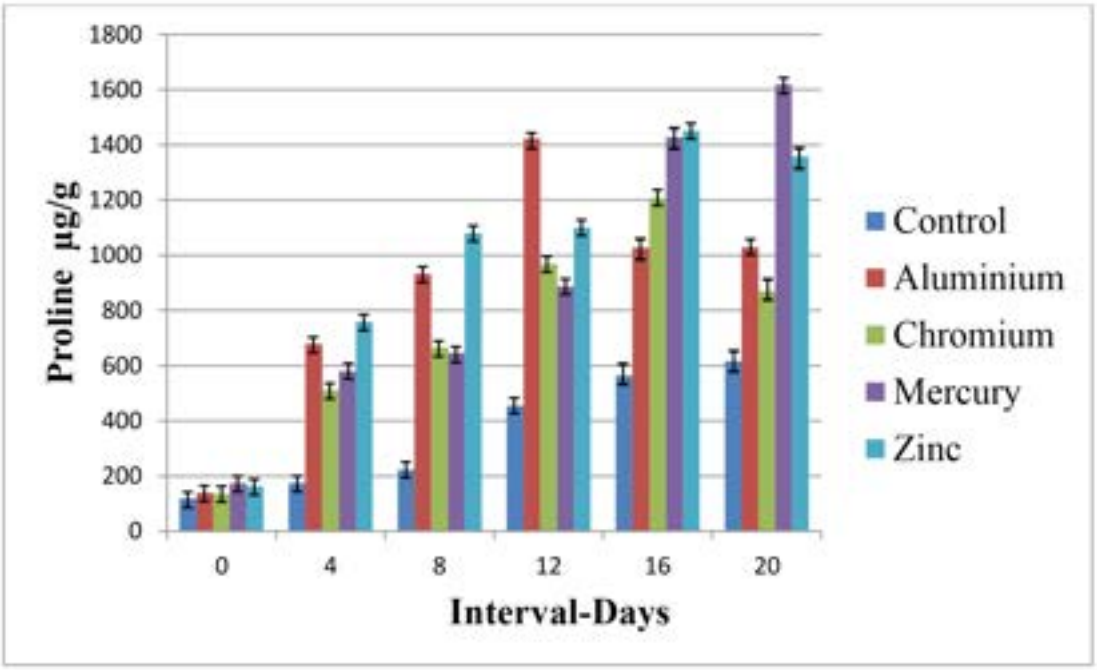
Values given are mean of 5 replicates ± SE

Figure- 36
Effect of heavy metals on distribution of total proline content in *Strobilanthes alternata*:A-Root,B-Stem,C-Leaf

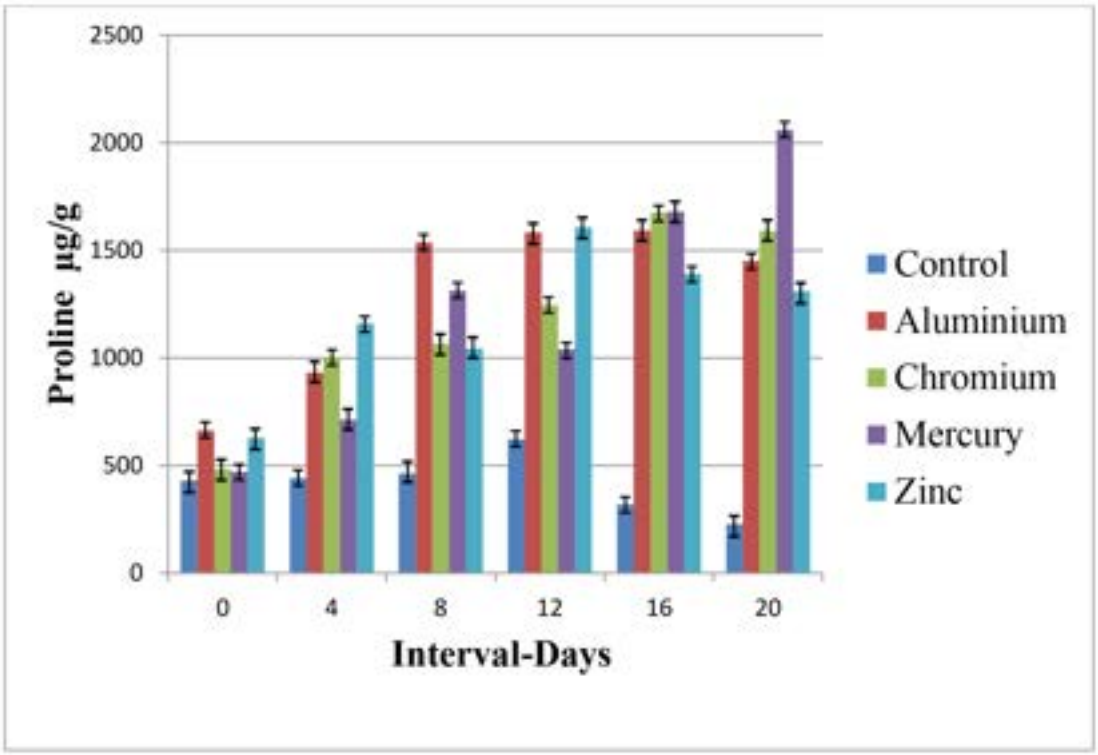
A



B



C


2500
2000
1500
1000
500
0
Proline µg/g
0
4
8
12
16
20
Interval-Days
Control
Aluminium
Chromium
Mercury
Zinc

Malondialdehyde (MDA) content

MDA content of control plants exhibited a linear increase during every stages of growth (20 days) in root, stem and leaves (Table-10:Fig-37). Root tissue of plants treated with aluminium showed significant increase in MDA content compared to the control root and the increase was significant in between the stages during entire period of growth. MDA content remained unchanged and the values were more or less similar to root except significant reduction during final stages of growth. MDA content of leaf tissue was higher than the root and stem upto 8^{th} day thereafter significant increase was observed compared to other tissues-root and stem. Chromium treatment resulted in increase in MDA content in all tissues compared to control. Plants treated with chromium showed only slight increase compared to the control whereas MDA content of the stem showed slight reduction in final stages of growth. Compared to the other tissues, leaf contained more MDA in all stages and between each stages increase was significant. Mercury treatment resulted in negligible changes in MDA content compared to control. Slight increase of MDA content was observed in 16^{th} and 20^{th} day of growth. MDA content of stem tissue showed an increase during 8^{th} to 20^{th} day compared to control. Very high MDA content was present in leaf tissues in all stages compared to root and stem and increase from stage to stage was significant ($P< 0.01$). Root tissue of plants treated with zinc showed significant increase compared to that of mercury treatment. But compared to control MDA content was very high in root tissue. Stem tissue of Zn treated plantsshowed comparitevely reduced MDA content than the root in all stages of growth. MDA content of leaf was very high during the initial stages and the increase from stage to stage was significant. Compared to root and stem Zn treatment resulted in synthesis of very high MDA content of leaf.

Table-10

Effect of heavy metals on MDA content of root, stem and leaf in *Strobilanthes alternata*

MDA µmol/g dry weight

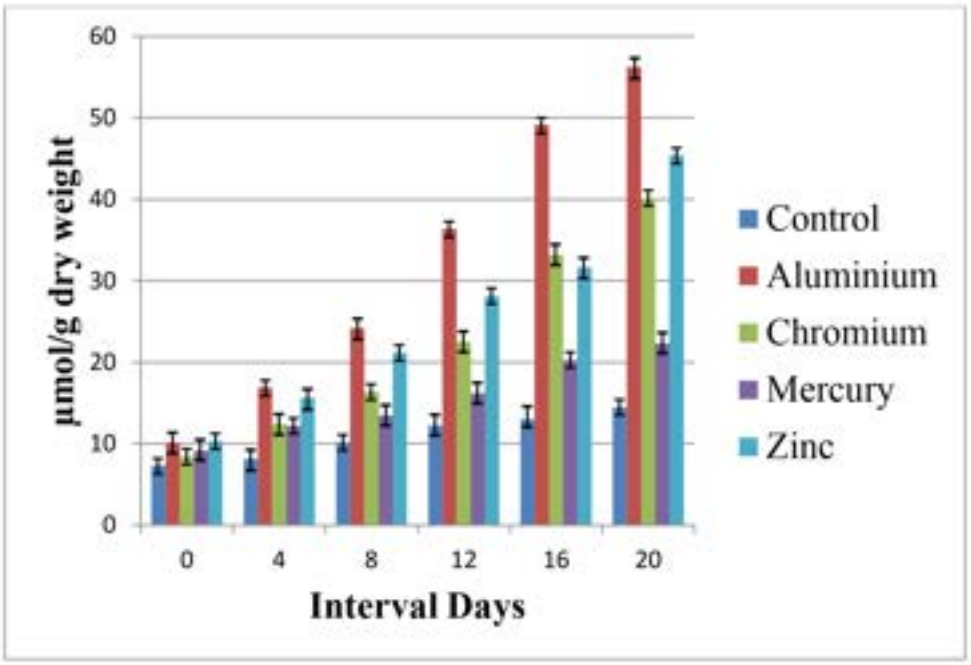
Treatments	Tissues	Intervals-Days					
		0	4	8	12	16	20
Control	Root	7.34±1.8	8.12±2.3	10.18±2.1	12.26±1.9	13.01±2.6	14.5±2.7
	Stem	9.21±3.1	10.18±2.7	11.17±3.2	14.32±2.4	19.63±3.6	21.37±3.9
	Leaf	15.13±1.5	18.22±2.5	21.32±2.2	25.13±2.7	28.68±0.10	26.91±1.7
Aluminium (400µM)	Root	10.13±0.24	16.91±0.79	24.19±2.1	36.38±1.67	49.13±0.81	56.21±0.79
	Stem	11.68±0.71	14.11±2.4	15.32±1.4	19.21±1.1	26.89±0.10	39.20±0.79
	Leaf	16.26±0.37	20.31±0.73	26.46±1.97	32.67±3.1	48.99±2.63	61.13±0.59
Chromium (70µM)	Root	8.41±0.77	12.41±2.7	16.39±0.94	22.51±1.9	33.14±1.83	40.11±3.6
	Stem	10.19±0.74	12.31±1.3	19.90±1.9	26.10±0.83	34.32±0.44	32.91±0.27
	Leaf	19.31±1.09	24.23±2.9	30.02±1.75	39.13±3.7	46.33±0.07	54.20±2.9
Mercury (20µM)	Root	9.13±0.16	12.21±2.02	13.45±2.36	16.18±0.75	20.21±1.17	22.31±1.93
	Stem	10.17±2.8	14.39±1.08	28.81±0.89	31.47±0.11	30.40±2.96	29.98±2.0
	Leaf	19.54±1.84	23.11±0.60	30.38±0.34	41.53±1.9	56.74±3.9	64.91±2.63
Zinc (250µM)	Root	10.34±2.7	15.61±0.92	21.17±1.23	28.14±1.7	31.63±0.09	45.41±2.98
	Stem	8.61±0.10	12.14±1.11	14.18±3.52	20.32±3.2	29.83±1.4	34.93±2.5
	Leaf	21.84±1.63	24.58±1.75	33.19±0.23	39.84±1.96	47.72±3.1	46.63±3.9

Values given are mean of 5 replicates ±S.E

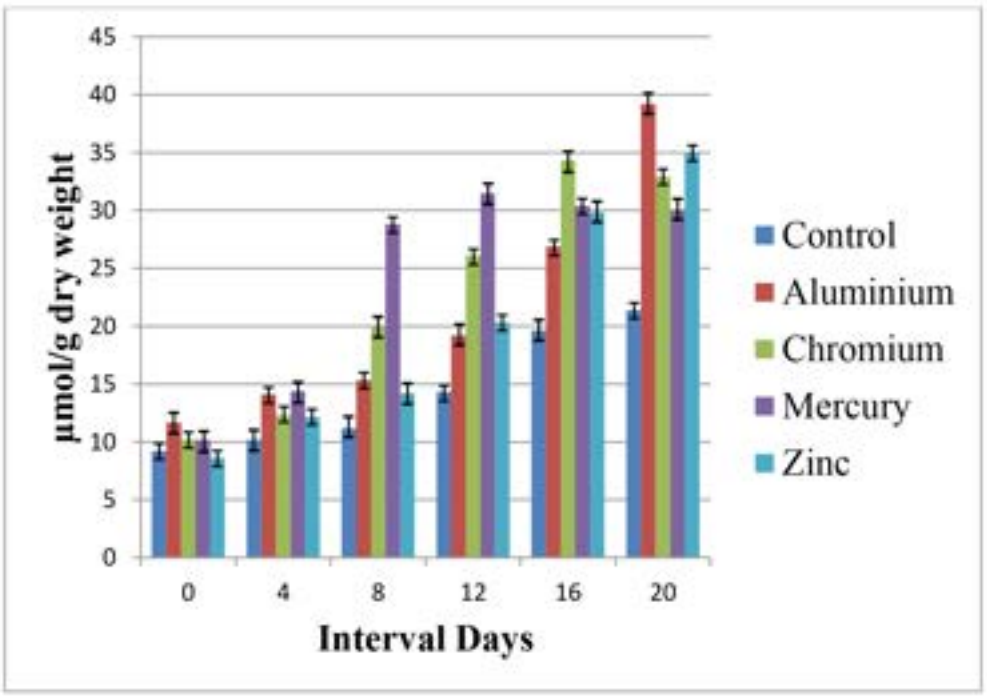
Figure - 37

Effect of heavy metals on MDA content of root, stem and leaf in *Strobilanthes alternata*: A-Root,B-Stem,C-Leaf

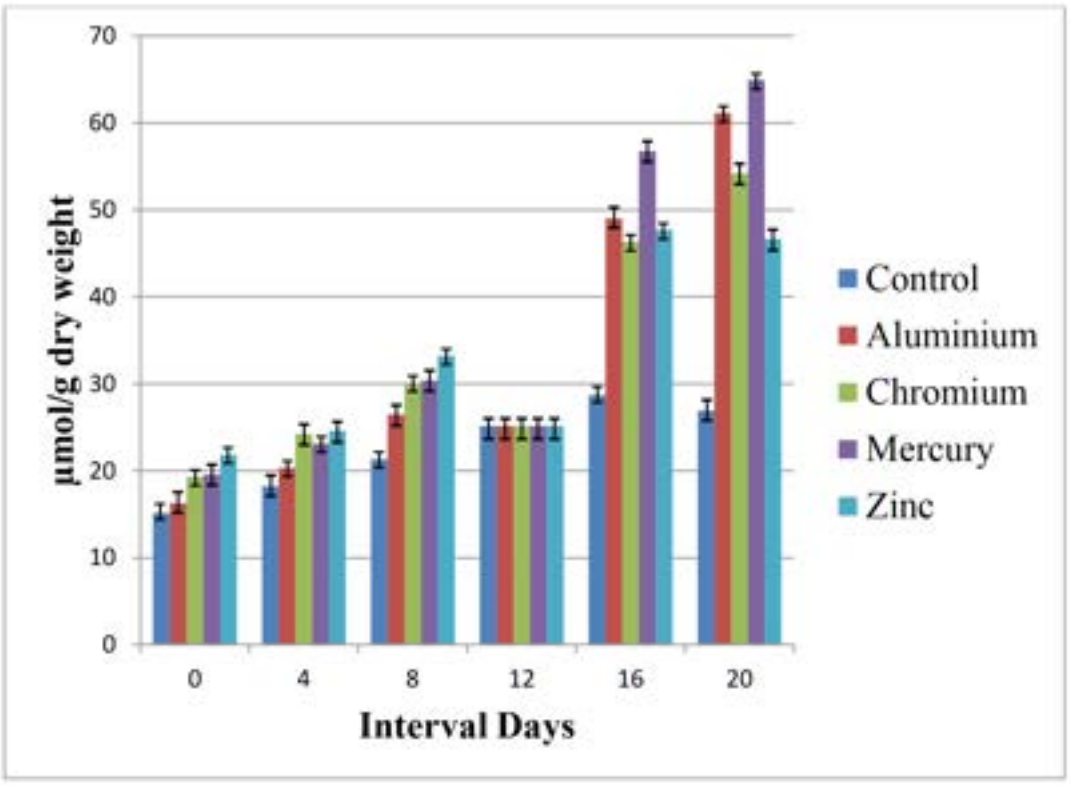
A



B



C


70
60
50
40
30
20
10
0
µmol/g dry weight
0
4
8
12
16
20
Interval Days
Control
Aluminium
Chromium
Mercury
Zinc

Phenolics

Phenolic content of the roots in the control plants showed a gradual increase but the increase was insignificant (Table-11,Fig-38). Stem tissue contained more phenolics content compared to the root and the content was continuously increased during growth. More phenolics content was present in the leaf tissue compared to the stem and root. Treatment with aluminium resulted in the enhancement in phenolic content in the root tissue compared to the control and the increase between each interval was not significant. Phenolics content of stem was more in Al treated in all stages compared to the control. Leaf tissue also exhibited increase in phenolics in all stages and increase between growth stages was increasing significantly($P< 0.01$). Plants treated with chromium showed significantly high phenolics content in the root compared to the control. Distribution of phenolics in the stem tissue of Cr treated plants was more or less similar to that of roots, whereas leaf tissues showed more phenolics in all stages compared to the root and stem, and significant. Phenolic content of plants treated with Cr showed significantly higher values than the control plants. Mercury treatment revealed an increase of phenolic content in the roots. Phenolic content of stem in plants treated with Hg showed slight reduction compared to roots but changes were insignificant compared to control. Leaf tissue exhibited significant increase of phenolic content in almost all stages compared to stem and root. Plants treated with zinc phenolic content was slightly increased compared to the control, in all stages. Phenolics content in stem tissue remained almost unchanged compared to root whereas leaf tissue showed very high phenolics content in all stages compared to control and other treatments.

Table-11

Effect of heavy metals on total phenolics content of root, stem and leaf in *Strobilanthes alternata*

mg/g dry weight

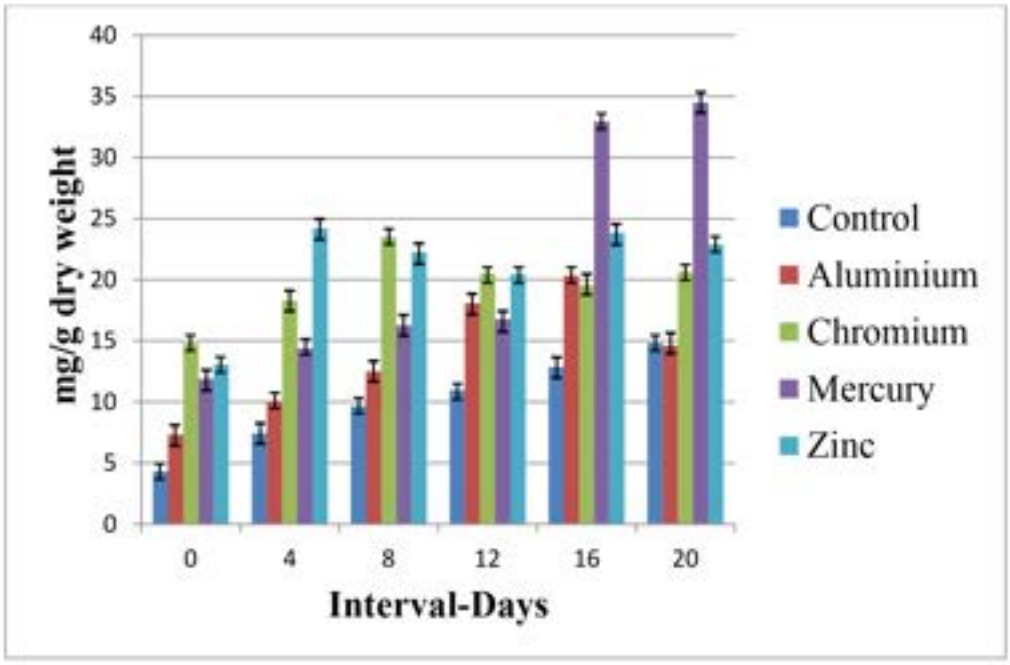
Treatments	Tissues	Intervals-Days					
		0	4	8	12	16	20
Control	Root	4.31±1.3	7.45±0.59	9.67±0.26	10.98±1.41	12.85±0.95	14.83±0.66
	Stem	2.39±0.02	7.04±0.52	11.30±0.46	24.50±1.51	16.65±1.32	19.15±0.11
	Leaf	10.16±0.08	15.07±0.24	19.75±0.89	26.42±1.07	20.48±1.71	24.83±0.09
Aluminium (400µM)	Root	7.34±0.17	10.07±0.19	12.49±3.5	18.08±2.36	20.33±0.52	14.61±2.6
	Stem	6.57±0.63	14.64±0.14	23.32±2.37	26.04±3.24	25.93±0.21	21.96±0.31
	Leaf	12.15±2.6	16.48±0.18	22.99±0.10	29.88±1.86	35.61±0.39	41.08±2.7
Chromium (70µM)	Root	14.81±1.2	18.30±2.3	23.50±0.09	20.50±2.24	19.54±0.13	20.63±0.14
	Stem	13.79±0.73	21.57±1.86	26.08±0.16	28.48±2.21	28.95±0.09	22.92±0.71
	Leaf	21.60±1.45	25.72±0.76	36.16±3.05	36.29±0.14	30.90±0.50	30.28±3.22
Mercury (20µM)	Root	11.88±0.08	14.38±0.82	16.26±2.34	16.77±0.26	32.94±1.82	34.50±0.69
	Stem	11.44±0.10	16.25±1.83	21.57±2.1	20.93±1.42	28.55±3.02	30.63±0.37
	Leaf	21.08±1.8	26.29±0.44	38.70±2.37	37.90±1.36	35.01±0.46	30.50±0.16
Zinc (250µM)	Root	13.06±0.42	24.20±1.02	22.26±1.85	20.46±2.94	23.83±1.79	22.86±2.47
	Stem	14.75±0.55	25.60±2.4	29.74±0.12	28.95±2.3	22.79±2.1	24.98±2.54
	Leaf	19.16±0.90	39.34±5.22	36.54±0.88	39.91±3.01	40.24±2.36	42.13±0.18

Values given are mean of 5 replicates ± SE

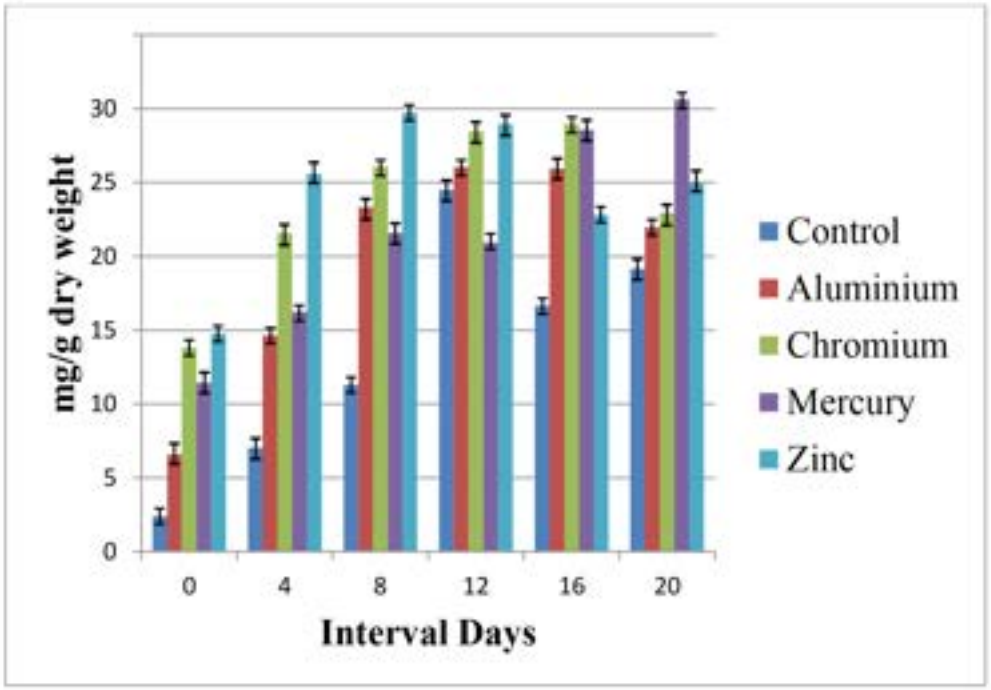
Figure-38

Effect of heavy metals on total phenolics content of root, stem and leaf in *Strobilanthes alternata*: A-Root,B-Stem,C-Leaf

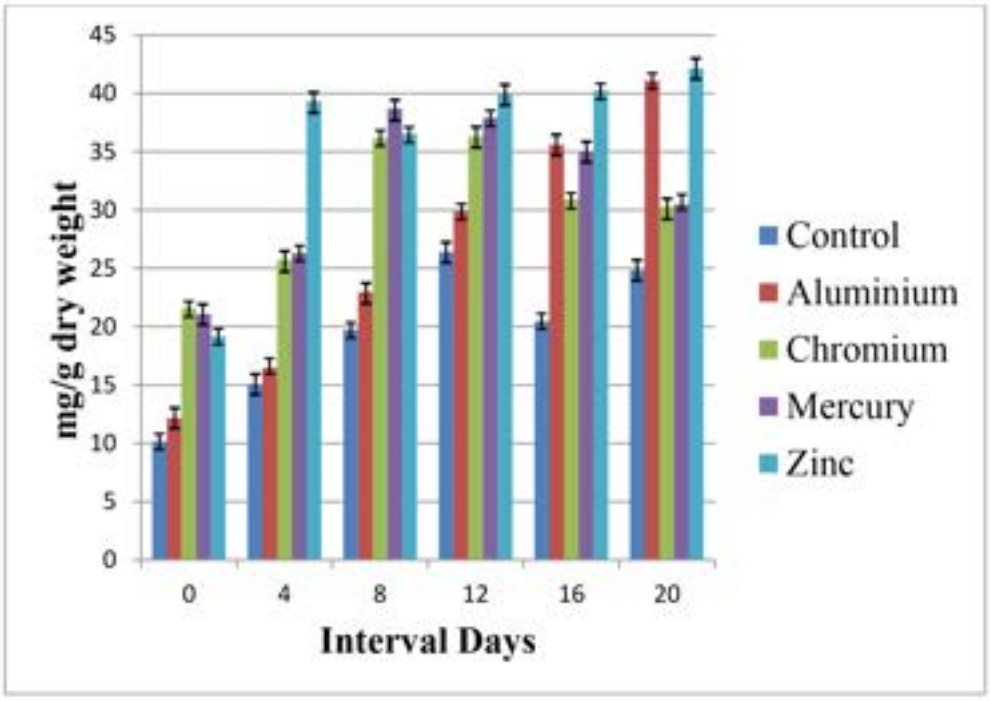
A



B



C


45
40
35
30
25
20
15
10
5
0
mg/g dry weight
0
4
8
12
16
20
Interval Days
Control
Aluminium
Chromium
Mercury
Zinc

Pigment distribution

Chlorophyll a, chlorophyll b, and total chlorophyll content of control leaves registered significant and linear increase during all stages of growth (Table-12,Fig-39). Leaves of *Strobilanthes alternata* treated with Al resulted in an increase of chl a content upto 4^{th} day followed by a gradual reduction. Chl b also was increased in comparison with the control in the earlier stages and decreased insignificantly during final stages of growth. Carotenoid content of plants treated with Al was reduced in all stages of growth compared to control. Chlorophyll a, chlorophyll b, total chlorophyll as well as carotenoid content of leaves of plants treated with chromium reduced on all stages of growth compared to control. The reduction was gradual and insignificant compared between the stages. Carotenoid content was least in Cr treated plant compared to other treatment and control. Plants treated with mercury showed an increase in chlorophyll a content upto 12^{th} day of treatment followed by a significant reduction upto 20^{th} day of treatment ($P< 0.01$). Same trend was followed by chlorophyll b also which shows an increase of chlorophyll b upto 12^{th} day and gradual reduction upto 20^{th} day of growth, which maintained the same trend in total chlorophyll also. Carotenoid content was significantly higher during initial stages and reduced during final stages. Zinc treatment resulted in decreased chlorophyll a, chlorophyll b and total chlorophyll content during all stages of growth compared to the control and other treatments. Carotenoid content also reduced significantly during all stages of growth.

Table-12

Effect of heavy metals on pigments distribution of leaf in *Strobilanthes alternata*

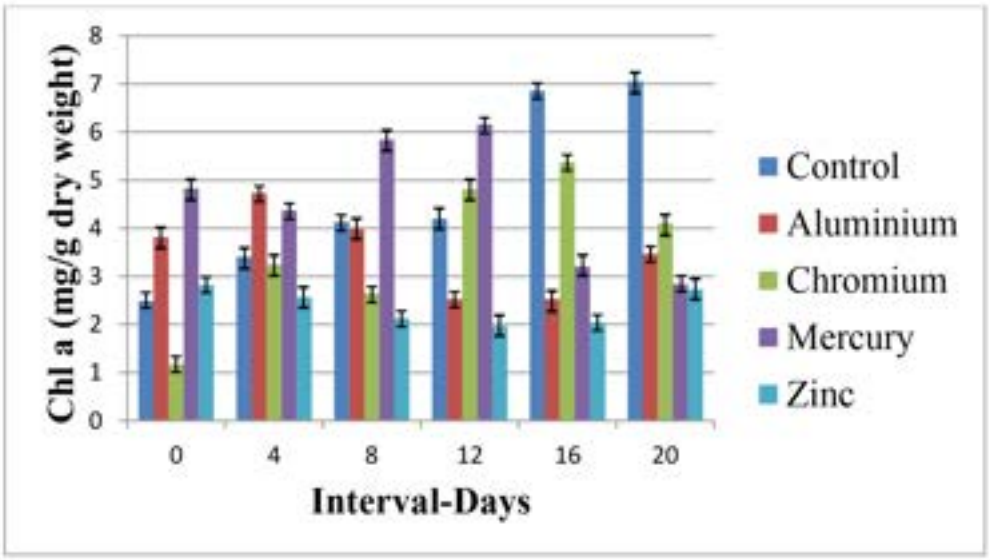
Chlorophyll content mg/g dry weight

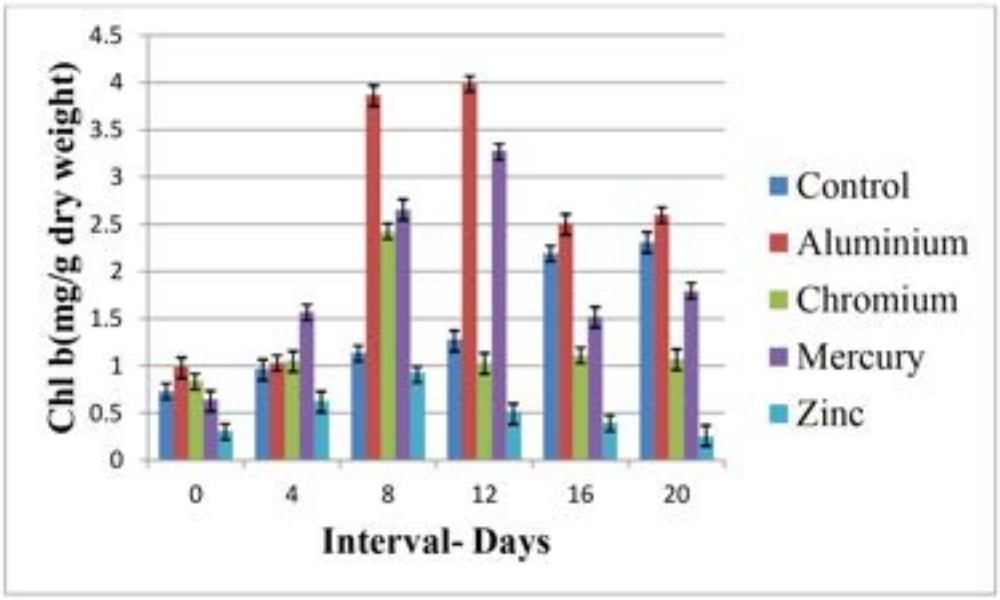
Treatments		Intervals-Days					
Control		0	4	8	12	16	20
	Chl a	2.49±0.001	3.41±0.013	4.13±0.004	4.20±0.009	6.86±0.08	7.06±0.02
	Chl b	0.73±0.008	0.97±0.005	1.14±0.012	1.28±0.024	2.19±0.026	2.31±0.002
	Total Chl	3.22±0.012	4.38±0.016	5.27±0.002	5.48±0.023	9.05±0.004	9.37±0.007
	Carotenoid	1.58±0.023	2.72±0.014	3.64±0.003	2.78±0.025	3.59±0.013	4.01±0.010
Aluminium (400µM)	Chl a	3.81±0.002	4.74±0.023	3.98±0.035	2.53±0.011	2.53±0.056	3.47±0.015
	Chl b	0.99±0.004	1.03±0.002	3.87±0.027	3.99±0.007	2.51±0.049	2.60±0.021
	Total Chl	4.80±0.015	5.77±0.013	7.85±0.009	6.52±0.004	4.84±0.031	6.07±0.025
	Carotenoid	2.73±0.116	2.89±0.019	1.87±0.018	1.53±0.015	1.96±0.003	2.03±0.036
Chromium (70µM)	Chl a	1.17±0.025	3.25±0.011	2.65±0.023	4.81±0.004	4.38±0.017	4.11±0.022
	Chl b	0.84±0.019	1.05±0.025	2.42±0.038	1.03±0.006	1.11±0.026	1.07±0.026
	Total Chl	2.01±0.015	4.31±0.08	5.07±0.013	5.84±0.015	6.49±0.063	5.18±0.007
	Carotenoid	2.73±0.001	1.98±0.010	1.56±0.026	2.51±0.026	1.03±0.018	1.01±0.005
Mercury (20 µM)	Chl a	4.83±0.008	4.37±0.028	5.84±0.02	6.16±0.043	3.21±0.001	2.84±0.045
	Chl b	0.64±0.017	1.57±0.025	2.65±0.057	3.28±0.032	1.52±0.005	1.79±0.057
	Total chl	5.47±0.022	5.94±0.004	8.49±0.031	9.44±0.003	4.73±0.017	4.63±0.004
	Carotenoid	1.97±0.031	4.57±0.011	3.12±0.042	3.18±0.009	2.46±0.031	2.07±0.053
Zinc (250 µM)	Chl a	2.82±0.007	2.57±0.013	2.13±0.002	1.96±0.008	2.04±0.006	2.73±0.027
	Chl b	0.31±0.006	0.63±0.026	0.93±0.010	0.52±0.009	0.40±0.011	0.25±0.046
	Total Chl	3.13±0.031	3.20±0.005	3.06±0.005	2.48±0.017	2.44±0.014	2.98±0.025
	Carotenoid	1.96±0.028	3.27±0.012	3.41±0.017	1.48±0.043	1.37±0.11	2.91±0.043

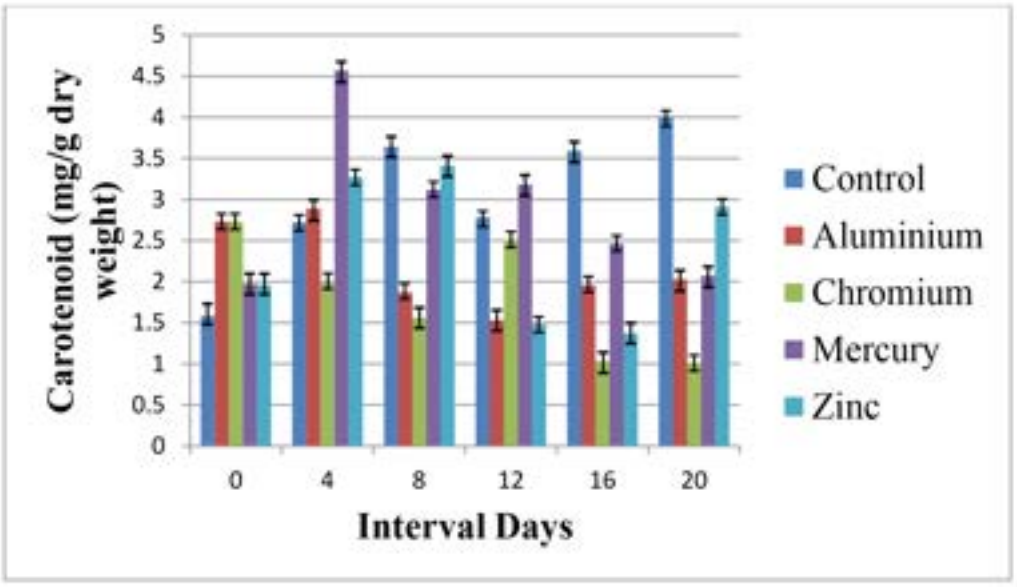
Values given are mean of 5 replicates ± SE.

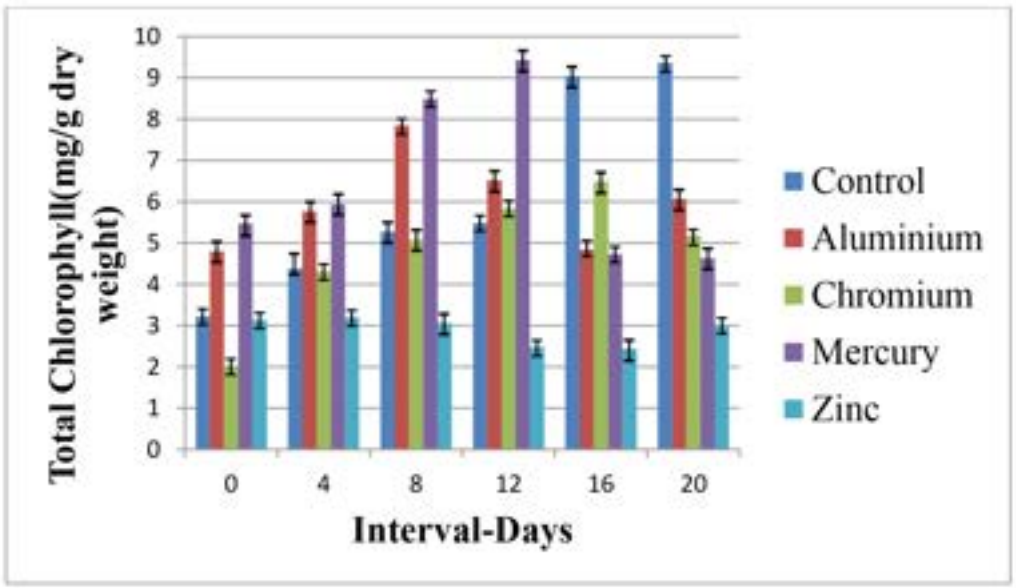
Figure- 39

Effect of heavy metals on pigments distribution of leaf in *Strobilanthes alternata*






Carotenoid (mg/g dry weight)
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
0
4
8
12
16
20
Interval Days
Control
Aluminium
Chromium
Mercury
Zinc


Total Chlorophyll(mg/g dry weight)
10
9
8
7
6
5
4
3
2
1
0
0
4
8
12
16
20
Interval-Days
Control
Aluminium
Chromium
Mercury
Zinc

Superoxide dismutase assay

Superoxide dismutase (SOD) activity showed an increasing trend of activity in all tissues of control plants during all intervals (Table-13, Fig-40). In aluminium treated plants the activity of SOD was more or less similar to that of control. Root, stem and leaves showed same trend of increase of SOD due to Al treatment. Root tissues of plants treated with chromium exhibited very high SOD activity initially on 4^{th} day and significantly reduced afterwards ($P< 0.01$). Stem tissue showed comparatevly high activity compared to control but the activity was less than that of root. Leaf tissues exhibited more or less same trend as that of root and stem. Least SOD activity was found at 20^{th} day of treatment. Among the tissues root showed highest variation in SOD compared to control. In the plants subjected to mercury toxicity the activity of SOD was decreased gradually compared to control. In all tissues the highest SOD activity were observed during the initial stages of treatments. This hike of SOD activity decreased gradually during further growth. Same trend of decrease in SOD was observed in all tissues due to mercury toxicity. Comparatively very low activity of SOD was shown in plants treated with mercury in root and stem tissues but leaf showed very high SOD activity which reduced gradually during further growth. SOD activity of all tissues of zinc treatment was significantly higher than control in all tissues in all intervals compared to control. During growth upto 20 days SOD activity significantly increased in all tissues in all intervals.

Table-13

Effect of heavy metals on SOD activity of root,stem and leaf in *Strobilanthes alternata*

SOD Unit activity/mg protein (Specific activity)

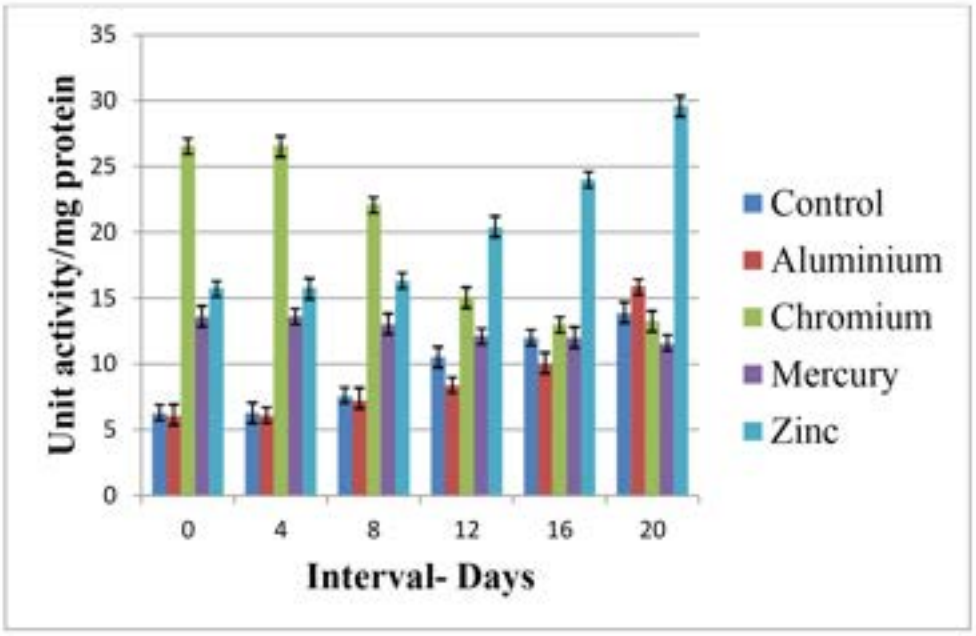
	Tissues	Interval days		
		4	12	20
Control	Root	6.27±0.12	10.54±0.21	13.86±0.15
	Stem	5.21±0.11	8.43±0.25	13.03±0.09
	Leaf	7.03±0.18	11.64±0.30	17.91±0.11
Aluminium	Root	6.07±0.17	8.36±0.12	15.90±0.17
	Stem	7.24±0.06	12.05±0.31	14.83±1.3
	Leaf	6.38±1.1	10.37±0.22	16.93±1.6
Chromium	Root	26.59±1.81	15.11±0.87	13.25±1.2
	Stem	19.98±2.1	12.56±0.85	16.65±0.78
	Leaf	21.01±1.03	19.83±0.73	14.24±1.6
Mercury	Root	13.61±0.12	12.13±0.08	11.54±0.15
	Stem	12.67±0.25	9.93±0.14	8.19±0.13
	Leaf	24.44±0.10	17.16±0.17	14.65±2.1
Zinc	Root	15.81±0.50	20.37±0.23	29.62±0.27
	Stem	16.53±0.19	21.56±0.31	33.87±0.36
	Leaf	14.77±0.21	18.10±0.35	25.72±0.21

Values given are mean of 5 replicants ±S.E

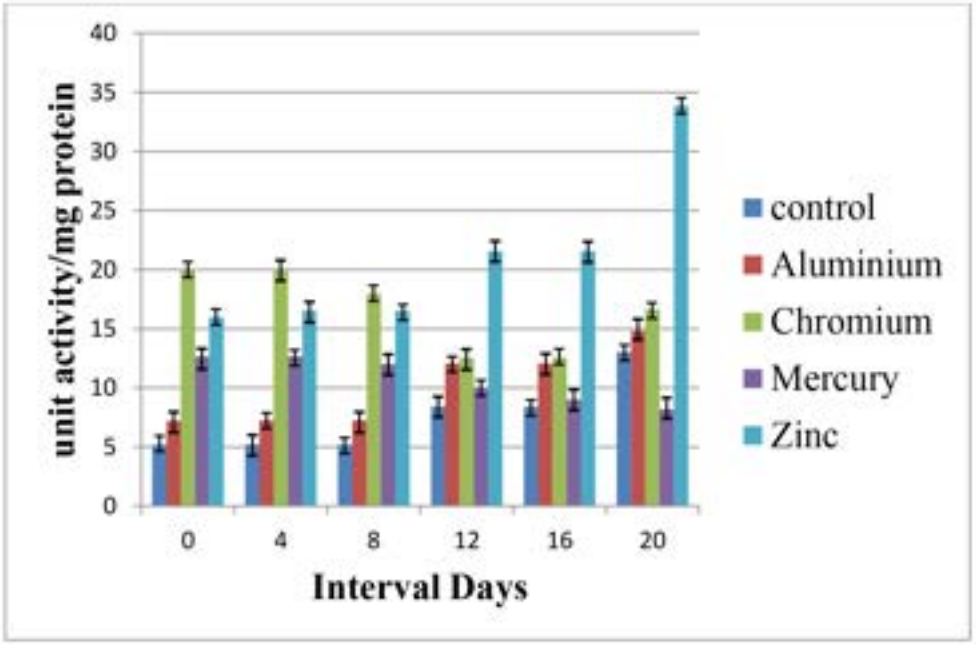
Figure-40

Effect of heavy metals on SOD activity of root, stem and leaf in *Strobilanthes alternata*: A-Root,B-Stem,C-Leaf

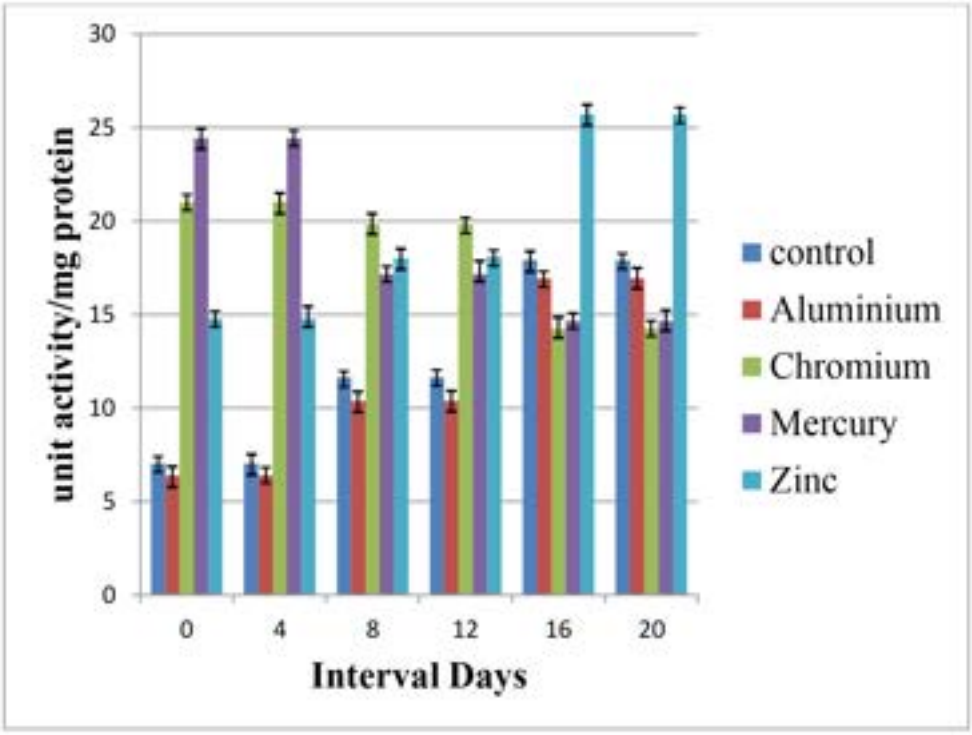
A



B



C


30
25
20
15
10
5
0
unit activity/mg protein
0
4
8
12
16
20
Interval Days
control
Aluminium
Chromium
Mercury
Zinc

Catalase activity

Catalase activity of *Strobilanthes alternata* showed an increasing trend in control plants (Table-14;,Fig- 41). Catalase activity of control plants showed more or less uniform activity in all tissues and slight CAT activity was observed in stem and leaf showed maximum CAT activity being maximum value on 20th day of treatment. Plant treated with aluminium showed significant reduction of CAT activity in all tissues. The leaf of aluminium treated plants exhibited more catalase activity in comparison with the other parts and the activity was slightly increasing during growth. Root, stem and leaves of chromium treated plants showed decrease in catalase activity compared to control. Eventhough the root tissues showed a decrease of catalase activity during the initial stages of growth at last stage of growth catalase activity increased than the control. Stem tissues of chromium treated plant showed only very low catalase activity compared to control, and during growth a gradual increase was observed. Leaf tissues exhibited more catalase activity compared to root and stem of Cr treated plants. Plants treated with mercury registered very feable activity in root and stem of *S.alternata* whereas leaf tissues exhibited increased activity compared to root and stem.Root and stem tissues exhibited more or less similar trend of decrease of catalase activity. Compared to other parts leaf tissues showed highest catalase activity and this is found to be increased with increase in growth. Due to zinc treatment catalase activity of root, stem and leaves decreased compared to control. Maximum reduction in catalase activity were found in root tissues followed by stem tissues. During each interval a gradual increase of catalase activity were observed. Catalase activity of leaf tissues is comparatevily higher than root and stem and also it is found to increase during each intervals.

Table-14

Effect of heavy metals on Catalase activity of root,stem and leaf in *Strobilanthes alternata*

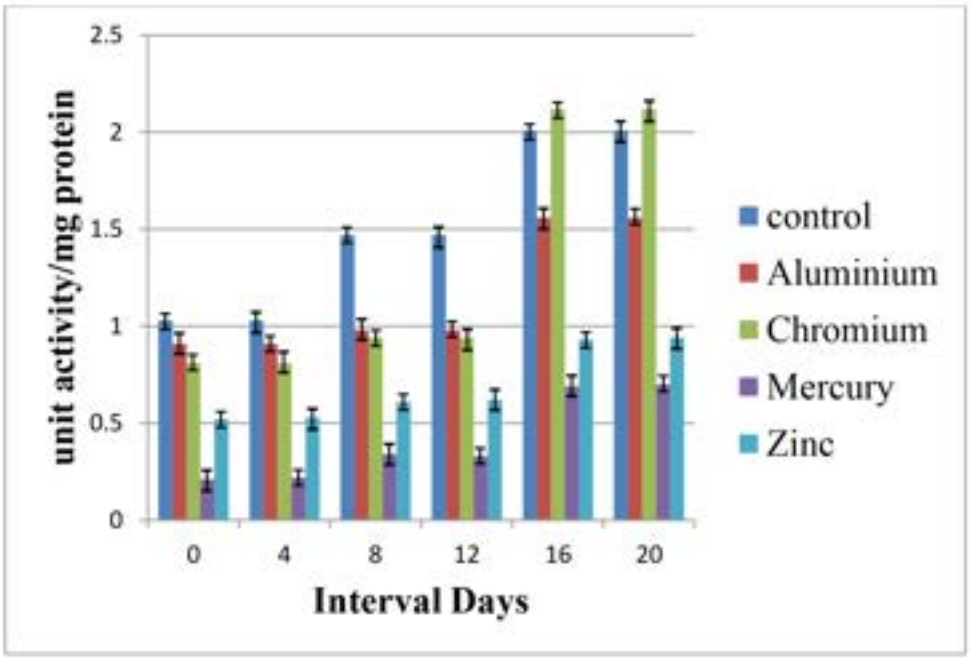
Unit activity/mg protein(Specific activity)

	Tissues	Interval days		
		4	12	20
Control	Root	1.03±0.19	1.47±0.13	2.01±0.19
	Stem	1.60±0.18	2.08±0.18	3.91±0.22
	Leaf	2.53±0.10	3.14±0.52	4.96±0.31
Aluminium	Root	0.91±0.03	0.98±0.04	1.56±0.05
	Stem	0.93±0.01	1.14±0.11	1.96±0.10
	Leaf	1.11±0.10	1.42±0.43	2.83±0.20
Chromium	Root	0.81±0.02	0.94±0.08	2.12±0.27
	Stem	0.77±0.01	0.84±0.10	0.91±0.09
	Leaf	1.44±0.33	1.49±0.09	2.12±0.13
Mercury	Root	0.21±0.09	0.34±0.01	0.70±0.06
	Stem	0.46±0.04	0.52±0.01	0.55±0.02
	Leaf	1.61±0.07	1.72±0.14	1.93±0.17
Zinc	Root	0.52±0.01	0.61±0.02	0.94±0.03
	Stem	0.74±0.09	0.85±0.04	1.08±0.19
	Leaf	1.55±0.10	1.84±0.17	1.96±0.24

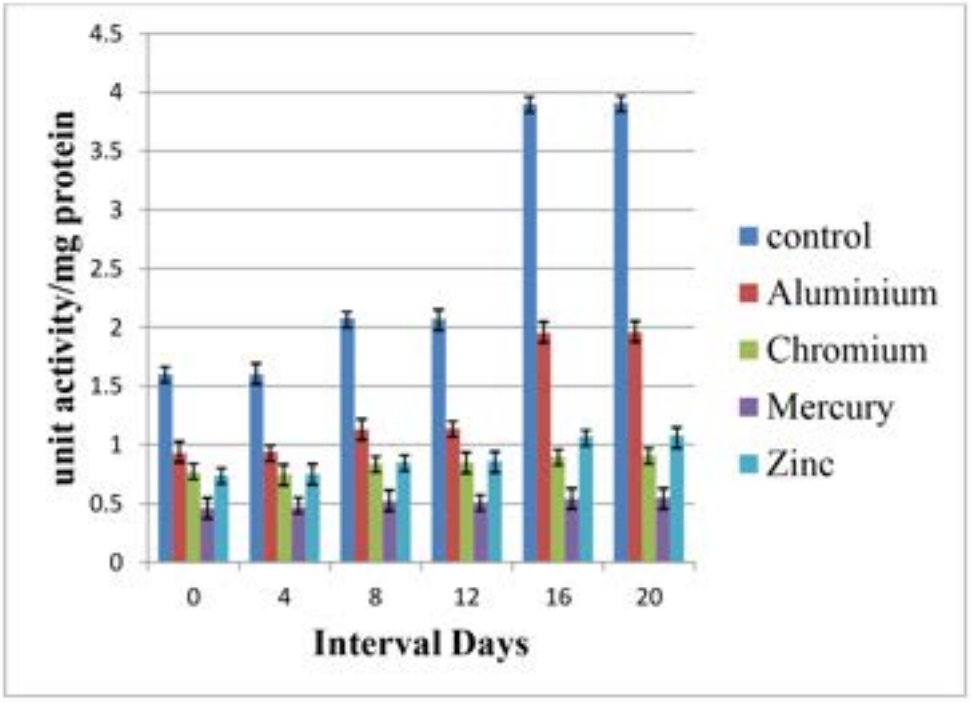
Figure-41

Effect of heavy metals on Catalase activity of in *Strobilanthes alternata*
A:root,B;stem andC: leaf

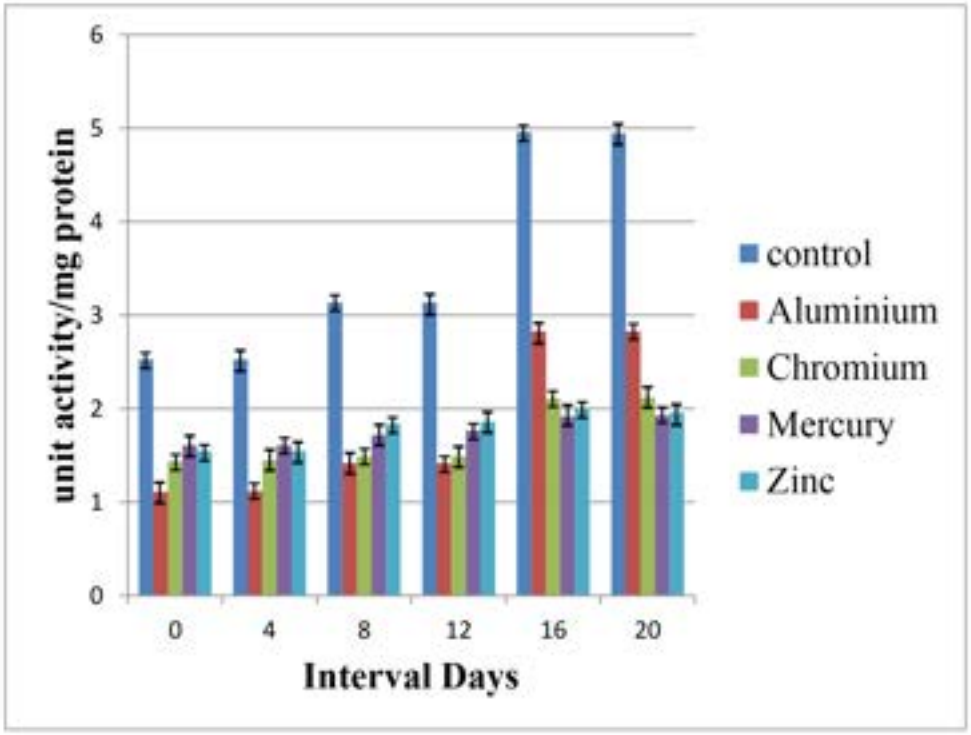
A



B



C


6
5
4
3
2
1
0
unit activity/mg protein
0
4
8
12
16
20
Interval Days
control
Aluminium
Chromium
Mercury
Zinc

Bioaccumulation

Bioaccumulation of aluminium, chromium, mercury and zinc were determined at different days of treatment. In the roots of *Strobilanthes alternata* treated with aluminium bioaccumulation was increasing gradually at each interval showing significant increase between 4^{th} and 12^{th} day ($P < 0.01$) (Table-15;Fig-42). Eventhough increase of aluminium content was gradual on all intervals, on 20^{th} day there occurred a four fold increase in aluminium content. Aluminium content of the stem was initially very low but showed significant increase during growth upto 20^{th} day. The leaf contained only meagre quantity of aluminium but it was insignificantly increased gradually upto final day of treatment. aluminium content in leaves was least compared to the other parts. aluminium content present in residual solution was comparatevily low and during each interval decrease in aluminium content was insignificant.

Plants treated with chromium showed significant increase in the bioaccumulation during all stages of growth (Table-15; Fig-42). Continuous and linear increase of chromium content was seen in the root tissues. Comparatively more chromium content was present on 20^{th} day of treatment in root, stem and leaves. Quantity of chromium was very low in leaf compared to root and stem and increase was insignificant between 12^{th} & 20^{th} day of treatment. Stem of plants treated with chromium contain only very low Cr content which showed more or less similar trend that of root. Even though the quantity of chromium was least in leaves compared to other parts linear increase of chromium content was observed. chromium content of residual solution was very meagre which showed gradual reduction during all interval.

Strobilanthes alternata treated with mercury resulted only a meagre bio accumulation in all tissues compared to the other – aluminium, chromium and zinc metals (Table-15). Bioaccumulation of mercury showed the trend as root

>Stem> leaf. Mercury content was continuously increasing in root tissues but the increase was gradual and insignificant. Stem tissues maintained the status same as root, but the quantity was very low compared to root. Accumulation of mercury in the leaf was comparatively very low and showed maximum on 20th day of treatment even though the mercury content was meagre in all plant parts a gradual increase of mercury content was exhibited. Residual solution contained only comparatively low quantity of mercury content during 4th day and afterwards content was below detectable level during further growth.

Plants treated with zinc resulted gradual and significant increase in root, stem and leaves during all stages of growth (Table- 15). Zinc content in the root tissues was increase significantly during the initial stages of growth and after 12th day the increase was insignificant. In the stem tissues increase was gradual and insignificant during initial stages of growth but there occurred zinc content at final stage of growth. Zinc content of leaf was maximum on 20th day of treatment and then increase was occurred gradually. Considerable quantity of zinc was present in residual solution on 4th day which significantly decreased on 12th day and remained unchanged during further growth.

Table-15

Bioaccumulation patterns of heavy metals on *Strobilanthes alternata* grown in Hoagland solution containing standardized concentrations of heavy metals

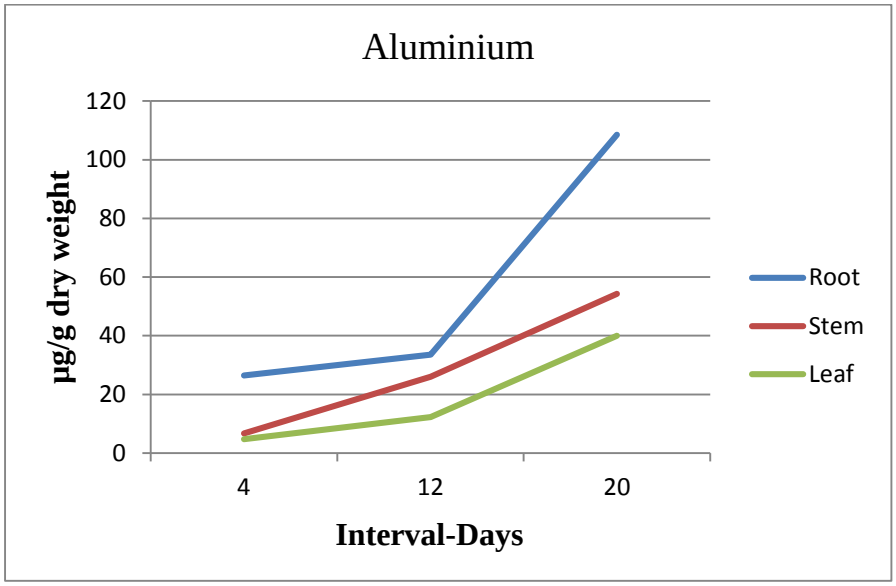
µg/g dry weight

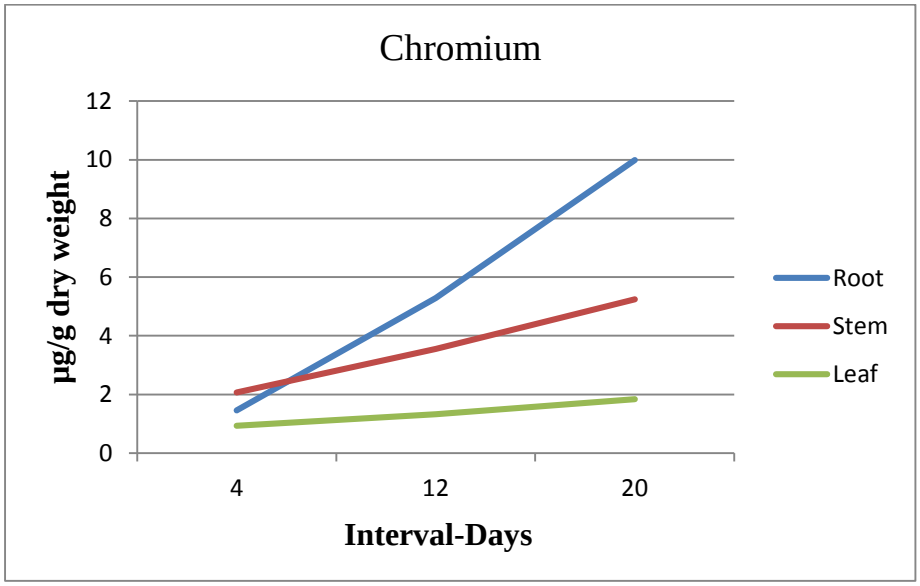
Treatments	Tissues	Interval days		
		4	12	20
Aluminium (400µM)	Root	26.5±1.23	33.51±3.1	108.5±5.2
	Stem	6.66±2.7	26± 2.87	54.3±4.32
	Leaf	4.71±0.63	12.3±1.5	40±3.54
	Residue	10.08±1.7	9.26±0.98	7.07±0.58
Chromium (70µM)	Root	1.46±0.007	5.28±0.76	9.99±1.03
	Stem	2.07±0.10	3.55±0.14	5.24±1.01
	Leaf	0.93±0.03	1.32±0.23	1.84±0.012
	Residue	0.26±0.06	0.76±0.002	0.29±0.01
Mercury (20µM)	Root	0.503±0.003	0.91±0.05	1.13±0.027
	Stem	0.012±0.009	0.02±0.001	0.56±0.03
	Leaf	0.01±0.001	0.16±0.005	0.24±0.06
	Residue	0.216±0.05	BDL	BDL
Zinc (250µM)	Root	5.42±0.12	8.03±0.11	8.61±0.16
	Stem	2.28±0.12	3.59±0.17	6.57±0.10
	Leaf	2.34±0.14	3.16±0.03	4.42±0.09
	Residue	9.27±0.11	4.77±0.12	3.03±0.08

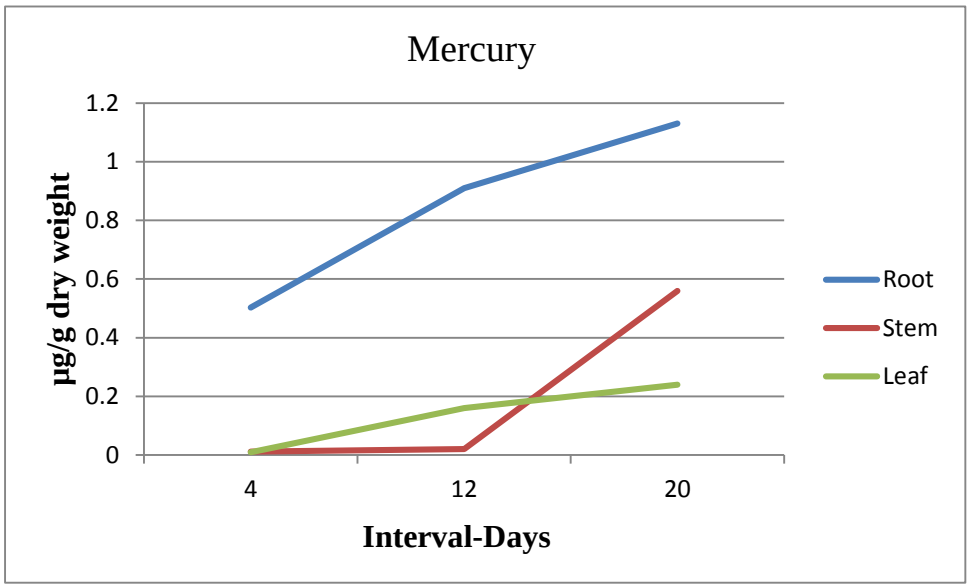
BDL- Below detectable level

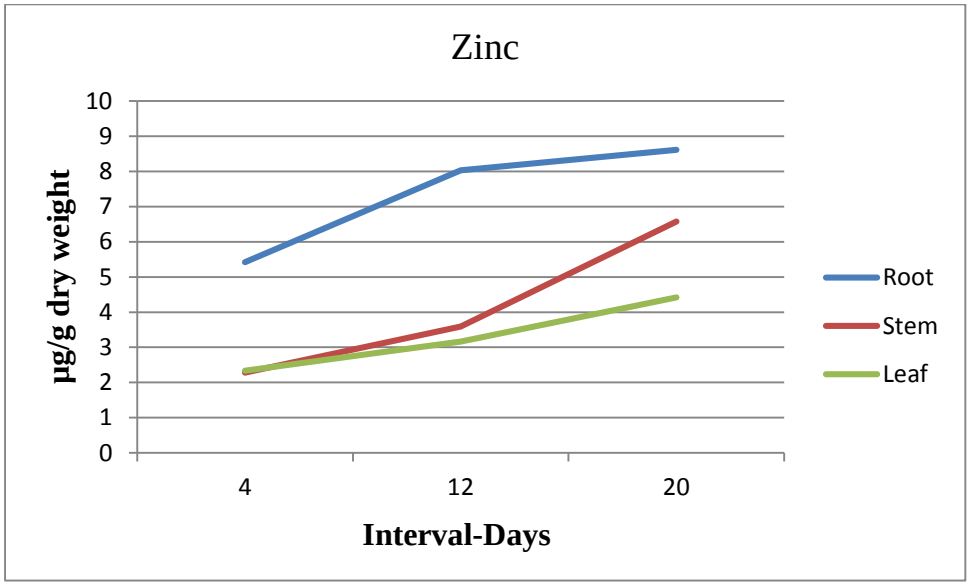
Figure-42

Bioaccumulation patterns of heavy metals on *Strobilanthes alternata* grown in Hoagland solution containing standardized concentrations of heavy metals






Mercury
1.2
1
0.8
0.6
0.4
0.2
0
µg/g dry weight
4
12
20
Interval-Days
Root
Stem
Leaf


Zinc
10
9
8
7
6
5
4
3
2
1
0
µg/g dry weight
4
12
20
Interval-Days
Root
Stem
Leaf

Bioconcentration factor and translocation factor

Potential of plants to accumulate metals from soils can be evaluated using the BCF (Bioconcentration factor) and the ability of plant to translocate metals from the roots to shoots is analysed using TF (Translocation factor) (Yoon *et al*., 2006). The ability of *Strobilanthes alternata* to absorb and translocate metals from soil to shoot was evaluated by comparing BCF and TF. BCF values of Al treated plants increased gradually throughout the growth and between the stages the difference in BCF values were significant (Table- 35). *S.alternata* showed highest BCF values in the treatment with Al at final stages of growth. TF values is less than one in all stages of Al treated plants. TF values is less than one in all stages of Al treated plants and hence S.alternata is not suitable for phytoextraction. TF values showed considerable reduction from stage 4 to 12 followed by an increase in the final stage of growth. BCF values of plant treated with chromium decreased during stages 4-12 of growth followed by a significant increase ($P < 0.01$) during last stage of growth. BCF value is greater than one during 20-th day of treatment. TF values of chromium treated plants is showed an increase on 20^{th} day only and TF factor is less than one in all stages. Mercury treatment showed negligible values/ below detectable range of BCF in all stages of growth. TF values showed gradual increase during all intervals but the value is less than one in all stages of growth. Eventhough increase of TF values are insignificant during the initial stages, during 20-th day of treatment, TF values increased significantly . BCF values of plants treated with zinc showed a gradual increase upto 20^{th} day of treatment and BCF were less than one during all stages of growth. TF showed a linear and significant increase during all stages of growth and values of Zn treated plants were less upto 12^{th} day followed by an increased TF value when a comparison is mede between the BCF factors of all metals in Strobilanthes alternata. Values of all metals except chromium are very low..

Table-16

Bioconcentration factor and translocation factor

Treatments	Interval-Days					
	4		12		20	
	BCF	TF	BCF	TF	BCF	TF
Aluminium (400μM)	0.09	0.42	0.12	1.14	0.39	0.86
Chromium (70 μM)	0.005	0.83	0.01	0.92	0.03	0.70
Mercury (20 μM)	0.004	0.04	0.008	0.19	0.004	0.70
Zinc (250 μM)	0.02	0.84	0.039	0.8	0.042	1.2

Gas Chromatography Mass Spectrometric analysis

Control

GCMS analysis of methanolic extract of *Strobilanthes alternate* resulted in the identification of 15 secondary metabolites(Table-17; Fig-43). Those compounds identified were Neophytadine (9.09%), 3,7,11,15 Tetramethyl 2 hexadecen 1ol (4.07%), Methyl palmitate (22.28%), Methyl octadeca 9,12 dienoate (7.86%), Methyl laurate (1.80%), Methyl myristate (1.55%), Hexahydrofarnesyl acetone (2.68%), 9-octadecenoic acid (2.69%), Isophytol(2.60%), 2,5-Dimethyl 4 hexen 3 ol (2.31%), Linolenic acid (16.05%), Phytol (20.57%), Methyl stearate (2.77%), 1,2-Benzenedicarboxylic acid (2.66%), and Squalene(1.02%) (Table-17). Quantitative expression of these secondary metabolites was done by considering the area percentage of GCMS profile. By considering the area percentage, least occurring secondary metabolite in control plants was squalene and abundantly occurring component was Methyl palmitate. Second abundantly occurring component was phytol followed by Linolenic acid. The pharmacological efficacy of the plant is found to be centered around the distribution of these secondary metabolites.

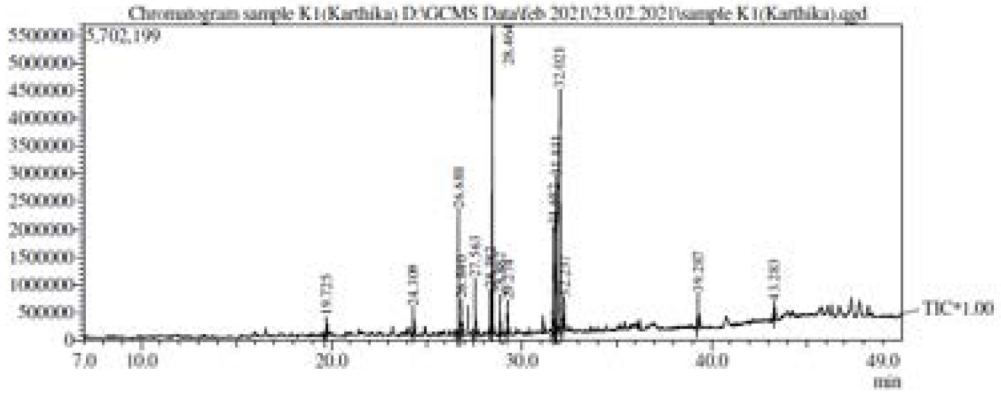
Table-17

GC-MS analysis of *Strobilanthes alternata*

SI.NO	SECONDARY METABOLITES	AREA PERCENTAGE
1	Neophytadine	9.09
2	3,7,11,15-Tetramethyl 2 hexadecen 1ol	4.07
3	Methyl palmitate	22.28
4	Methyl octadeca 9,12 dienoate	7.86
5	Methyl laurate	1.80
6	Methyl myristate	1.55
7	Hexahydrofarnesyl acetone	2.68
8	9-Octadecenoic acid	2.69
9	Isophytol	2.60
10	2,5-Dimethyl 4 hexen 3ol	2.31
11	Linolenic acid	16.05
12	Phytol	20.57
13	Methyl stearate	2.77
14	1,2-Benzene dicarboxylic acid	2.66
15	Squalene	1.02

Figure -43

GC-MS analysis of *Strobilanthes alternata*



Effect of aluminium

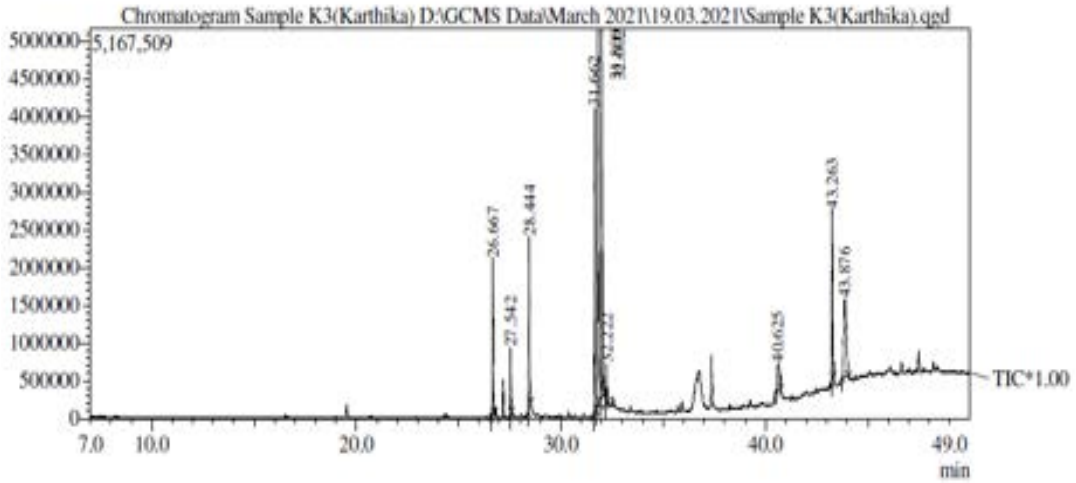
GCMS analysis of the plant treated with aluminium resulted in the occurrence of ten secondary metabolites (Table-18;Fig-44). They were Neophytadine (6.66%), 3,7,11,15-Tetramethyl 2 hexadecen 1 ol (2.82%), Methyl Palmitate (8.99%), Methyl Octadeca 9,12 Dienoate (14.52%), Linolenic acid(22.85%), Phytol (17.44%), Methyl stearate (2%), Squalene (8.635), Gamma sitosterol (3.75%), and Lupeol (12.34%).when compared to control there occurred a deletion of secondary metabolites along with the formation of two more components which were not present in control. Gamma sitosterol and Lupeol were the newly identified secondary metabolites in comparison with the control. In the plants treated with aluminium, Linolenic acid was the abundantly occurring secondary metabolite. Phytol is the second abundantly occurring component and the least occurring component is 3,7,11,15, tetramethyl 2-hexadecen-1ol. The quantity of squalene and Linolenic acid increased in comparison with the control. Due to aluminium treatment all other secondary metabolites were decreased when compared to the control. Methyl laurate, methyl myristate, hexahydrofarnesyl acetone, 9-octadecenoic acid, isophytol, 2,5-Dimethyl 4-Hexen-3 ol and 1,2-Benzenedicarboxylic acid were deleted due to the effect of aluminium.

Table-18

GCMS analysis-plant treated with aluminium

SI.NO	SECONDARY METABOLITES	AREA PERCENTAGE
1	Neophytadine	6.66
2	3,7,11,15 Tetramethyl 2 hexadecen 1 ol	2.82
3	Methyl palmitate	8.99
4	Methyl octadeca 9,12 Dienoate	14.52
5	Linolenic acid	22.85
6	Phytol	17.44
7	Methyl stearate	2.0
8	Squalene	8.63
9	Gamma sitosterol	3.75
10	Lupeol	12.34

Figure-44
GCMS analysis-plant treated with aluminium



Effect of chromium

Neophytadine (10.68%), 3,7,11,15 tetramethyl 2 hexadecen 1 ol (5.01%), methyl palmitate (9.91%), methyl octadeca 9,12 dienoate (13.71%), linolenic acid(23.56%), phytol(24.11%), methyl stearate (2.22%) and squalene (10.80%) were present in *S.alternata* subjected to chromium treatment in comparison with the control(Table-19;Fig-45). Methyl laurate, Methyl myristate, Hexahydrofarnesyl acetone, 9-octadecenoic acid, Isophytol, 2,5-Dimethyl 4-hexen 3ol and 1,2-Benzene dicarboxylic acid were absent in comparison with that of control. Quantitativly squalene increased about ten fold compared to control. Linolenic acid content showed slight increase than control,while Methyl palmitate was reduced to one half than control.

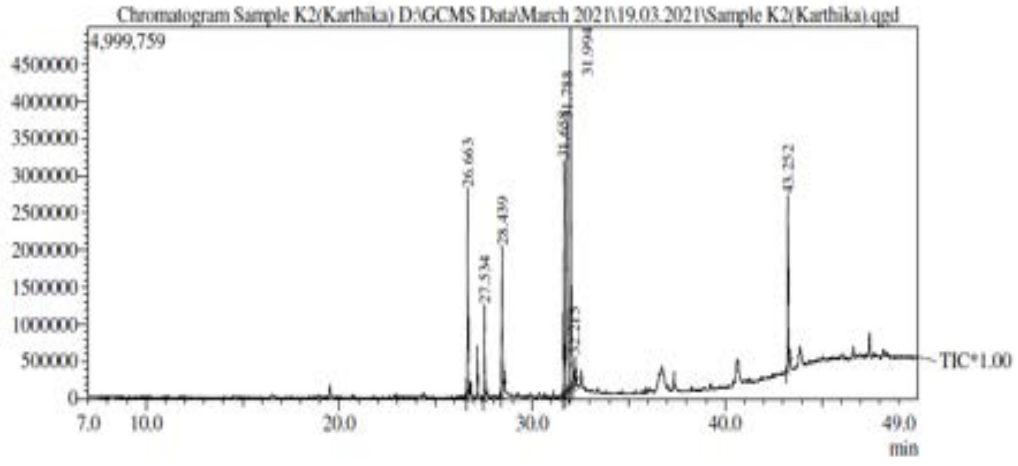
Table-19

GCMS analysis- plants treated with chromium

SI.NO	SECONDARY METABOLITES	AREA PERCENTAGE
1	Neophytadine	10.68
2	3,7,11,15 Tetramethyl 2 hexadacen 1 ol	5.01
3	Methyl palmitate	9.91
4	Methyl octadeca 9,12 Dienoate	13.71
5	Linolenic acid	23.56
6	Phytol	24.11
7	Methyl stearate	2.22
8	Squalene	10.80

Figure-45

GCMS analysis- plants treated with chromium



Effect of mercury

Strobilanthes alternata treated with mercury showed the presence of 8 secondary metabolites. The components present are Neophytadine (6.39%), 3,7,11,15 tetramethyl 2 hexadecen 1ol(2.46%), Methyl palmitate (14.48%), Methyl octadeca 9,12 dienoate (20.80%), Linolenic acid (34.34%), Phytol (12.50%), and Squalene (6.62%) (Table-20;Fig-46). The secondary metabolites absent due to mercury toxicity were similar that of plants exposed to chromium. They are methyl laurate, methyl myristate, hexahydrofarnesyl acetone, 9-octadecenoic acid, isophytol, 2,5-Dimethyl-4-hexen-3-ol, and 1,2-benzenedicarboxylic acid. Abundantly occurring component due to mercury treatment was linolenic acid (34.34%) and least occurring component was methyl stearate(2.43%). Quantity of methyl octadeca 9,12 dienoate (20.80%) increased 2 fold than control, and squalene was increased upto 3 fold compared to control (6.62%).

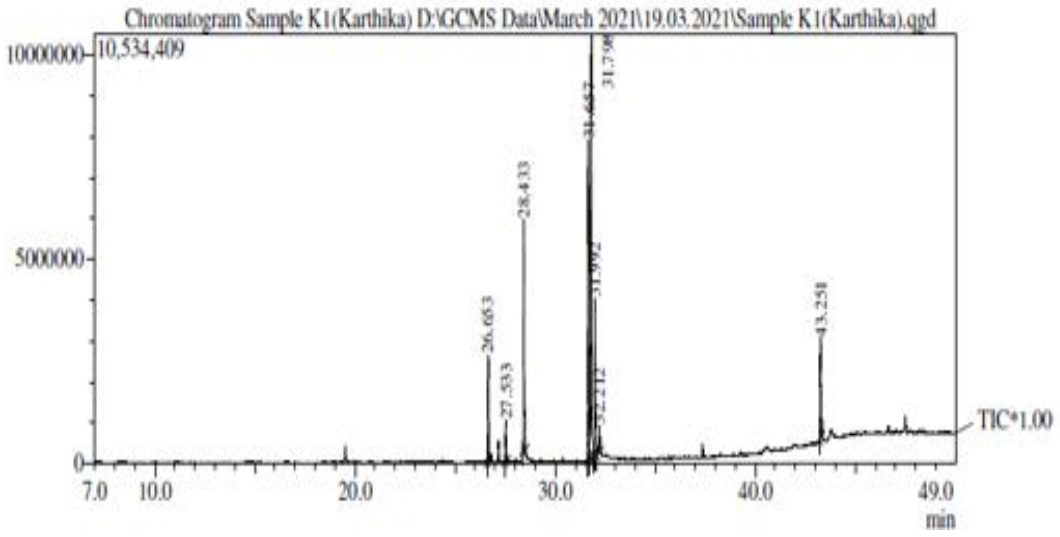
Table-20

GCMS analysis-plants treated with mercury

SI.NO	SECONDARY METABOLITE	AREA PERCENTAGE
1	Neophytadine	6.39
2	3,7,11,15 tetramethyl 2 hexadecen 1ol	2.46
3	Methyl palmitate	14.48
4	Methyl octadeca 9,12 dienoate	20.80
5	Linolenic acid	34.34
6	Phytol	12.50
7	Methyl stearate	2.43
8	Squalene	6.62

Figure-46

GCMS analysis-plants treated with mercury



Effect of zinc

Out of 15 secondary metabolites identified in the control, plant nine were disappeared and two members were formed newly in plants treated with zinc (Table-21;Fig-47) .Neophytadine (8.24%), 3,7,11,15-Tetramethyl 2-hexadacen 1-ol(3.53%), Linolenic acid(22.92%), Phytol (16.38%), squalene (4.70%) are the components present. The disappeared secondary metabolites were Methyl octadeca 9,12 dienoate, Methyl laurate, Methyl myristate, Hexahydrofarnesyl acetone, 9-octadecenoic acid, Isophytol, 2,5-dimethyl 4 hexen 3 ol, Methyl stearate and 1,2-benzen dicarboxylic acid. Newly formed components were Lupeol (19.68%) and Methyl linolaidate (14.66%). Abundantly occurring components were Linolenic acid (22.92%) followed by Lupeol and Phytol. Least occurring components was 3,7,11,15 Tetramethyl 2-hexadecen 1 ol(3.52%). Quantity of most of the secondary metabolites were found to be decreased compared to control.

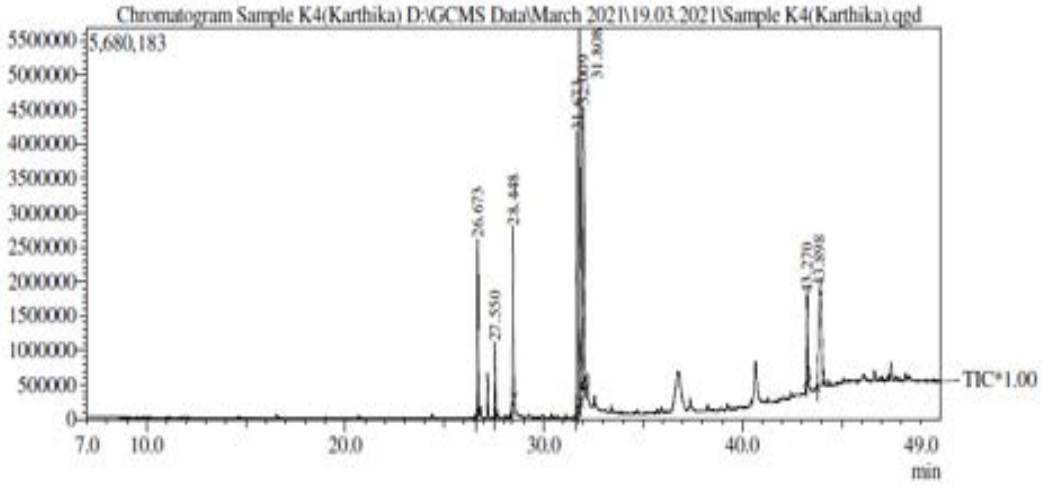
Table-21

GCMS analysis-plants treated with zinc

SI.NO	SECONDARY METABOLITES	AREA PERCENTAGE
1	Neophytadine	8.24
2	3,7,11,15 Tetramethyl 2 hexadacen 1ol	3.52
3	Methyl palmitate	9.88
4	Methyl linolelaidate	14.66
5	Lupeol	19.68
6	Linolenic acid	22.92
7	Phytol	16.38
8	Squalene	4.70

Figure-47

GCMS analysis-plants treated with zinc



DISCUSSION

To investigate the effect of heavy metals such as aluminium, chromium, mercury and zinc on *Strobilanthes alternata* screening experiments were conducted with different concentrations of the salts of aluminium ($AlCl_3$), chromium($K_2Cr_2O_7$), mercury ($HgCl_2$) and zinc ($ZnSO_4$) and it was confirmed that 400 µM, 70 µM, 20µM and 250 µM concentrations of the aluminium, chromium, mercury and zinc respectively exhibited toxicity symptoms retaining the survival of the plants. In an excellent review, Foy *et al.,* (1978) stated that sensitivity and/or tolerance of plants towards heavy metals vary from species to species and metal to metal. According to those authors for the investigations on the effect of heavy metals toxicity, optimal concentrations are to be selected based on trial experiment with various concentrations which show about 50% growth retardation retaining growth and survival of the plants.

Strobilanthes alternata is a vegetatively propagated herb and hence rooted cuttings were cultivated in Hoagland nutrient solution artificially contaminated with the standardized concentration of aluminium, chromium, mercury and zinc for a period of 20 days. Important visible effect of these metals was morphological changes exhibited as reduced root and stem length and leaf area in comparison with the control (Table-2,3,4)

Effect of different heavy metals impart specific and nonspecific changes which vary from species to species and metals to metals. Since the primary toxicity mechanisms of different metal ions may be as different as their chemical properties vary. Primary characteristic features of heavy metal stress are membrane damage, inhibition of root growth, alteration of enzyme activities which leads to secondary effects like alteration in water relation, deficiency of essential nutrients, inhibition of physiological aspects such as, photosynthesis, respiration and photo assimilate translocation (Huang and Cunningham, 1996;

Singh, 2005; Solanki and Dhankhar, 2011). Even though morphological changes such as growth rate of root, shoot and leaf area due to presence of heavy metals can be observed within days of exposure to the metals. 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, signaling, movement and long-distance transport of nutrients (Pantoja, 2021).

Among the heavy metals selected in the present study for treatments on *Strobilanthes alternata*, morphological parameters to assess growth pattern include root length, shoot length and leaf area. Aluminium treatment resulted in only negligible differences compared to control (Table-2,3,4). At lower concentrations (*i.e.*, 400µM) aluminium imposes no retardation on growth, rather leaf growth is found to increase slightly during early days of growth revealing slight stimulating effect of aluminium and this observation is corroborated with the view of Kochian, (1995); Taiz *et al.*,2015) who suggested slight beneficiary effect of aluminium on plant growth at lower concentrations. However, root and shoot growth was reduced insignificantly due to aluminium treatment (Tables-2,3). Nevertheless, aluminium phytotoxicity is associated primarily with disruption of roots structure, physiology and functions of plants in general and roots in particular. (Taylor, 1998; Kochian and Shaff, 1991).

In *Strobilanthes alternata*, primary effect of aluminium on root anatomy is appearance of densely stained rhizodermal area in which cellular details are obscure and root hairs also appear damaged (Fig-8) whereas, protoxylem cells are more in number and developmental pattern (Fig-8) indicating the lack of aluminium toxicity in root growth and it seems that metal forms a thick rhizodermal region plausibly checking the entry of more Al^{3+} to stelar region.

However, stained particles are seen scattered all over the cortical cells. Anatomy of stem and leaf also affected by aluminium treatment in such a way that the stelar area become more conspicuous compared to the control (Fig-8) and primary xylem increase in number resulting in reduced pith region. This observation is in consonance with the view of Batista *et al*., (2013); Ozyigit *et al*., (2013) who stated that in corn and cotton which exhibited more or less similar anatomical features.

In general, the anatomical features of stem and leaf of *Strobilanthes alternata* remained unaltered except the stelar region which imparted slight stimulation due to the treatment. Development of epidermal hairs on the leaf (Fig-8) is found to be an effect of aluminium treatment and significance is not clear.

Treatment with chromium and mercury resulted in a significant reduction of growth in *Strobilanthes alternata* estimated as root and stem length, leaf area and dry weight per plant (Table-2,3,4,7) compared to the control. Growth retardation due to chromium toxicity has been reported in *Medicago sativa* (Peralta *et al*., 2001), *Brassica juncea* (Han *et al*., 2004), *Phaseolus vulgaris* (Barcelo *et al*., 1986), *Triticum aestivum* (Dotaniya and Meena, 2014), Maize (Anjum *et al*., 2017). Root structure of chromium treated plant exhibited completely damaged and densely stained rhizodermis and completely torn root hairs whereas stele showed more elaborated xylem which deeply stained with toluidine blue (Fig-9). Anatomy of stem and leaf did not show much variation due to chromium compared to control.

Mercury treatment resulted in reduction of root length, shoot length and leaf area (Tables-2,3,4,7). Another observation was thinning of root diameter than the control and other treatments (Fig-10). But rhizodermal cells were clearly seen since densely stained outer layer but root hairs were totally absent

due to mercury phytotoxicity. Inhibition of root growth was reported as one of the most important as well as rapid responses of toxic concentration of mercury (Wilkins,1978). Wong and Bradshaw, (1982) suggested that root growth is frequently used in many tolerance tests of plants. Dark stained masses were seen scattered all over the root section indicating the interference of mercury in the root development. Stelar cylinder also was reduced and cellular details were not clearly seen. An interesting observation was the development of many epidermal hairs on the stem and leaf of *Strobilanthes alternata* subjected to mercury treatment. These hairs are indicative of the role of epidermal hairs/ appendages which play vital role in the process of phytovolatilization of mercury as reported in *Brassica juncea* (Moreno *et al*., 2007), *Arabidopsis thaliana* (Gao *et al*., 2021).

In *Strobilanthes alternata* treated with zinc, the rhizodermal layer was densely stained and hence the cellular details were not seen. Stele and cortex region remained almost unchanged except stained patches scattered along the stele in comparison with the control. Anatomy of stem and leaf did not register any structural changes. Even though zinc is an essential nutrient, the concentration selected was 250µM which imposed about 50% growth inhibition (by visual observation) but the plants survived. During further growth in the same medium containing 250 µM the growth retardation is seemed to be due to prolong exposure to zinc, resulting deficiency of the metal and impaired growth.

Morphology/anatomy of roots of *Strobilanthes alternata* are slender, control (root) exhibited an undulated rhizodermal layer consisting of cells with irregular shape and arrangement (Fig-7), whereas the roots of plants with aluminium showed densely stained rhizodermal layer with thickened walls due to the deposits of aluminium complexed with cell walls (Fig-8) and hence the diameter of the roots found increased resulting in stunted growth and brittle

texture of the root. The undulated rhizodermal layer is plausibly due to the flimsy structure of the roots, since the roots are not meant for anchorage as the plants grow in nutrient solution. Stained particles distributed all over the root section indicates the presence of aluminium presumably due to some complex formation with cellular aluminium content stained with toluidine blue (Fig-8).

Inhibition of root growth is one of the effects of metal toxicity particularly cadmium and mercury in plants (Patra *et al*., 2004). According to Afraas, *et al.*, (2016) mercury treatment results in cell degeneration and thickening of cell walls in rice plants. Lignin synthesis is considered as an effect rather than a defensive mechanism of heavy metal toxicity.

Structure of roots in plants treated with chromium also rhizodermal cells are with thick walls and the cell layer appear thick and stained dark due to adsorption or formation of colored complexes with chromium (Fig-9). Dark deposits in the endodermal cells and vascular cylinder as well as rupturing of parenchyma tissues due to mercury has been reported in rice (Patra *et al*., 2004). According to Shah and Nongkynrich, (2007) and Rascio and Navari-Izzo, (2011) several plant species have been identified that extract, tolerate and/or hyperaccumulate metal pollutants from soil in their above ground tissues. Concentration of metal bio accumulator plants sometimes exceed environmental field concentration of common plant species (Maestri, *et al*., 2010).

Standardization of the optimal quantity of metals to impart inhibitory effects retaining and maintaining the survival showed significant difference in the concentrations of metals. Aluminium 400µM concentration is optimum to impart toxicity symptoms in *Strobilanthes alternata* while 70 µM Chromium was optimum. 20 µM mercury and 250 µM Zinc showed growth retardation symptom. Earlier studies reported that 60-70 µM Aluminium imparted toxicity symptoms in *Bacopa monneri* (Sinha, 1999). 12-24 mg/l Chromium in *Medicgo*

sativa (Shanker *et al*., 2003). Mercuric Chloride ($HgCl_2$) imparted toxicity symptoms at 5-10 µM Mercuric Chloride concentration in *Pisum sativum* (Beauford, 1977), 1-2 µM in *Chromalaena odorata* (Velaseo-Alinsug *et al*, 2005), *Bacopa monnieri (*Hussain, 2007).

Root is the first plant part to encounter heavy metal pollution concerning the uptake and translocation of heavy metals and the relationship between the metal and root tissue organization and development is very complex and vary metal to metal in one and the same plant and/or vary plant to plant also (Vaculk *et al.*, 2012). In *Strobilanthes alternata,* root growth, shape and structural variations as seen in anatomical study clearly indicates the differences in the structural organization shown as rhizodermal cells with thickened cell walls and densely stained outer layer ruptured cortex and distinct xylem cells with bright blue coloured cell wall in root section treated with aluminium(Fig-8), whereas structure of roots in *Strobilathes alternata* treated with chromium showed densely stained rhizodermal layer with damaged root hairs and a yellow colour was spread all over the section probably imparting the hew of orange coloured salt, potassium dichromate (Fig-9). Structural changes of *Strobilanthes alternata* roots subjected to mercury treatment exhibited striking differences such as thinning of entire root, complete loss of root hairs, rhizhodermal cells with thick cell wall indicating more hazardous impact of mercury compared to other metals (Fig-10). Zinc treatment also resulted characteristic structural changes in *Strobilanthes alternata* such as thick-walled xylem vessels, thickened cell wall, wide pith cells, and reduction in size of vasculature (Fig-11) in comparison with control roots (Fig-7). Localization of stained deposits indicating the presence of aluminium, chromium, mercury and zinc in the root also vary in locality, size, shape and darkness among the metals (Fig-7-11).

Shrunken nature of roots and presence of deposits on the rhizodermis is presumably due to the accumulated and/or adsorbed mercury/chromium (Fig-9). This observation is in consonance with the findings of Beauford *et al*., (1977) who suggested that *Pisum sativum* and *Mentha spikata* exposed to mercuric chloride showed the presence of mercury bound to cell wall components. Velasco-Alingsung, (2005) reported that in *Chromolena odorata* treated with mercuric chloride granular dark deposits were present on the rhizodermal layer of roots.

Tolerance index calculated on the basis of root length of the treatment in comparison with the control (Turner, 1994) showed the pattern as Chromium>zinc>aluminium>mercury (Table-6). Significant differences are not seen between the metals whereas during growth, tolerance index considerably differenced in the plants treated mercury and zinc. 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 *Strobilathes alternata* exhibit growth retardation soon after the exposure of aluminum, chromium, mercury and zinc. Tolerance index of *Strobilanthes alternata* towards excess metal concentration also be correlated to the bioaccumulation potential of the plant which will be explained with in detail under the section on bioaccumulation in the forthcoming pages.

Stomatal distribution showed significant differences between the stomata of lower and upper epidermis and more values were showed by stomata of lower epidermis (Table-5). Stomatal index values for *Strobilanthes alternata* treated with aluminum and zinc are more or less similar to control and of each other. Mercury effect resulted in an increase stomatal index compared to control and other treatments. Chromium treatment also showed considerable increase in

stomatal index value. The distribution of stomatal index values of upper epidermis remained unchanged except mercury where the values are slightly high.

More stomatal index in upper epidermis due to the effect of heavy metals in general, cadmium in particular in plants like *Arabdiopsis thaliana* has been shown to render stomatal conductance by osmoregulation of guard cells- water relations (Perfus- Barbeoch *et al*., 2002). Increased stomatal index in both upper epidermis and lower epidermis due to the exposure of aluminium, chromium, mercury and zinc may cause enhanced transpiration rate and resulted water stress. A significant role of increased stomatal index in the detoxification of mercury is apparent as it is related to the bioaccumulation pattern and phytovolatilization of mercury from the leaf through the stomata as reported in *Brassica juncea* (Moreno *et al*., 2004, 2007).

Inhibition of growth in general and that of root system in particular due to heavy metal toxicity as reported in many plants like *Oryza sativa* (Kim *et al*, 2002), *Vigna* species (Ratheesh-Chandra *et al*, 2010), lead in *Brassica* species (Shakoor *et al*, 2014), chromium in *Amaranthus viridis* (Zou *et al.*, 2006), mercury in *Triticum arestivum* (Setia and Bala, 1994) and in *Bacopa monneri* (Hussain, 2007).

Scanning electron micrographs of *Strobilanthes alternata* stomatal distribution showed significant differences in number of stomata per unit area (cm^2). Stomatal distribution of aluminium treated plants and the control appeared more or less similar (Fig-17) whereas chromium, mercury and zinc treatment resulted in slight increase. Stomatal opening also found to be affected due to heavy metal treatment. In the control, almost all stomata appeared closed while in the treatment's stomata remained maximum opened (Fig-17). More opening of stomata was found in the lower epidermis of mercury treated plants

(Fig-18) whereas in the treatments with chromium, aluminium and zinc the stomata opening was not uniform. Some stomata are widely opened, other partially closed. Stomatal index values are indirectly related to water relations, particularly transpiration (Meidner and Mansfield, 1968). Increased stomatal index in *Strobilanthes alternata* due to chromium and mercury treatment indirectly related to the water stress tolerance.

The increased stomatal index values in *Strobilanthes alternata* due to chromium treatment is in consonance with the view of Barcelo *et al.,* (1985) who suggested, that leaf water potential in chromium treated Phasolus *vulgaris* (Barcelo *et al*.,1986) remain unchanged. Treatment of Strobilanthes *alternata* with mercury showed increased stomatal index and opening of stomata also.

Dry weight distribution of the roots of *Strobilanthes alternata* is found to be more compared to the stem and leaf (Table-7). Maximum dry weight was observed in zinc treatment compared to other elements and control. Mercury treatment resulted in maximum dry weight content. Irrespective of these significant differences in the optimal concentration of aluminium, chromium, mercury and zinc on *Strobilanthes alternata* to impart toxicity maintaining survival, dry weight distribution in the root showed gradual increase and varied between the metals. Dry weight content of stems exhibited reduction only in plants treated with mercury while other treatments and control the dry matter remained unaltered. Dry matter content of leaves of plants treated with zinc exhibited increase compared to control and other treatments in which the increase of dry weight was negligible. Increased dry weight 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 level and tolerance vary from plant to plant (Reichman, 2002) according to whom plant responses both tolerance and toxicity phases both causing significant growth retardation.

Protein content of root exhibited maximum value than stem and leaf (Table-8). Roots of mercury treated plant contain maximum protein followed by chromium. Protein content is almost similar in aluminium with that of control. Impaired protein synthesis has been reported in plants under heavy metal stress (Reddy and Prasad, 1992; Prasad, 1997). Those authors reported inhibitory effect of heavy metals on protein synthesis. According to Prasad, (1997) one of the reasons for inhibited protein synthesis may be unavailability of essential amino acids. Inhibition of amino acids mobilization in the site of protein synthesis has been reported in *Pisum sativum* by Bishnoi *et al*., (1993).

Unlike the effect of chromium and mercury, resulted in increased protein content continuously during growth due to zinc treatment showed a decrease in protein content. Since zinc is an essential metal involved in metabolic process in plants, related to growth and development, reduced protein content in zinc treatment is presumed to be related to a stimulated effect of protein metabolism unlike inhibitory effect of toxic metals. (Taiz *et al* ,2015). More or similar content of protein in control and aluminium rather beneficiary effect as reported by Kochian, (1995).

One of the reasons for increased protein content in *Strobilanthes alternata* treated with chromium and mercury 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 chromium and mercury (Grill, *et al*., 1985; Verkleij *et al*., 1990; Salt *et al*., 1998; Chowdary and Panda, 2005).

Despite the increased protein in roots of chromium and mercury in plants, growth retardation was negligible in *Strobilanthes alternata* indicating the contribution of phytochelatin synthesis reported to occur in many plants under heavy metal stress. In otherwards, partial sequestration of heavy metals (mercury and chromium) toxicity is found to be due to the phytochelatin synthesis which is known to sequester toxicity in many plants (Rauser,1987; Reddy and Prasad,1990; Kubota *et al*., 2000; Cobbett and Goldsbrough, 2002).

In the leaves of *Strobilanthes alternata* more protein content due to all metals during growth up to 20 days reveals the variation and pattern and level of toxicity among aluminium, chromium, mercury and zinc. Increased protein content of leaves of mercury treated plants can be correlated with very low mercury content in leaves because toxicity of mercury is meager to inhibit protein synthesis resulting in more protein content. Leaves of aluminium treated plants showed high metabolic activity since toxicity of aluminium was very feable as already mentioned earlier. Since zinc is an essential element of plant growth increase in protein in leaves of *Strobilanthes alternata* cannot be ruled out (Taiz *et al*., 2015). Increased protein synthesis induced under heavy metal stress to sequester toxicity was reported in plants (Chandra *et al*., 2009; Zhang, *et al*., 2002).

Proline content is increased continuously during growth up to 20 days in control and treatments (Table-9). Due to all treatment proline content of leaf is more in all intervals than the respective controls. Proline has multiple functions and the important one is regulation of osmoticum change imposed by drought (Saradhi and Saradhi, 1991; Rout *et al*., 1997). The enhanced proline content of root compared to the control indirectly reveals the water deficient in the growth medium due to the presence of metal ions and hence proline play the role of osmoregulation in the roots. Nevertheless, proline content of roots in aluminium

and zinc treatments is comparatively lower than chromium and mercury presumably due to the more toxicity of chromium and mercury than aluminuim and zinc as reported in chromium treated tomato, (Moral *et al.*,1995) *Caesalpinia pulchermia* (Iqbal *et al.*, 2001) and a mercury treated *Rubia Tindorium* (Maitani *et al.*, 1996) .According to Costa and Morel, (1994) water potential reduction due to metal ions get alleviated by proline accumulation in plants.

Proline concentration of the stem also vary from metal to metal in *Strobilanthes alternata* compared to the control and the difference in distribution of proline in the stem of all treatments is found to be mainly due to the presence of toxic metal ions rather than osmoregulation because the dry weight content (Table-9) reciprocally reveals the moisture content which remained almost unaltered in the stem tissue of all treatments. Leaf of *Strobilanthes alternata* contained more proline compared to the root and stem in all treatments. Occurrence of proline in all treatments is almost alike with minor fluctuations between them. Bioaccumulation pattern of *Strobilanthes alternata* plants treated with aluminium, chromium, mercury and zinc (in the leaf, stem or root) is related to proline distribution. According to Hopkins (2000), Rai *et al* (2004) accumulation of proline in *Ocimum tenuiflorium* is related to the strategies adopted by the plant to cope up with heavy metal toxicity. Since proline has multiple functions such as osmoticum maintenance, scavenging of free radicals, stabilization of membranes etc. Accumulation of proline due to heavy metal toxicity have been reported in plant *Phaseolus vulgaris* (Zengin and Munzuroglu, 2005). Matysik *et al.,* (2002) unequivocally interpreted the molecular mechanism of quenching of reactive oxygen species by proline under stress in plants. Among the treatment chromium and mercury induces comparatively more proline synthesis and the toxic effect of these metals

seemed to be mitigated by proline as suggested by Zengin and Munzuroglu (2005).

An important role of proline is the function of the amino acid as a compatible solute which is an osmotically active organic compound but do not interfere with metabolic enzymes activator even at higher concentration unlike organic and inorganic ion (Taiz *et al*., 2015). So, in *Strobilanthes alternata* subjected to toxic metals like aluminium, chromium, mercury and zinc that is mostly metal ions get accumulated in vacuole to alleviate the stress and to maintain the equilibrium of water potential between vacuoles and cytosole compartments, the compatible proline increases in the plants.

Phenolic content of root exhibited only slight increase in *Strobilanthes alternata* subjected to aluminium treatment (Table-11) whereas stem and leaf contained significantly high amount of phenolics. Distribution of phenolics in the root, stem and leaves was significantly increased in plants treated with chromium and mercury. Zinc treatment resulted in only slight increase in the roots.

Phenolics come under an important group of secondary metabolites and constitute a number of compounds/molecules. Harborne (1980) reported enhanced phenolic synthesis in plants under environmental stresses. Root growth inhibition is reported as one of the effects of chromium (Kahle, 1993), mercury (Godbold and Huttermann,1986) and aluminium (Goldbold and Kettner,1991). Toxic effect of heavy metals generally results in morphological and anatomical changes such as woody nature, slender growth and cell wall thickening (Cseh, 2002). The woody structure of roots indicates enhanced lignification of cell wall (Fahn,1982). According to Buchanan *et al*., (2000) phenolics are the precursor of lignin. *De novo* synthesis of soluble phenolics occur under heavy metal stress and these phenolics act as intermediate in lignin

biosynthesis (Michalak, 2006). The anatomical modification with in the root of *Strobilanthes alternata* treated with mercury and chromium are positively correlated with increased phenolics.

Increased phenolics of roots particularly plants subjected to chromium and mercury stresses is found to be indirectly correlated with woody structure of roots with thickened and lignified cell wall (Table-11). 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).

In *Strobilanthes alternata* plants treated with aluminium, phenolics of root showed only moderate increase compared to chromium and mercury in which a hike of phenolics occurred in the roots. Induction of phenolics compounds biosynthesis has been reported in maize in response to aluminium (Winkel-Shirley, 2002) and in *Phaseolus vulgaris* exposed to cadmium (Dietz *et al*., 1999).

Significant increase of phenolics in the stem and leaf of plant treated with zinc, presumably indicate the tolerance towards zinc since phenolics synthesis known to be related to mitigation of stress impact and superimposed with the defensive mechanism of phenolics against metal toxicity (Grace, 2007; Michalak, 2006; Chalker-Scott and Fuchigami, 2018). Since zinc is an essential metal for plant growth, behavior of *Strobilanthes alternata* in terms of proline accumulation (Table-11; Fig-38) root morphology/ anatomy (Fig-2,11) due to zinc treatment is more or less similar to the control plants in spite of significantly increased phenolics in zinc treated plants.

Another important property attributed to phenolics is antioxidant potential functioning in plants under stressful condition (Michalak, 2006). According to the author flavonoid group of phenolics are able to directly scavenge molecular species of ROS due to their ability to bind with phenolic molecules. Flavonoids constitute an enormous class of phenolics and commonly occur in natural products (Buchanan *et al*., 2000). According to Hossain and Rahman (2016) flavonoids isolated from the leaves of *Orthosiphos staminens* scavenge free radicals and ROS and also inhibit lipid peroxidation. Significant increase of phenolics in almost all tissues in general and that of roots in particular of *Strobilanthes alternata* particularly treated with chromium and mercury either due to the antioxidant potential and/or ROS scavenging property. Morgen *et al*., (1997) suggested that antioxidant property of phenolics is due to their high tendency to chelate metals because phenolics processes hydroxyl and carboxyl groups which are able to bind metals. According to Lavid *et al*., (2001) direct chelation of chromium and mercury by binding to phenolics occur in *Nymphaea alba*. Coincident of abundant phenolics and maximum bioaccumulation of metals aluminium, chromium, mercury and zinc in the roots indicate the chelation of these metals by phenolics (Table-15) revealing in sequestration and reduced translocation to the shoot.

Malondialdehyde production (MDA) is closely associated with lipid peroxidation and MDA content is considered as an indicator of peroxidation of membrane lipids in plants (Buchanan *et al*., 2000). Oxidative stress is the most powerful impact of heavy metal stress as reported by Dietz *et al*., (1999), Singh *et al*., (2004) and Singh and Swena (2006). MDA content of roots of Strobilanthes *alternata* is found to be very high in treatment with all metal (Table-10). Leaf also exhibited very high MDA content and stem showed only moderate increase. Generation of free radicals and ROS is an established impact of stresses and their synthesis is stimulated by the presence of heavy metals in

plants (Halliwell and Gutteridge, 1999) and hence normal metabolism is disrupted by lipid peroxidation of membrane system resulting in MDA production. Maximum MDA content is present due to lipid peroxidation in the roots of *Strobilanthes alternata* treated with aluminum, chromium and zinc compared to other tissues (Table-10). Lipid peroxidation in the leaf tissue of plants treated with chromium and mercury also increased significantly. MDA is routinely produced as a result of lipid peroxidation under stressed condition and is used as an index of stress states (Zhang *et al*., 2007). Increased MDA content has been reported in the roots and leaves of *Brassica juneca* and *Cajanus cajan* treated with zinc (Abia *et al.*, 1995). Exactly similar behavior is shown by *Strobilanthes alternata* treated with zinc. Enhanced rate of MDA synthesis is shown in *Triticum aestivum* and *Brassica campertis* due to toxicity of chromium and cadmium (Chandra *et al.*, 2009). Maximum MDA content in the leaves of *Strobilanthes alternata* treated with mercury and chromium indicate lack of tolerance since *Strobilanthes alternata* is sensitive to comparatively low concentrations of 20µM and 70 µM respectively compared to the concentration of aluminium (400 µM) and zinc (250 µM) to which the plants are exposed.

According to Bradley and Min (1992) oxidation of unsaturated fatty acids of membrane lipids by singlet of oxygen produces different products inclusive of Malondialdehyde hydroxyl radicals one of the components of ROS generated in response to heavy metal stress also induce lipid peroxidation. Increased MDA content in the roots of *Oryza sativa* treated with chromium has been reported by Panda (2007). Enhanced production of MDA in the roots of *Strobilanthes alternata* subjected to aluminium and zinc compared to stem and leaf presumably due to close contact of the roots to comparatively high concentration of aluminium and zinc (400µM and 250 µM respectively) present in the growth medium.

Superoxide dismutase activity of root, stem and leaf remain unaltered compared to the control in *Strobilanthes alternata* subjected to aluminium treatment (Table-13). A dramatic increase of SOD activity occurs in plant treated with chromium comparted to other metals and control. SOD is the first enzyme to detoxify highly reactive oxygen species in plants by converting O_2^- radicals to H_2O_2 (Giannopolitis and Ries, 1977). The product of SOD activity, H_2O_2 is still toxic and must be eliminated by conversion to water in subsequent reaction. In plants, stress enzymes like catalase and peroxidase are considered as most important in scavenging H_2O_2 (Noctor and Foyer, 1998; Zhang *et al.*, 2007). Catalase eliminates H_2O_2 by breaking it directly to water and oxygen.

In *Strobilanthes alternata* feeble activity of both SOD and catalase in aluminium treated plants compared to control indicates the reduced toxic impact of aluminium and this behavior of in agreement with the toxicity symptoms shown by parameters like, lack of growth retardation (Fig-2,3,4), impaired metabolism, anatomical variation (Fig-8) etc. in aluminium treated plants. SOD activity of root, stem and leaves of plants subjected to chromium treatment increased two to three-fold compared to the control presumably indicating the enhanced stress induced by chromium is mitigated by this enzyme whereas catalase activity of chromium treated plants is feeble and no complimentary effect of this enzyme or SOD activity is seen in *Strobilanthes alternata* under chromium stress. Catalase is less efficient than other peroxidases in scavenging H_2O_2 because of its low substrate affinity (Zhang *et al*., 2007). So, in *Strobilanthes alternata* one of this specific reason for feeble catalase activity may be the reduced substrate affinity and this view is conceivable because increased activity of SOD in all treatments particularly in chromium treated plants results in the exorbitant production of H_2O_2 which is not properly functioned as substrate for catalase activity. Effect of metal stresses due to aluminium, chromium, mercury and zinc in *Strobilanthes alternata* exhibited as

structural changes, modifications, increased synthesis of MDA and proline clearly indicate the occurrence of only moderate stress impact. So, it seems that since the stress is not much strong for defense potential. SOD activity is efficient to cope with metal activity. According to Siedlecka and Krupa (2002) as long as heavy metal stress is not too strong for the plants defense capacity the maximum response to heavy metal is an increase in SOD. Shanker *et al.,* (2004) suggested that combined activity of SOD and catalase is critical in mitigating the effect of oxidating stress imposed by chromium in *Vigna radiata*. But in *Strobilanthes alternata* the feeble activity of catalase is not coordinated with SOD activity. So, plant shows reduced tolerance towards chromium which have already been demonstrated in different parameters adopted in the present study.

Bioaccumulation pattern of aluminium, chromium, mercury and zinc vary significantly between the metals (Table-15). Maximum accumulation of all metals occurs in the roots than other parts (Table-15) but depending on the optimum concentration given to the plants to impart slight toxicity, thus accumulated quantity of each metal cannot be compared because significant differences do occur in the concentrations of treatments. However, slight proportionality can be observed between the concentration of treatments and contents accumulated in various plant parts-root, stem and leaves. Nevertheless, the variations in the bioaccumulation pattern of aluminium, chromium, mercury and zinc are found to be due to the specificity of individual metals in the process of absorption, translocation and accumulation.

Aluminium accumulation in the roots of *Strobilanthes alternata* is very high and stem and leaf also exhibited significant aluminium content during all intervals of growth. Huge percentage of aluminium is observed to enter the plant retaining some percentage in the residual solution. The morphology, anatomy and distribution pattern of metabolites of Strobilanthes *alternata* plants

treated with aluminium showing negligible stress symptoms and impact of toxicity (Table:2-6; Fig:2-11), rather a positive influence in growth and metabolism is apparent. Hence the tolerance potential of *Strobilanthes alternata* towards aluminium is very high since the availability of considerable quantity of aluminium in the nutrient solution never result in any inhibitory effect of aluminium. Aluminium absorption, translocation and accumulation have been reported in Delhaize and Ryan, 1995, Kochian, 1995, Taylor, 1998.

Strobilanthes alternata shows comparatively low content of chromium in the roots, stem and leaves in the order root < stem > leaf and the same trend follows throughout the growth period and retaining low chromium content in the residual solution (Table-15). Progressive accumulation of chromium with more content in the roots (10 – 200 times) than the shoots have been reported in *Lettuce sativa* (Singh, 2001) and *Nelumba nucifora* (Vajpayee *et al* ,1999). *Veronica becanga* and several hydrophytes have been reported to show high chromium removal from soil (Zurayk *et al*., 2001). According to Kabata-pendias and Pendias (2001) progressive increase of chromium accumulation occur in the roots and shoots of *Helianthus annus*, *Zea mays* and *Vicia faba*. The bioaccumulation pattern shown by *Strobilanthes alternata* in various tissues – root, stem and leaves are similar to the reports of Kabata-pendias and Pendias (2001).

Another correlation can also be drawn between the root anatomy and heavy metal accumulation behavior of *Strobilanthes alternata*. Presence of chromium depicted in various tissues in the form yellow tinge appearing all over the roots and dark localized particles and damaged cellular structure, cell wall thickening etc. indicate the presence and/or impact of chromium (Fig-9). Although the anatomical aspects have been studied only in one interval of the growth period, a positive correlation can be drawn between the root structure

and chromium accumulation pattern in *Strobilanthes alternata*. Accumulation of chromium in the stem tissue also is found to be related to the stem anatomy which showed development of epidermal hairs as trichomes (Fig-9). Trichomes have been reported to involve in the sequestration of heavy metals like cadmium in Tobacco (Choi *et al*., 2001), *Arabidopsis thaliana* (Dominguez solis *et al.*, 2004), *Arabidopsis thaliana* (Gao *et al*., 2021).

Bioaccumulation of mercury also reveals maximum content in the roots and get increased significantly during growth (Table-15). Mercury content of stem tissue is comparatively low but during growth significant increase occurs. Accumulated mercury content in the roots and stem tissue of *Strobilanthes alternata* display the absorption and translocation of the metal and resultantly only meager amount reaches the leaf during early days of growth and get reduced to below detectable level.

Anatomy of root shows thick walled rhizodermal layers. Root hairs vanished and vasculature become indistinct stele. Structure of stem shows considerable anatomical changes such as localization of stained spots and development multicellular epidermal hairs and trichome like appendages on the epidermis (Fig-7-11). The epidermal modification of the stem is found to be related to the accumulation and for sequestration of mercury as reported in *Arabidopsis thaliana* in which cadmium is sequestrated in trichomes (Dominguez-solis *et al*., 2004). In *Chromolena odorata* the accumulation pattern of mercury is quite different (Velasco-Alinsug *et al*., 2005). The plant accumulate mercury in a progressive order in the root, stem and leaves (root<stem<leaves) and which this plant has been recommended as a phytoremediant because, inside the plant's mercury and Sulphur content of stem forms a stable product 'Cinnabar' which is insoluble and get removed along with the disposal of the plant for phytoremediation purpose. Given no plants

have yet been reported as natural hyper-accumulator of mercury (Henry, 2000; Raskin and Ensly, 2000), transgenic plants such as *Arabidopsis thaliana* and *Nicotiana tabacum* are capable of accumulating and converting methyl mercury. Studies on mercury accumulation to evaluate the capacity in removing mercury from water containing different concentrations of mercury in four aquatic plants, *Eichhornia* species, *Pistia stratiotes* and *Colocasia esculents* revealed that accumulation is maximum in plants treated with more amount of mercury salts (Zimmels *et al*., 2004). According to Skinner *et al*., (2007) loss of mercury has been reported to occur by volatilization from plants. The process of volatilization of mercury from aerial part of plants has been reported in *Bacopa monneiri* (Hussain and Nabeesa, 2012), *Phaseolus mungo* (Hussain *et al*., 2010) take place through epidermal trichomes developed in the stem and leaves. In *Strobilanthes alternata* also the epidermal layer of stem and midrib on adaxial side of the leaf exhibit the presence of multicellular epidermal hairs and trichomes (Fig-7) which are entitled to take part as passage for the volatilization of mercury. It is clearly seen that in the leaves of *Strobilanthes alternata* mercury accumulation is very low, presumably due to the loss of mercury from the leaves and stem through trichomes by means of phytovolatilization. The concentration of mercury in which *Strobilanthes alternata* shows toxicity symptoms is comparatively very low (20 µM). Nevertheless, considerable accumulation is observed in the roots and shoots and hence retaining of mercury is below detectable level (BDL) (Table -16) whereas in the case of aluminium and zinc residual amount of these metals comparatively high and proportional to their respective concentrations i.e.; 400 µM and 250 µM respectively. Hence it can be presumed that the bioaccumulation potential of *Strobilanthes alternata* is dependent on the concentration of the metals in the growth medium, mode of translocation and toxicity level of individual metals. A comparison can also be made between the total amount (i.e.; accumulation in the root, stem and leaf) of

each metal content in the plant and the quantity given in each treatment in the nutrient medium.

Strobilanthes alternata treated with aluminium resulted in very high accumulation of the metal in the root exhibiting a progressive increase from stage to stage. Aluminium content accumulated in the stem and leaf also was comparatively higher than that of the roots and the values were less than one half of the aluminium accumulated in the root (TF) and considerable aluminium content was retained in the residual solution. The translocation potential of aluminium from root to stem and from stem to leaf is found to be very high compared to chromium, mercury and zinc. Aluminium comes under the redox inactive group of metals (Hossain *et al*., 2012) and causes oxidative stress resulting in the interaction with antioxidant defense system at a reduced level compared to the other metals which are highly involved in the formation of ROS (Dietz *et al*.,1999). Generally, all heavy metals get absorbed more in the root and partially only translocated to the shoot in order to regulate or minimize the adverse effect of heavy metals (Qureshi *et al*.,1995; Wheeler and Power, 1995). According to Tice *et al*., (1992) occurrence of more metals in the root system is an exclusion method against toxicity. In wheat plants aluminium stress is reduced by exclusion from the root (Kochian,1995).

In the present study, the plant is cultivated in nutrient solution containing known quantities of metals and hence the bioaccumulation potential of the plant observed cannot be comparable or equalize to comply with the accumulate pattern in the soil system because in the soil the elemental concentration and pattern are very complex and antagonistic and synergistic effect cannot be ruled out. An important impact of mineral nutrition in plants is antagonism and/or synergism between ions as suggested by many authors (Orcutt and Nilsen, 2000; Cseh, 2002; Hopkins *et al*., 2004; Taiz *et al*., 2015). According to those

authors, absorption/ accumulation of one element may be antagonistic or synergistic to another element and depend on the availability and/or interaction of other ions as well as several scenarios of nutrition and these two phenomena are species specific also.

The distribution pattern of chlorophyll a, chlorophyll b and total chlorophyll of leaves is found to be altered due to the treatment with different metals- aluminium, chromium, mercury and zinc (Table-12). Chlorophyll is often estimated in order to assess the impact of environmental stress since the changes with pigments are linked with visual symptoms of growth disorder and photosynthetic productivity (Parekh *et al*., 1990). Aluminium treatment resulted in significant, linear increase of all the components of chlorophyll compared to the control. Aluminium treatment resulted in the maintenance or slight stimulation in the chlorophyll contents and the observation is indicative of the lack of toxicity in *Strobilanthes alternata* due to aluminium as observered in the other parameters adopted in the present study as mentioned earlier. A general trend of reduced chlorophyll contents shown by plants subjected to chromium is found to be another important inhibitory effect of chromium in plants and photosynthetic activities. Reduction of chlorophyll synthesis and inhibited photosynthetic rate have been reported in wheat as a result of chromium stress (Mukhopadhyay and Aery, 2000). According to Vajpayee *et al.,* (2000) chromium inhibits biosynthesis of chlorophyll by impaired γ amino leucine acid dehydrogenase activity leading to reduced pigments in *Nymphaea alba*. *Strobilanthes alternata* plants treated with mercury showed an increase in chlorophyll content up to 12^{th} day and followed by a reduction on 20^{th} day. Inhibition of chlorophyll synthesis due to mercury 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- copper, cadmium, mercury, lead, zinc etc. interfering with chlorophyll synthesis by

substituting Mg^{2+} of chlorophyll molecules and resultant inhibition of photosynthesis. According to Ahmed and Tajmir-Riahi (1993) mercury interact 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. Notwithstanding, in *Strobilanthes alternata* drastic reduction of chlorophyll a, b and total chlorophyll is not observed presumably due to the reduced concentration given to the plant which are found to impart only slight alteration or concentration of metabolites and regulatory molecules such as phenolics, pholine, MDA etc. resulting in the overall growth and survival of the plant under the given concentration of aluminium, chromium, mercury and zinc.

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 lead and Cadmium.

Carotenoids are integral constituents of the thylakoid membrane and are usually associated intimately with many of the protein that makeup the photosynthetic apparatus. Carotenoid content of *Strobilanthes alternata* leaves registered significant increase only in plants treated with mercury (Table-12). All other pigments increase in the treatment of aluminium and zinc, whereas carotenoid content of aluminium treatment remained unaltered. Carotenoid pigments play an essential role in photoprotection when the photosynthetic membrane get 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, mercury induces maximum toxicity in *Strobilanthes alternata* in the terms of parameters like reduced SOD activity (Fig-13), proline and MDA (Fig-9,10) content etc. resultantly production of comparatively more ROS can be noticed and hence the ROS scavenging activity of carotenoid cannot be ruled out in *Strobilanthes alternata*.

Responses of Strobilanthes *alternata* towards mercury stress is not similar to other metals. MDA content was comparatively more whereas SOD activity registered slight reduction compared to chromium treatment maintaining comparatively high activity of SOD in plants treated with other metals (Table-13). This behavior of Strobilanthes *alternata* towards mercury stress can also be interpreted by comparing the toxicity level and concentration of metals given to the plants. Among the four metals, *Strobilanthes alternata* shows more sensitivity towards both chromium and mercury. But the molar concentration of mercury given to the plants is the least (20 µM) whereas the concentration of chromium is more than three times (70 µM). The SOD activity of mercury treated plants shows significant reduction during growth up to 20 days and this observation was confirmed more toxicity of this metal than other metals. So, a comparison of stress response of Strobilanthes *alternata* towards chromium and mercury shows the plant is more sensitive to mercury than chromium as well as other metals. Given the differences with responses of *Strobilanthes alternata* towards the four metals - aluminium, chromium, mercury and zinc in the distribution of biomolecules, antioxidant enzymes, bioaccumulation potential etc., the tolerance level rather mechanism is found to be more or less alike with obvious fluctuations. Notwithstanding, the marked differences in the molar concentration of aluminium, chromium, mercury and zinc opined on the basis of more or less similar morphological manifestation reveal that each metal ion inclusive of essential and non-essential elements

exhibit specificity in the metabolic role, interference and for impact on *Strobilanthes alternata*.

SEM-EDX study was conducted on *Strobilanthes alternata* 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. SEM study conducted on Strobilanthes *alternata* root, stem and leaf of plants treated with aluminium, chromium, mercury and zinc provided more details of cellular structure and localization of the metals in the section (Fig-19-33). 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 (Fig 19-33). A combination of Scanning Electron Microscopy and Energy Dispersive X-ray analysis 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. Aluminium exists not as free form; in combination with O_2, silicon, fluoride etc. (Silva, 2012). Aluminium forms irreversible macromolecular complexes in the cytoplasm (Poschanrider *et al*., 2008). Aluminium prevents root tip elongation, bind with cell wall mucilage in root tip due to the secretion of aluminium chelating ligands, binding of aluminium with cell wall (Delhaize and Ryan, 1995., Matsmoto, 2000).

Based on EDX analysis, the weight percentage of ions in root, stem and leaves vary widely between the metal and plant parts (Figure-19-33). Generally increased values are observed in the case of potassium and calcium in aluminium treated plants than the control. Chromium treatment showed only

increased calcium and boron in the root, stem and leaf. Mercury treatment resulted in more ions of potassium, calcium and boron.

The accumulation profile in terms of BCF and TF (Table-16) of all the metals (Aluminium, chromium, mercury and zinc) is found to be almost similar to the weight percentage obtained in GCMS-EDX. Notwithstanding, EDX values of the other ions present in the nutrient solution seems to be incomparable to each other and the increased weight percentage of calcium and potassium invariably occurs in almost all treatments.

When a comparison is made between the weight percentage of all metals in the SEM-EDX profile (Table-19-33) the distribution pattern implies more or less similar pattern in the case of ions generally present in the plant which is naturally obtained from the nutrient medium. But weight percentage of aluminium, chromium, mercury and zinc are significantly high in their respective treatments and this pattern is more or less similar to the bioaccumulation of these elements estimated quantitatively using biochemical methods. Hence EDX analysis is confirmatory to the biochemical analysis rather indicating more accuracy and distinction. It seems that SEM-EDX image/tables shows the occurrence of ions of only insoluble components of the cell since the values are very low probably pertaining to the small quantity of tissues subjected to EDX profiling and soluble ions may be lost during processing the tissues for SEM.

The EDX profile of all treatments are characterized by another observation that occurrence of more weight percentage of calcium compared to other elements in all treatments. Even though calcium is an integral component of Hoagland nutrient medium (Epstein, 1972) the quantity shown by EDX is very high compared to the nutrient solution (Fig-19-33). A plausible explanation for this finding is the occurrence of various forms of calcium

crystals (cystoliths) which have been reported in many members of Acanthaceae family (Gabel *et al.*,2021) to which *Strobilanthes alternata* belongs. According to those authors, occurrence of cystolith have been reported in *Strobilanthes alternata.* Hence it can be speculated that since the cystolith are made up of calcium carbonate the crystals remain insoluble and contributes to accumulation of more calcium than other elements as shown by EDX profile (Fig-19-33). However, weight percentage of calcium in all treatments with all metals and control plants exhibit only negligible variations and effect of these elements can impose no significant changes in the metabolism of *Strobilanthes alternarta.*

Accumulation of heavy metals in root, stem and leaves can also be interpreted in comparison with the quantity of each metal given to the plant in Hoagland nutrient medium. The distribution of metals in the root and stem can be evaluated using BCF (Bioconcentration factor) and TF (Translocation factor) as per the view of Yoon *et al., (*2006). According to those authors, hyperaccumulation of metals occurs when a contaminant taken up by a plant is not degraded rapidly resulting in high accumulation.

Tolerant plants tend to restrict soil-root and root-shoot transfer and therefore have more or less similar metal concentration in their biomass while hyperaccumulator plants absorb and translocate metals into their above ground biomass. Plants exhibiting TF and BCF values less than one (<1) are unsuitable for phytoremediation (Fitz and Wenzel, 2002). BCF indicates the bioavailability of the metal from the soil (medium of growth) and the value is also related to the absorption potential of each element (Yoon *et al*., 2006).

Potential of plants to accumulate metals from soils can be evaluated using the BCF (Bioconcentration factor) and ability of plant to translocate metals from the roots to shoots is analyzed using TF (Translocation factor) (Yoon *et al*.,2006). The ability of *Strobilanthes alternata* to absorb and translocate metals

from the growth medium to shoot was evaluated by comparing BCF and TF. BCF values of aluminium treated plants increased gradually throughout the growth and between the stages the difference in BCF values were significant (Table-35). *Strobilanthes alternata* showed highest BCF values in the treatment with aluminium at the final stages of growth. TF values are less than one in all stages of aluminium treated plants and hence *Stroboilanthes alternata* is not suitable for phytoextraction with the view of Yoon *et al., (*2006). TF values showed considerable reduction from stage 4 to 12 followed by an increase in the final stage of growth. Increased TF values of aluminium treated leaf during the last stage of growth indicate the enhanced translocation of aluminium from root to shoot and this observation is in agreeable with the concept of Kochian, (1995) who suggested aluminium ions get translocated to various parts of the plant without interfering the metabolism.

BCF values of *Strobilanthes alternata* treated with chromium are very low but increase during growth period while TF values remain almost unchanged indicating absorption pattern is not proportional to translocation with chromium mobilization pattern since a significant increase of chromium is observed in the roots (Table-16). Progressive accumulation of chromium content in the root than the shoots have been reported in *Lactice sativa* (Singh,2001) and *Nilumbo nucifera* (Vajpayee *et al*., 1999).

Bioaccumulation pattern of mercury in *Strobilanthes alternata* shows significant increase only in TF values revealing enhanced translocation to the shoots compared to the roots. Accumulation pattern of zinc in *Strobilanthes alternata* is different from that of aluminium, chromium and mercury. BCF factor registered only very low change. But the TF values show exorbitant increase observed in the final stage of growth is indicative of the enhanced translocation of zinc to the shoot meanwhile in the root system comparatively

the zinc content remained unchanged presumably due to the exhaustion of the metal in the medium. Since the zinc is an essential metal translocation to the areal part is essential for growth.

Considering the area percentage of GC-MS chromatogram (Fig-43) of alcohol soluble compounds revealed the presence of 15 bioactive components in *Strobilanthes alternata* were identified and methyl palmitate is the most abundantly occurring molecule. Methyl palmitate is characterized by anti-inflammatory activity (Usha and Nazarine, 2003: Wang *et al*., (2009). According to (Usha and Nazarine, 2003) methyl palmitate possesses antifibrotic activity also and this quality can be considered as a contributory factor to the process of wound healing. Phytol is the second abundant component which is having antimicrobial, antinociceptive, antioxidant and immunostimulant potential (Ryu *et al*., 2011: Santos *et al*., 2013). In addition to methyl palmitate and phytol, neophytadine and 3,7,11,15 tetramethyl 2 hexadecen 1 ol also are present in *Strobilanthes alternata* which have already been reported to have anti-inflammatory activity (Venkataraman *et al*., 2012). Antibacterial and antifungal property of *Strobilanthes alternata* has already been reported due to the presence of neophytadine, sesquiterpene (Hexahydrofarnesyl acetone), isophytol and phytol (Venkataraman *et al*., 2012). *Strobilanthes alternata* contains comparatively very small quantity of squalene which is a triterpene and a biochemical intermediate for the synthesis of sterols, hormones and vitamins which are present in almost all vegetable oils (Lozano-granta *et al*., 2018).

Medicinal property of *Hemigraphis colorata* (Synonym: *Strobilantes alternata*) is known earlier and has been used as a medicine for wound healing as a folk medicine. Research publications on the medicinal use of this plant reveals the antimicrobial (Anitha *et al*., 2012) and anti-inflammatory properties (Subramoniam *et al.,*2001). Antioxidant activity has also been mentioned

recently (Megha *et al*., 2013; Akhil and Prabhu, 2013). All these investigations are based on experiments with microbes and or test animals subjected to treatment with aqueous and alcoholic extracts of *Strobilanthes alternata*. However, the principles and/or phytochemical roles of the component of the extracts have not yet been delineated.

As mentioned above, GC-MS study on alcohol extract of *Strobilanthes alternata* (*Hemigraphis colorata*) revealed the occurrence of a number of bioactive components (Table- 17) of which many molecules possess active principles pertaining to the medicinal use for wound healing. Methyl palmitate is abundantly occurring in *Strobilanthes alternata* and this component is having anti-inflammatory property (Goswami and Nazarine, 2003; Wang *et al*., 2009). Anti-inflammatory potential of this plant is found to be due to the presence of neophytadine, 3,7,11,15-Tetramethyl 2 hexadecen 1 ol because these molecules are known to play anti-inflammatory role in accordance with the view (Venkataraman *et al*., 2012). Another bioactive molecule present in *Strobilanthes alternata* is phytol which is the second abundant component possessing antimicrobial, antinociceptive, antioxidant and immunostimulant activities as opined by Ryu *et al*., 2011 and Santos *et al*., 2013. Hexahydrofarnesyl acetone (sesquiterpene), Isophytol are potential molecules characterized by antibacterial and antifungal properties as reported by Venkataraman *et al*., (2012). Methyl stearate is present in the leaf extract of *Strobilanthes alternata*. Ukwubile *et al*., (2019) stated that methyl stearate isolated from *Melastromatum capitatum* play a vital role in health care systems and according to those authors presence of methyl stearate and fatty acids in the leaves of the plant might be responsible for its biological activity in traditional medicine.

Antifungal activity of fatty acid methyl esters of vegetable oils has been demonstrated and evaluated in case studies on fungus (Pinto *et al*., 2017). Antioxidant potential was demonstrated by scavenging effect of these fatty acid methyl esters on 2,2-diphenyl 1- picryl hydroxyl (DPPH) radicals.

Antioxidant activities of many secondary metabolite molecules is well known and excellently interpreted in the process of wound healing (Shetty *et al*., 2007; Suntar *et al*., 2012). 3,7,11,15-tetramethyl 2 hexadecane 1 ol, 9-octadecenoic acid, phytol, fatty acid methyl esters and squalene are the bioactive components present in *Strobilanthes alternata* which are characterized by antioxidant potential (Venkataraman *et al*., 2012; Santos *et al*., 2013; Pinto *et al*., 2017; Mishra *et al*., 2020). Antioxidant potential of *Strobilanthes alternata* extract has been demonstrated by DPPH assay (Akhil and Prabhu, 2013; Megha *et al*., 2013).

Houghton *et al*., (2005) reported the involvement of antioxidant components in wound healing process in *Secamone alzeli* and *Spathodea campanulata*). In addition, a number of plants that have been reported to show antioxidant potential coming under different families have also been shown to have wound healing property (Suntar *et al*., 2012).

Squalene is a triterpene that also occurs comparatively in very small quantity in *Strobilanthes alternata* (Table- 17). According to Lozano-Granta *et al*., (2018) squalene is a biochemical intermediate possessing antioxidant activity present in all vegetable oils. Aioi *et al*., (1995) reported the scavenging role of squalene on ROS produced by oxidative stressors and emphasized the protective role of this molecule in combination with SOD. Squalene is abundant in skin (Micera *et al*.,2020) which is the target tissue exposed to environmental stresses leading to oxidative stress and hence squalene plays an antioxidant role in protecting the skin due to the specific role of squalene (Micera *et al*.,2020).

Shetty *et al*., (2007) interpreted that wound healing is the process of repair that follows injury to the skin and initial stages of wound healing involves an acute inflammatory phase. According to those authors, the antioxidant properties of *Ocimum sanctum* is found to be responsible for faster wound healing in rats. Incision wounds in rats resulted in the increased antioxidant enzymes and *Ocimum* extract treatment showed decreased activity indirectly establishing antioxidant potential of *Ocimum sanctum* in wound healing (Shetty *et al*., 2007).

According to Dissemond *et al*., (2002) in the therapy of chronic wounds overproduction of ROS results in the oxidative stress, thereby causing cytotoxicity and delayed wound healing. Antioxidant enzymes like SOD, catalase, glutathione etc. hasten the process of wound healing by destroying the ROS (Shetty *et al*., 2007). It is known that the wound healing process can be aided by the presence of antioxidants and 36 plants have already been reported to possess wound healing and antioxidant activity (Suntar *et al*., 2012). Antioxidant property is one of the important characteristics of bioactive components of plants (Shetty *et al*., 2007; Suntar *et al*., 2012). Antioxidants are needed to prevent the formation and quench the action of reactive oxygen species.

As mentioned earlier, besides antibacterial and antifungal properties, antioxidant potential is another important property of squalene (Lozano-Grande *et al*.,2018). According to those authors, in human body squalene is secreted by sebaceous glands for skin protection and forms 10-15% of lipid on skin surface and on internal organs such as liver. This behavior of squalene is found to be correlated with the practice of topical application of leaf juice of *Strobilanthes alternata* to fresh wounds for immediate healing (Subramoniam *et al*., 2001) and their view is consistent with the finding of Subramoniam *et al*., (2001). Antioxidant property of squalene is also related to the protection of wounds by

scavenging the ROS produced due to wound- induced stress whereas antimicrobial activity of those components of the leaf juice provides an aseptic condition to enhance wound-healing indicating an antiseptic property of squalene present in *Strobilanthes alternata* resulting in immediate healing of wounds.

The antioxidant properties of squalene and use as a topical skin lubricant and protectant can also be correlated with wound healing property of *Strobilanthes alternata* and it in consonance with the view of Micera *et al*., (2020) according to whom squalene possess antioxidant activity and is abundant in skin of animals which is the target tissue exposed to wounds or other incisions.

The vital role of antioxidants in the process of wound healing is found to be due to the presence of phytol, squalene, 9-octadecenoic acid, 3,7,11,15 tetramethyl 2 hexadacen 1 ol, methyl palmitate and other fatty acid methyl esters of *Strobilanthes alternata*. This observation is in accordance with the view of Suntar *et al*., (2007) according to whom wound healing and antioxidant activity coexist in many plant species of a variety of families. As mentioned earlier, wound healing capacity of *Strobilanthes alternata* is already known and presumed to involves antimicrobial, anti-inflammatory and antioxidant activities of leaf juice (Subramaniam *et al*., 2001). The phytochemical functions of each bioactive component of leaf extract of *Strobilanthes alternata* play a vital role in the process of wound healing which is found to be a concerted activity of molecules present in the leaf extract such as fatty acid methyl esters, squalene, phytol, Neophytadine,9-octadecenoic acid and isophytol superimposed with other established functions of the leaf extract containing 3,7,11,15-Tetramethyl 2 hexadacen-1ol, methyl palmitate, methyl octadeca 9,12 dienoate, methyl laurate, methyl myristate, hexahydrofarnesyl acetone, 2,5 dimethyl-4 hexen 3

ol, linolenic acid, phytol, methyl stearate, 1,2 benzene dicarboxylic acid and squalene . Nevertheless, the supplementary role of hexahydrofarnesyl acetone, methyl stearate, linolenic acid and 9-octadecenoic acid compounds in the process of wound healing and their complementary roles cannot be ruled out.

Aluminium treatment resulted in remarkable deviations in the distribution of secondary metabolites of *Strobilanthes alternata* (Table-18). Even though *denovo* occurrence of gamma sitosterol and lupeol was observed due to aluminium treatment in *Strobilanthes alternata*, their therapeutic uses are not studied in detail so far. According to Olofssom *et al*., (2014), lupeol is an effective inhibitor in laboratory models of prostate and skin cancers and an anti-inflammatory agent, lupeol functions primarily on the interleukin system. Saleem (2009) wrote an exhaustive review and the author explains the role of lupeol in alleviating inflammation and cancer. According to Fernandez *et al*., (2001) lupeol rich extract of *Pimenta racemosa* plant exhibits significantly high anti-inflammatory activity in animal models. Lupeol was reported to exhibit significantly high wound healing in dead space wound healing mouse model (Harish *et al*., 2008). Medicinal property of gamma sitosterol is not yet investigated in the current pharmaceutical practice in general and wound healing process in particular. Hence, the treatment with aluminium resulting in the synthesis of gamma sitosterol is not contributing any positive impact in *Strobilanthes alternata*.

The components methyl laurate, methyl myristate, hexahydrofarnesyl acetone, 9-octadecenoic acid, isophytol, 2,5-dimethyl 4 hexen 3ol and 1,2-benzenedicarboxylic acid are absent in *Strobilanthes alternata* due to aluminium treatment. Among the components present, only hexahydrofarnesyl acetone and isophytol are involved antibacterial and antifungal properties (Venkataraman *et al*.,2012) as described earlier. Absence of the above bioactive

components, particularly hexahydroxyfarnesyl acetone and isophytol indicates the adverse or negative effect of aluminium on *Strobilanthes alternata* since the important antimicrobial property which is essential for wound healing is lost due to aluminium treatment.

Treatment with chromium and mercury on *Strobilanthes alternata* resulted in the removal of methyl laurate, methyl myristate, hexahydrofarnesyl acetone, 9-octadecenoic acid, isophytol,2,5-dimethyl 4 hexen 3ol and 1,2 benzene dicarboxylic acid of which hexahydrofarnesyl acetone, isophytol, 9-octadecenoic acid are directly involved in wound healing process according to Suntar *et al*., (2007) and Venkataraman *et al.*, (2012). Absence of above-mentioned secondary metabolites due to chromium as well as mercury indicates the loss of wound healing property of *Strobilanthes alternata* because almost all of these components are directly involved in the process of wound healing as described earlier and as opined by Suntar *et al*., (2007) and Venkataraman *et al.*, (2012).

Strobilanthes alternata treated with zinc resulted in the *denovo* synthesis of abundant quantity of lupeol and methyl linolelaidate whereas of eight secondary metabolilites of which fatty acid methyl esters, 9-octadecenoic acid, hexahydrofarnesyl aceton, and isophytol were absent. Most of these lost biomolecules play vital/major roles in wound healing due to their anti-inflammatory, antioxidant and antimicrobial activities as discussed earlier (Venkataraman *et al.*,2012; Santos *et al*., 2012; Pinto *et al*., 2017; Mishra *et al*.,2020). Hence it is evident that zinc treatment causes deterioration of the medicinal potential *i.e*., wound healing property of *Strobilanthes alternata*. Even though the newly synthesized component, methyl linolelaidate plays no direct role in wound healing process, the other new secondary component lupeol play a direct involvement in wound healing as described earlier (Olofssom *et*

al., 2014; Fernandez *et al*., 2002; Harish *et al*., 2008). An interesting observation is the presence of significant quantity of lupeol in *Strobilanthes alternata* subjected to aluminium and zinc while it is absent in the control. Given the role of lupeol has been reported as a component involved in the mechanisms of wound healing it is absent in the control plants which is entitled to possess wound healing potential (Subramoniam *et al.*, 2001). Nevertheless, but in spite of the occurrence of considerable quantity of lupeol many important bioactive components get lost due to aluminium and zinc revealing the partial loss of wound healing capacity.

In conclusion, *Strobilanthes alternata* subjected to aluminium, chromium, mercury and zinc exhibits significant structural, metabolic changes and tolerance mechanisms which vary from metal to metal and toxicity responses also is different among metals. Notwithstanding, the celebrated potential of *Strobilanthes alternata i.e.,* wound healing is found to be deteriorated due to all the four metals. These findings indicate that *Strobilanthes alternata* plants are intolerant to these metals particularly the synthesis of secondary metabolites is concerned and hence the plants grown in soil or water fields polluted with heavy metals should not be used for medicinal purposes. Since *Strobilanthes alternata* is a wild plant naturally growing under shady and marshy areas, the plants seldom get exposed to heavy metal pollution and the medicinal property *i.e.,* wound healing potential remains intact under natural habitat.

SUMMARY AND CONCLUSION

Strobilanthes alternata (Burm.f.) E. Moylan ex J.R.I. wood (syn: *Hemigraphis colorata*) belongs to the family Acanthaceae. In kerala, the plant is popular in the name "Murikootti" and "Murianpacha", due to its incredible potency to heal fresh wounds (Subramoniam *et al*., 2001).

In the present investigation, an attempt is made to analyze the effect of selected heavy metals – Aluminium, Chromium, Mercury and Zinc on the medicinal plant *Strobilanthes alternata*. 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 zinc cultivated under nutrient culture method in 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. Effect of heavy metals on growth, development and metabolism was done following different parameters such as growth measurements of roots, stem and leaves. Tolerance index also was calculated and taken as a parameter to assess tolerance potential. Impact of heavy metals was analyzed in terms of stomatal index also. In addition, qualitative and quantitative analyses of protein, phenolics, proline, chlorophyll and carotenoid pigments were done. Since heavy metal toxicity induces production of ROS, the role of scavenging enzymes like catalase, superoxide dismutase activity as well as MDA production etc. also were analyzed in various organs like root, stem and leaves. As a confirmatory step to elaborate the anatomical changes in the various parts of the plant Scanning Electron Microscopic study (SEM) also was done. To pinpoint the involvement and distribution pattern of heavy metal ions added to the nutrient medium, SEM-EDX (SEM attached to Energy Dispersive X-ray analysis) technique also

was done. Bioaccumulation potential of *Strobilanthes alternata* towards the heavy metals also was analyzed using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES). Since *Strobilanthes alternata* is a medicinal plant, the occurrence and distribution of bioactive secondary metabolites was analyzed using GC-MS (Gas Chromatography-Mass Spectrometry).

Standard most referred protocols were employed for the all the methodology adopted for the investigation.

Morphological changes due to the treatment with all metals was slight growth retardation in root length compared to the control and between the metals root length variation was insignificant. Aluminium alone showed enhanced growth of stem almost similar to control and all other treatments resulted in inhibited root length. Leaf area also exhibited a slight increase in plants treated with aluminium whereas all other metals induced reduction of leaf area and maximum reduction was shown by zinc treated plants. Effect of aluminium and mercury on stomatal index was shown only negligible differences compared to control while plants treated with chromium and mercury resulted in significantly increased stomatal index during 12^{th} day onwards in the lower epidermis of leaves. Tolerance index percentage of all the treatments registered significant reduction compared to the control and between the metals the difference in tolerance index was negligible.

Anatomical changes such as undulated rhizodermal layer resulting reduced root length, size and shape are shown by plants treated with chromium and mercury while aluminium and zinc did not register much structural changes of roots. This may be due to negligible stimulatory effect of aluminium and/or zinc as essential metal. Stunted nature and brittle texture of roots are caused due to mercury treatment. In comparison with control stem, localization of stained masses all distributed in all treatments particularly in roots of chromium and

mercury treated plants. Cell wall thickening is seen as densely stained. In addition shrinkage, loss of rhizodermal hairs also is another characteristics of mercury and chromium. Damage of cells are seen in cortical and pith cells.

Significant anatomical changes of stem occurred in plants treated with mercury and chromium. But aluminium and zinc treatment showed only negligible changes in the stem. Stimulatory effect of aluminium and zinc showed more elaborated structure of the stem.

SEM-EDX studies revealed more clear and distinct structure of root, stem and leaf. Damaged rhizodermal and cortical cells are seen in all treatments. Most of the cells appear as empty but many one of them show cell cavity filled with masses in the stem and leaves of all treatments. In the roots many cells contain deposits of different shape and size. Chromium and mercury treatment showed most of the cells of stem and leaf appear as filled with uniformly distributed structure which are not distinct. Effect of zinc on the anatomy of root, stem and leaf was very feeble and hence cellular structure is not very clear. Some cells appear empty while others appeared fully filled.

SEM-EDX studies showed an overall picture of the distribution of mineral ions as weight percentage. The SEM image represent almost the entire section and hence specific localized structures are lacking. However, the weight percentage values shows difference in the distribution of elements aluminium, chromium, mercury and zinc given as treatments exhibited their values corresponding to the quantity treatment. In the control those elements are absent. Occurrence of all the elements as more or less similar pertaining to their concentrations. In addition to the heavy metal significant values of calcium was observed in all treatments and control.

SEM study on stomata of lower epidermis of control plants appeared almost closed and plants treated aluminium resulted in wide opening of stomata. Stomata of chromium treated plants also remained open. Stomatal number and opening were more in plants treated with mercury. Zinc treatment resulted in even distribution of stomata and stomatal apertures were fully opened.

The observation data are interpreted, compared and discussed with current appropriate literature and the following conclusions are drawn.

- Different concentrations of aluminium, chromium, mercury and zinc imparted more or less similar growth retardation and morphology of *Stroboilanthes alternata* inspite of significant variation in the concentration given to impart visible toxicity symptoms and hence tolerance potential of the plant varies from metal to metal.
- In spite of the difference in concentrations of aluminium, chromium, mercury and zinc, morphological performances are almost uniform and sustainable, but fluctuations are observed in many parameters among individual metals.
- Even though morphological changes imposed by heavy metals are negligible, anatomical observations such as impaired growth, removal of root hairs, disruption of roots are observed in the treatments of mercury and chromium indicating more toxicity of these elements than aluminium and zinc.
- Occurrence of dark stained bodies which are seen scattered all over the sections. Roots of plants treated with mercury reveals localized stained masses. In chromium treatment an overall yellow colour throughout the root section indicating the presence of chromium.
- Anatomical variations of stem and leaves also are different among metals.

Development of many epidermal hairs in midrib of leaves of *Strobilanthes alternata* subjected to mercury treatment is found to be correlated with removal of mercury by phytovolatilization through the multicellular epidermal hairs which functions as trichomes for the exit of volatile form of mercury to mitigate the toxicity.

- Increased stomatal index observed in *Strobilanthes alternata* treated with mercury is considered as another mechanism to tolerate metal toxicity.
- Distribution of stomata and size of stomatal opening are more in plants treated with mercury and zinc and this characteristics are directly related to the exit of volatile forms of both the metals as a measure of toxicity sequestration.
- Although the anatomical details of root, stem and leaves in SEM images are not clear or obscure presumably due to metal toxicity damage, in comparison with the light microscopic images, SEM-EDX analysis reveals the distribution of metal and mineral ions as weight percentage in specific selected areas of the sections.
- Exorbitant increase in the weight percentage of aluminium, chromium, mercury and zinc in their respective treatments, compared to the distribution of other elements, affirms the precision of SEM-EDX study.
- Increased values of weight percentage of calcium and silicon in the control plants confirms the abundant occurrence of these elements as components of cystoliths earlier reported in *Strobilanthes alternata* and these changes are not related to heavy metal toxicity compared to other elements.
- Dry weight of plants remain almost unaltered in the selected concentrations of metals highlighting almost similar responses of *Strobilanthes alternata* towards different concentrations of aluminium,

chromium, mercury and zinc revealing similar growth and biomass production.

- Physiological and biochemical changes as a result of heavy metal toxicity analyzed by estimating total protein, proline, phenolics and chlorophyll pigment distribution registered only negligible variations among treatments in general, but significant alterations are shown in the function of protein and proline.
- Increased protein reveals the possibility of phytochelatin synthesis which is considered as a major effect of heavy metals in general and chromium and mercury in particular, since phytochelatins are involved in heavy metal sequestration.
- Continuous increase of proline in the roots of plants treated with chromium and mercury indicates the compatible role of proline and osmoregulation in mitigating the toxicity of heavy metal ions in the roots. Functions of proline in quenching the ROS also occurs in *Strobilanthes alternata* since the activity of antioxidant enzymes register only very feeble activity.
- Increased production of total phenolics in cell tissues of *Strobilanthes alternata* in all treatments is directly related to induction of tolerance to metal toxicity particularly in zinc treatment where the metals play role as a mitigator superimposed with defensive mechanism of phenolics against toxicity.
- Antioxidant potential of phenoloics also is worth mentioning because occurrence of phenolics and antioxidant enzymes activity are not directly proportional to each other.
- Stress impact of the four heavy metals in the photosynthetic efficiency is linked with visual symptoms and distribution of chlorophyll pigments is seen such a way that aluminium, chromium and mercury induces reduced

chlorophyll contents since these metal ions inhibit enzyme activities involved in chlorophyll synthesis, whereas aluminium treatment results in negligible changes of chlorophyll pigment.

- Carotenoid contents of *Strobilanthes alternata* leaves show a general increase in all treatments and particularly in mercury treatments. The role of carotenoids as ROS scavenging molecules is found to be correlated with comparatively reduced activity of SOD and catalase thereby protecting the plant from the toxicity of metals.
- Comparatively very low increase in the SOD activity and hence the defense mechanism against ROS production induced by metal toxicity is very feeble in *Strobilanthes alternata* due to aluminium while slightly increase activity of SOD in all other metals indicates the increased activity of SOD in other metals in which other defense mechanism like catalase activity is very low.
- Accumulation of MDA content, as a product of lipid peroxidation due to highly reactive ROS induced toxicity of all the metals is an important strategy of *Strobilanthes alternata* and the difference in magnitude of this process confirms the specificity of each metal in such a way that chromium and zinc induced more MDA production particularly in leaves than other parts of the plant.
- Bioaccumulation profile of all the four metals differed and found to be dependent on process of absorption, translocation and accumulation in *Strobilanthes alternata.* Maximum accumulation of aluminium and minimum accumulation of mercury can be interpreted in terms of concentration of metals since in the treatments mode of loss through different passages of the plant body. Despite the increased content of mercury in the root system and mercury content is below detectable level in the leaves. Loss occurs by phytovolatilization through modified hairs

on the midrib of leaves as well as through open stomata. Translocation potential of aluminium is very high and this observation can also be correlated with the maximum concentration given in the treatment.

- The tolerance and/or sensitivity of *Strobilanthes alternata* towards aluminium, chromium, mercury and zinc can be compared between the concentrations of metals given to impart almost similar visual observations and different parameters used to assess the stress impact. Given the significant variation in the molar concentrations of each metals, defensive mechanisms/strategies in terms of various metabolites distribution, antioxidant potential of specific primary and secondary metabolites, antioxidant enzymes and bioaccumulation potential comply with the tolerance/ sensitivity of *Strobilanthes alternata* cultivated under simulated experimental setup.
- Fifteen bioactive secondary metabolites present in varying concentrations in methanolic extract of leaves are reported to possess antibacterial, antifungal and anti-inflammatory activities and many molecules are characterized by antioxidant potential also. The process of wound healing in which *S.alternata* known to play vital role is found to be due to the combined activity of each and every secondary metabolites and the involvement of these molecules in the wound healing process is related to the celebrated character of the plant wound healing process is found to be due to the concerted activities of 15 bioactive molecules and many of them possess antimicrobial, anti-inflammatory particularly, antioxidant potential since wound healing and antioxidant activity of many of the bioactive molecules coexist in *Strobilanthes alternata*.
- Effect of heavy metals resulting in the absence of many secondary metabolites which is vital for wound healing found to deteriorate the potential of *Strobilanthes alternata* in wound healing whereas occurrence

of some new bioactive components especially the presence of lupeol in plants treated with aluminium and zinc treated plants. Notwithstanding none of these *denovo* synthesized secondary metabolites of *Strobilanthes alternata* possess any potential role for wound healing process.

REFERENCES

Abdussalam, A.K., RatheeshChandra,P., Hussain, K. and Nabeesa-Salim(2013). Response and bioaccumulation potential of *Boerhavia diffusa* towards different heavy metals. *J.Stress Physiol.Biochem*.**9**:23-36.

Abid, R., Mahmood, S., Ghaffar, S., Zahra, S. and Noreen, S.(2019). Tannery effluent induced morphobiochemical expressions and chromium accumulations in *Jatropha curcas* L. and *Pongamia pinnata* L. *Sains Malays*. **48**:927-936.

Abid,S. and Touqueer, S.(2015). Antimicrobial and antioxidant activity of *Breynia disticha* and *Vernonia elaeagnifolia*. *J.App.Pharm*.**7**:178-182.

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. *Ecotoxicol. Environ. Saf*. **122:**1-8.

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. *J.Inorg.Biochem*. **50**:235-243.

Aioi A, Shimizu T, and Kuriyama K. (1995) Effect of squalene on superoxide anion generation induced by a skin irritant, lauroylsarcosine. *Int. J. Pharm*. **113**:159-164.

Aiyegoro,O.A. and Okoh,A.I. (2010). Preliminary phytochemical screening and invitro antioxidant activities of the aqueous extract of *Helichrysum longifolium* DC.*BMC Complement.Altern.Med*.**10**:1-8.

Akhil, P. and Prabhu, P. (2013). Evaluation of antioxidant, anti-inflammatory and cytotoxicity potential of *Hemigraphis colorata*. *Int.J.Pharm.Sci.Res*.**4**:3477-3483.

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.). *Environ. Sci. Pollut. Res*. **22**:10669-10678.

Ali, G. and Ghasemzadeh. (2011). Flavanoids and phenolic acids: Role and biochemical activity in plants and human. *J.Med.Plants Res*.**5**:6697-6703.

Alia, K.V., Prasad, S.K. and Saradhi, P.P. (1995). Effect of zinc on free radicals and proline in *Brassica* and *Cajanus. Phytochem*. **39**:45-47.

Alloway, B.J. (2004). *Zinc in Soil and Crop Nutrition*. Brussels, Belgium:International zinc association.

Alloway,B.J. (2008). Micronutrients and crop production: An Introduction. In: *Micronutrient Deficiencies in Global Crop Production*. 1-39.

Al-Owaisi,M., Al-Hadiwi,N. and Khan,S.A.(2014).GCMS analysis,determination of total phenolics, flavonoid content and free radical scavenging activities of various crude extracts of *Moringa peregeina* (Forssk.) Flori leaves. *Asian Pac.J. Trop. Biomed*.**4**:964-970.

Ambler, J.E., Brown, J.C. and Gauch, H.G. (1971). Sites of Iron reduction in Soyabean plants. *Agron. J*. **63**:95-97.

Amit,K.G., Johal,E.S. and Rath,G. (2011). Nanotechnology for water treatment. *Curr.Nanoscience*.**7**:640-654.

Anitha,V.T.,Antonisamy,J.M. and Jeeva,S.(2012). Antibacterial studies on *Hemigraphis colorata* (Blume) H.G.Hallier and *Elephantopus scaber* L. *Asian Pac. J. Trop. Med*. **5**:52-57.

Arnon, D.I. (1949). Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris. Plant Physiol*. **24**:1-5.

Aubert, S., Bligny, r. and Douce, R. (2001). Contribution of glutamate dehydrogenase to mitochondrial metabolism studied by 13C and 31P nuclear magnetic resonace. *J. Exper. Bot.*. **52**:37-45.

Avoseh, O.N., Lebbeck,A., and Zygia, A.(2021). *Albizia lebbeck* and *Albiziza zygia* volatile oils exhibit anti-nociceptive and anti-Inflammatory properties in pain models. *J.Ethanopharmacol*. **268**:1-8.

Awasthi, M. and Rai, L.C. (2005). Toxicity of nickel, zinc and cadmium to nitrate uptake in free and immobilized cells of *Scenedesmus quadricauda*. *Ecotoxicol. Environme. safety*. **61**:268-272.

Azzi,V.S., Kanso, A., Kobeissi,A., Kazpard, V., Lartiges,B. and Samrani, A.El. (2015). Effect of cadmium on *Lactuca sativa* grown in hydroponic culture enriched with phosphate fertilizer. *J.Environ.Prot*. **6**:1337.

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. *Toxicolo. Environ. Chemi*. **103**:162-183.

Badea, D.N. (2015). Determination of potentially toxic heavy metals (Pb, Hg, Cd) in popular medicinal herbs in the coal power plant area. *Rev. Chim*. **66**:1132-1136.

Baker, R., Lavie, B. and Novo, E. (1985). Natural selection for resistance to the pollution. *Experientia*. **41**:697-699.

Baker,A.J.M. and Walker,P.L. (1989). Physiological responses of plants to heavy metals and the quantification of tolerance and toxicity. *Chem.Speciat. Bioavailab*.**1**:7-17.

Baker,A.J.M. and Walker,P.L.(1990). Ecophysiology of metal uptake by tolerant plants. In:A.J.Shaw(Ed.). *Heavy Metal Tolerance in Plants*. *Evolutionary Aspects.* CRC Press,Florida.156-177.

Bakiyaraj, R., Baskaran, L., Chidambaram, A., Mahakavi, T. and Santhoshkumar, M. (2014). Bioremediation of chromium by *Bacillus subtilis* and *Pseudomonas aeruginosa. Int. J. Curr. Microbiol. Appl. Sci*. **3**: 715-719.

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 Physiol. Biochem*. **118**:653-666.

Balogun,O.S., Ajayi,O.S. and Adeleke,A.J.(2017). Hexahydrofarnesyl acetone rich extractives from *Hildegardia barteri*. *J. Herbs Spices Med. Plants.* **23:**393-400.

Balsberg-Pahlsson,A.M.(1990).Influence of aluminium on biomass, nutrients, soluble carbohydrates and phenols in beech (*Fagus sylvatica). Physiol.Plant.***78**:79-84.

Barcelo,J., Poschenrieder,C.,Ruano,A and Gunse,B.(1985). Leaf water potential in Cr (VI) treated bean plants (*Phaseolus vulgaris* L.). *Plant Physiol.Suppl*.**77**:163-164.

Basak, M., Sharma, M. and Chakraborty, U. (2001). Biochemical responses of *Camellia sinensis*(L.) O kutenze to metal stress. *J. Environ. Biol*. **22:**29-36.

Bates,L.S.,Waldren,R.P. and Teare,I.D.(1973). Rapid determination of free proline for water stress studies. *Plant Soil*. **39**:205-208.

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 morpho-anatomy of root and leaf. *Revista Brasileira de Ciencia do Solo*. **37**:177-187.

Beauford, W., Barber, J. and Barringer, A.R. (1977). Uptake and distribution of mercury within higher plants. *Physiol. Plant*. **39:**261-265.

Bennet,R.J.,Breen,C.M. and Fey,N.V.(1987). The effects of aluminium on root cap functions and root development in *Zea mays* L. *Environ.Exp.Bot*.**27**:91-104.

Bere,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).*Environ.Ecol*.**17**:958-961.

Bernier, M., Popovic, R. and Carpentier, R. (1993). Mercury inhibition at the donor side of photosystem II is reversed by chloride. *FEBS let*. **321**: 19-23.

Bhargavi,C.H.S., Kumar, N.V.S.P.P. and Babu, V.R. (2011). Ancient and modern view of wound healing: Therapeutic Treatments.*Res.J.Pharm.Biol.Chem.Sci.* **2**:474-479.

Bishnoi,N.R., Sheoran, I.S., and Singh, R. (1993). Effects of chromium on photosynthesis, respiration and nitrogen fixation in pea (*Pisum sativum* L.) seedlings. *J. Plant Physiol*. **142**:25-30.

Bizily,S., Rugh,C., Summers,A.O. and Meagher,R.B. (1997). Phytoremediation of methyl mercury pollution:Mer B expression in *Arabidopsis thaliana* confers resistance to organo mercurial. *Proc.Nate.Acad.Sci.USA*. **96**:6808-6813.

Boening.(2000). Ecological effects, transport and fate of mercury : a general review. *Chemosphere*. **40**: 1335-1351.

Borovik, A.J. (1990). Characteristics of metals in biological systems. In:A.J.Shaw (Ed.). *Heavy Metal Tolerance in Plants*: *Evolutionary aspects*. CRC Press. Florida, 3-5.

Bowler,C.H.,Montagu,M.V. and Inze,D.(1992). Superoxide dismutase and stress tolerance. *Annu.Rev.Plant Physiol.Plant Mol.Biol*. **43**:83-116.

Bradley, D.E. and Min, D.B. (1992). Singlet oxygen oxidation of foods. *Cat. Rev. Food Sci. Nutr*. **31**:211-236.

Breckle,S.W., Waisel, Y., Eshel, A., and Kafkafi,X.(1991). Growth Under Stress:Heavy Metals, Plant Roots. Marcel Dekker:New York. 351-373.

Broadley, M. R., White, P.J., Hammond, J.P., Zelko, I., Lux, A. (2007). Zinc in plants. *New Phytol*. **173**:677-702.

Brzyska, M., Ginalska, G. and Lobarzewski, J. (1991). Combined influence of lead, mercury and cadmium ions in the presence of magnesium on endopolygalacturonase activity. *Biotechnol. Appl. Biochem*. **13**:83-89.

Buchanan, B.B., Gruissem, W. and Jones, R.L. (2000). *Biochemistry and Molecular Biology of Plants*. American society of Plant Physiologists. Rockville, Maryland.

Budikova,S.(1999). Structural changes and aluminium distribution in maize root tissues. *Biol.Plant*.**42**:259-266.

Cakmak,I.(2002). Plant nutrition research:Priorities to meet human needs for food in sustainable ways. *Plant and soil*. **247:**3-24.

Cakmak, I., Welch, R.M., Hart, J., Norvell, W.A., Ozturk, L. and Kochian, l.V. (2000). Uptake and retranslocation of leaf-applied cadmium in diploid, tetraploid and hexaploid wheat. *Exp. Bot*. **51**:221-226.

Cakmak,I. and Horst,W.J.(1991). Effect of aluminium on net efflux of nitrate and potassium from root tips of soyabean (*Glycine max* L.). *J.Plant Physiol*.**130**:400-403.

Caldas, E.D. and Machado, L.L. (2004). Cadmium, mercury and lead in medicinal herbs in Brazil. *Food Chem. Technol*. **42**:599-603.

Caldelas, C., Bort, J. and Febrero, A. (2012). Ultrastructure and subcellular distribution of Cr in *Iris pseudacorus* L. using TEM and X-ray microanalysis. *Cell Biol. Toxicol.* **28:**57-68.

Cargnelutti,D., Tabaldi,L.A., Spanevello,R.M., Jucoski,G.O., Battisti,V., Redin,M., Linares,C.E.B., Dressler,V.L., Flores,E.M.M., Nicoloso,F.T., Morsch,V.M. and Schetinger,M.R.C.(2006).Mercury toxicity induces oxidative stress in growing cucumber seedlings. *Chemosphere*.**65**:999-1006.

Caroli,M., Furini, A., DalCorso,G., Rojas,M. and Sansebastiano,G.P. (2020). Endomembrane reorganization induced by heavy metals. *Plants*.**9**:482.

Cervantes, C., Campos-Garcia, J., Devars, S., Gutlerrez-corona, F., Loza-Tavera, H., Torres-Guzman, J.C. and Moreno- Sanchez, R. (2001). Interactions of chromium with microorganisms and plants. FEMS. *Microbiol. Rev*. **25**:335-347.

Chandra, R., Bharagava, R.N., Yadav, S. and Mohan, D. (2009). Accumulation and distribution of toxic heavy metals in wheat (*Triticum aestivum* L.) and Indian mustard (*Brassica campestris* L.) irrigated with distillery and tannery effluents. *J. Hazard. Mater*. **162**:1514-1521.

Chandra,P. and Garg,P. (1992). Absorption and toxicity of chromium and cadmium in *Limnanthemum cristatum*. Griseb. *Sci. Total Environ*. **125**:175-183.

Chaney,R.L.(1993).Zinc phytotoxicity. In A.D.Robson(Ed.).*Zinc in Soils and Plants. Kluwer Academic publishers*.135-150.

Chang,H.Y.,Sun,B.Y. and Liu,C.S.(2000). Advances in the study of plants copper toxicity. *J.Shandong Agr.Univ.(Nat.Sci.)***31**:227-230.

Chatterjee,J. and Chatterjee,C.(2000). Phytotoxicity of cobalt, chromium and copper in cauliflower. *Environ.Pollut*.**109**:169-174.

Cho, U.H. and Park, J.O. (2000). Mercury-induced oxidative stress in tomato seedlings. *Plant Sci.* **156**:1-9.

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.

Choudhury,S. and Panda,S.K. (2005). Toxic effect, oxidative stress and ultra structural changes in moss *Taxitheelium nepalense* (Schwaegr.) Broth.under lead and chromium toxicity. *Water Air Soil Pollut*.**167**:73-90.

Chung,K.T.,Wong,T.Y.,Wei,C.,Huang,Y. and Lin,Y.(1998).Tannins and human health:a review. *Crit. Rev. Food Sci. and Nutr*.**38**:421-464.

Ciamporova,M.(2002).Morphological and structural responses of plant roots to aluminium at organ,tissue and cellular levels. *Biol.Plant*.**45**:161-171.

Clemens, S. (2001). Molecular mechanisms of plant metal tolerance and homeostasis. *Planta.* **212**:475-486.

Clemens, S. and Ma, J.F. (2016). Toxic heavy metal and metalloid accumulation in crop plants and foods. *Annu. Rev. Plant Biol*. **67**:489-512.

Clemens, S., Palmagren, M.G. and Kraemer, U. (2002). A long way ahead: understanding the engineering plant metal accumulation trends. *Plant Sci*. **7**:309-315.

Clijsters, H. and Van Assche, F. (1985). Inhibition of photosynthesis by heavy metals. *Photosynth. Res*. **7**: 31-40.

Cobbett,C.S. (2000). Phytochelatins and their roles in heavy metal detoxification. *Plant physiol*.**123**:825-832.

Cobbett,C.S. and Goldsbrough,P.B.(2002). Phytochelatins and metallothioneins: Roles in heavy metal detoxification and homeostasis. *Annu.Rev.Plant Biol.* **53**: 159-182.

Corradi,M.G.,Bianchi,A.K. and Albasini,A.(1993). Chromium toxicity in *Salvia sclarea*:1.Effect of hexavalent chromium on seed germination and seedling development. *Environ.Expt.Bot*.**33**:405-413.

Costa, G. and Morel, J. L. (1994). Water relations, gas exchange and amino acid content in Cd- treated lattuce. *Plant Physiol. Biochem*. **32**:561-570.

Cseh,E.(2002). Metal permeability, transport and efflux in plants.In: M.N.V.Prasad and K.Strzalka (Eds.). *Physiology and Biochemistry of Metal Toxicity and Tolerance in plants*. *Kluwer Academic.Dordrecht*.1-36.

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. Res. Int*. 2014.

Davies,F.T., Puryear,J.D., Newton,R.J., Egilla,J.N. and Grossi, J.A.S. (2002). Mycorrhizal fungi increase chromium uptake by sunflower plants: influence on tissue mineral concentration, growth and gas exchange. *J.Plant Nutr*.**25**:2389-2407.

De Grado,W.F., Summa,C.M., Pavone,V.,Nastri,F. and Lombardi,A. (1990). *De novo* design and structural characterization of proteins and metalloproteins. *Annu.Rev.Biochem*.**68**:779-819.

De Vos, C.H. R., Vonk, M.J. and Schat, H. (1992). Glutathionine depletion due to

copper induced phytochelatin synthesis causes oxidative stress in *Silena cucubalus*. *Plant Physiol.* **98**:853-858.

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 Pollut*. **24**:351-360.

Delhaize,E. and Ryan,P.R.(1995). Aluminium toxicity and tolerance in plants. *Plant physiol*.**107**:315-321.

de-Olivera,L.M., Ma, L.Q., Santos, J.A., Guilherme, L.R. and Lessl, J.T. (2014). Effects of arsenate, chromate and sulfate on arsenic and chromium uptake and translocation by arsenic hyperaccumulator *Pteris vittata* L. *Environ. Pollut*. **184**:187-192.

de-Olivera, L.M., Gress, J., De, J., Rathinasabapath, B., Marchi, G., Chen, Y. and Ma, L.Q. (2016). Sulfate and chromate increased each other's uptake and translocation in As-hyperaccumulator *Pteris vittata*. *Chemosphere*. **147**: 36-43.

Dey,U., and Mondal,N.K.(2016). Ultrastructural deformation of plant cell under heavy metal stress in gram seedlings. *Cogent Environ.Sci*.**2**:1-12..

Dietz, K.J., Baier, m. and Kramer, M. (1999). Free radicals and reactive oxygen species as mediator of heavy metal toxicity in plants. In:M.N.V. Prasad and J. Hagemeyer(Eds.). *Heavy Metal Stress in Plants:from Molecules to Ecosystem*. *Springer Berlin*. 73-79.

Diwan, H., Khan, I., Ahmed, A. and Iqbal, M. (2010). Induction of phytochelatins and antioxidant defence system in *Brassica juncea* and *Vigna radiate* in response to chromium treatments. *Plant growth Regul*. **61**:97-107.

Dixit,V.,Pandey,V. and Shyam,R.(2002). Chromium ions inactivate electron transport

and enhance superoxide generation *invivo* in pea (*Pisum sativum* L.cv.Azad) root mitochondria. *Plant Cell Environ.***25**:687-690.

Dominguez-Solis, J.R., Lopez-Martin, M., Ager, F.J., Ynsa, M.D., Romero, L.C. and Gotor, C. (2004). Increased cysteine availability is essential for cadmium tolerance and accumulation in *Arabidopsis thaliana. Plant Biotechnol. J.* **2**:469-476.

Dubey,N.K.,Kumar,R. and Tripathi,P.(2004). Global promotion of herbal medicine:India's opportunity.*Curr. Sci*.**86**:37-41.

Ebinghaus, R., Tripathi, R.M. and Wallschlager, L.S.E. (1999). Natural and anthropogenic mercury sources and their impact on the air surface exchange of mercury on regional and global scales. In:R.Ebinghaus, R.R., Turner, L.D., Lacerd, O., Vasilev and W. Salomons: (Eds.). *Mercury Contaminated Sites*: *Characterization, Risk Assessment and Remediation*. New York, *Springer-Verlag*, Berlin Heidelberg. 76-89.

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*. *Int. J. Mol. Sci*. **16**:15852-15871.

Epstein,E.(1972). *Mineral Nutrition of Plants, Principles and Perspectives*. *John Wiley & Sons*,New York.

Fagria,N.K.(1982). Differential tolerance of rice cultivars to aluminium in nutrient solution.*Pesq.Agropec.Bras*.**17**:1-9.

Fahn, A. (1982). *Plant Anatomy* (3 rd Ed.). Oxford: Pergamon Press.

Falchuk, K.H., Goldwater,L.J., and Vallee,B.L.(1977). The biochemistry and toxicity of mercury. In: C.A.Mc Auliffe (Ed.). *The Chemistry of Mercury*. *Mac Millan*, London,259-283.

Fergusson,J.E.(1990). *The Heavy Elements: Chemistry, Environmental Impact and Health effects*. *Pergamon Press*, Oxford.

Fernandez,A., Alvarez,A., Garcia,M.D. and Saenz,M.T. (2001). Anti-inflammatory effect of *Pimenta racemosa* var. ozua and isolation of the triterpene lupeol. *Farmaco*. **56**:335-338.

Fitter, A.H. and Hay, R.K.M. (1983). *Environmrntal Physiology and Plants*. *Academic press*, London.

Fitz,W.J. and Wenzel,W.W. (2002). Arsenic transformations in the soil-rhizosphere-plant system: fundamentals and potential application to phytoremedition. *J.Biotechnol*. **99**:259-278.

Fodor, F. (2002). Physiological responses of vascular plants to heavy metals. In:M.N.V. Prasad and K. Strzalka (Eds.). *Physiology and Biochemistry of Metal Toxicity and Tolerance in Plants. Kluwer Academic Dordrecht*. 149-177.

Folin, O and Denis, W. (1915). A calorimetric method for the determination of phenols (and phenol derivatives) in urine. *J.Biol.Chem*. **22**:305-308.

Foy, C.D., Chaney, R.L. and White, M.C. (1978). The physiology of metal toxicity in plants. *Annu. Rev. Plant Physiol*. **29**:511-566.

Foy,C.D. (1992). Soil chemical factors limiting plant root growth. *Adv.Soil Sci*.**19**:97-131.

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.,Madison,WI* 57-97.

Friedland, A.J. (1990). The movement of metals through soils and ecosystems. In: A.J. Shaw (Ed.). Heavy meta tolerance in plants: Evolutionary aspects. *CRC Press*, Florida. 7-19.

Gabel, N.H., Wise, R.R., and Rogers, G.K. (2021). Distribution of cystoliths in the leaves of Acanthaceae and its effect on leaf surface anatomy. *Blumea***. 65**:224-232.

Gadallah, M.A.A.(1994). Interactive effect of heavy metals and temperature on growth and chlorophyll, saccharides and soluble nitrogen contents in *Phaseolus* plants. *Biol.Plant.* **36**:373-382.

Gao,W., Guo,C., Hu,J., Dong,J. and Zhou,L.H. (2021). Mature trichome is the earliest sequestration site of Cd ions in *Arabidopsis thaliana* leaves. *Heliyon*. **7**:e7501.

Gardea-Torresdey,J., Peralta-Videa, J., Montes, M., De la Rosa, G., Corral and Diaz, B.(2004). Bioaccumulation of cadmium, chromium and copper by *Convolvulus arvensis* L. :impact on plant growth and uptake of nutritional elements. *Bioresour. Technol*. **92**:229-235.

Garg,P., Chandra,P. and Devi,S. (1994). Chromium (VI) induced morphological changes in *Limnanthemum cristatum* Griseb: A possible bioindicator. *Phytomorphology*.**44**:201-206.

Ghasemzadeh, A. and Jaafar, H.Z.E. (2011). Anticancer and antioxidant activities of Malaysian young ginger (*Zingiber officinale* Roscoe) varieties grown under different CO_2 concentration. *J.Med. Plant Res*. **5**:3247-3255.

Giannopolits, C.N. and Ries, S.K. (1977). Superoxide dismutase occurrence in higher plants. *Plant physiol*. **59**:309-14.

Gill, R.A., Zang, L., Ali, B., Farooq, M.A., Cui, P., Yang, S., Ali, S. and Zhou, W.

(2015). Chromium- induced physio-chemical and ultrastructural changes in four cultivars of *Brassica napus* L.. *Chemosphere.* **120**:154-164.

Godbold, D.L (1994). Aluminium and heavy metal stress: From the rhizosphere to the whole plant.In: D.L.Godbold and A.Hutterman (Ed.). *Effects of acid rain on forest processes.Wiley-Liss*, New York. 231-264.

Godbold, D.L. and Huttermann, A. (1986). The uptake and toxicity of mercury and lead to spruce seedlings. *Water Air and Soil Pollut.* **31**:509-515.

Goldbold, D.L. and Kettner, C. (1991). Use of root elongation studies to determine aluminium and lead toxicity in *Picea abies* seedlings. *J. Plant Physiol*. **5**:138-231.

Gomathipriyadharsini,T., Prabhu,K. and Sathiyanarayanamurthy, S. (2019). Preliminary phytochemical analysis of leaf extract of one medicinal plant *Premna tomentosa*. *Int.J.Phar.Biol.Sci.***9**:271-274.

Goswami,U. and Nazarine,F. (2003). Bioactivity of methyl palmitate obtained from mangrove plant *Salvadora persica* L. *US Patent*.**6**: 638,546.

Grace,S.C.(2007). Phenolics as antioxidants. In: Smirnoff N. (Ed.) *Antioxidants and reactive oxygen species in plants.* Blackwell, Hoboken 141:168.

Grill, E., Coffler, S., Winnacker, F.L. and Zenk, M.A. (1989). Phytochelatins, the heavy metal binding peptides of plants are synthesized from glutathione by a specific γ-glutamyl cysteine dipeptidyl transpeptidase (Phytochelatin synthase). *Proc. Natl. Acad. Sci.* USA. 86:6838-6842.

Grill, E., Winnacker, E.L. and Zenk, M.H. (1985). Phytochelatins, the principal heavy metal complexing peptides of higher plants. *Sci*. **230**:674-676.

Gupta, N., Ram, H. and Kumar, B. (2016). Mechanism of zinc absorption in plants :

uptake, transport, translocation and accumulation. *Reviews in environmental science and Bio/Tecghnology.* **15**: 89-109.

Gupta,S.D.(2010). Metal toxicity, oxidative stress and antioxidative defense system in plants. In; *Reactive oxygen species and antioxidants in higher plants*.CRC Press. 193-220.

Hall, G.E.M., Pelchat, J.C., Pelchat, P., Vaive, J.E. (2002). Sample collection, filteration and preservation protocols for the determination of "total dissolved" mercury in waters. *Analyst*. **127**:674-680.

Halliwell,B. and Gutteridge, J.M.C.(1999). *Free Radicals in Biology and Medicine*. Oxford,UK,Clarendon Press.

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 Phytol*.**162**:489-499.

Harborne, J.B. (1980). Plant phenolics. In: E.A. Bell and B.V. Charlwood (Eds.). . *Secondary Plant Products*. *Encyclopedia of Plant Physiology*,Vol.8 (New series) springer-verlag Berlin. 329-395.

Harish,B.G., Krishna, V., Santhosh Kumar,H.S., Khadeer Ahamed,B.M., Sharath,R., Kumara swamy,H.M.(2008). Wound healing activity and docking of glycogen-synthase-kinase-3-beta-protein with isolated triterpenoid lupeol in rats. *Phytomed*. **15**:763-767.

Harmens, H., Gusinao, Hartog, D., Verkleij and Ernst. (1993). Uptake and transport of zinc in zinc sensitive and zinc tolerant *Silene vulgaris*. *J.Plant Physiol*.**141**:309-315.

Haydon, M.J. and Cobbett, C.S. (2007). Transporters of ligands for essential metal ions in plants. *New Phytol*. **174**: 499-506.

Heath, R.L. and Packer, L. (1968). Photo-peroxidation in isolated chloroplast. I. Kinetics and stoichiometry of fatty acid peroxidation. *Arc. Biochem. Biophys*. **125**:189-198.

Henry, J.R. (2000). Phyutoremediation of mercury. In: An overview of the phytoremediation of Lead and Mercury: A Report for the U.S. Environ. Protect. Agency Washington, D.C. USA,44-46.

Hoagland,D.R. and Arnon,D.I.(1950). The water culture method of growing plants without soil. *Colif.Agric.Expal.Stn.Circ*.**347**:461-465.

Hopkins,W.G., Norman,P.A. and Huner.(2004). *Introduction to Plant Physiology,* IIIrd Edn.John-Wiley & Sons, Inc.New York.

Hopkins, L., Malcolm, J. and Hawkensford, J. (2000). S-supply, free sulfur and free aminoacids pools in potato (*Solanum tuberosum* I.(V.Desiree) In:C.Brunold (Ed.) *Sulfur Nutrition And Sulfur Assimilation In Higher Plants*. 259-261.

Hossain, M.A., Piyatida, P., Teixeira da Silva, J.A. 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 methyl glyoxal and in heavy metal chelation. *J.Bot.* **2012**:1-37.

Hosseini,R.H.,Khanlarian,M. and Ghorbanli,M.(2007). Effect of lead on germination, growth and activity of catalase and peroxidase enzyme in root and shoot of two cultivars of *Brassica napus* L. *J.Biol.Sci*.**4**:592-598.

Houghton,P.J., Hylands,P.J., Mensah,A.Y., Hensel,A. and Deters,A.M. (2005). Invitro tests and ethnopharmacological investigations. Wound healing as an example. *J. Ethanopharmacol*..**100**:100-107.

Huang,B., Hatch,E. and Goldsbrouh,P.B. (1987). Selection and characterization of cadmium tolerant cells in tomato. *Plant Sci*. **52**:211-221.

Huang,J.W. and Cunningham,S.D.(1996). Lead phytoextraction:species variation in lead uptake and translocation. *New Phytol*.**134**:73-84.

Huang,J.W.,Pellet,P.M.,Papernik,L.A. and Kochian,L.V.(1996). Aluminium interactions with voltage dependent calcium transport on plasma membrane vesicles isolated from roots of aluminium sensitive and resistant wheat cultivars. *Plant Physiol*.**110**:561-569.

Hussain, K. (2007). Ecophysiological aspects of *Bacopa monnieri* (L.) Pennell. Ph.D. Thesis submitted to the University of Calicut.

Hussain, K. and Nabeesa-salim. (2012). Bioaccumulation pattern of mercury in *Bacopa monneiri* (L.). Pennel. *J. Stress Physiol. Biochem.*. **8**:10-21.

Hussain, K., Abdussalam, A.K., Ratheesh-Chandra, P. and Nabeesa-Salim,. (2010). Bioaccumulation of heavy metals in *Bacopa monneiri* (L.) Pennell growing under different habitat. *Inte. J. Ecol. Develop*. **10**:66-73.

Ichihara,K.I. and Fukubayashi,Y. (2009). Preparation of fatty acid methyl esters for gas-liquid chromatography. *J.Lipid Res*.**51**:635-640.

Igwe,K.K., Nwankudu,O.N., Ijoma, S.N., Madubuike, A.J. and Achi, N.K. (2016). Sreening of secondary metabolites in *Huru crepitans* bark ethanol extract using GC-MS analysis : A preliminary study approach. *J. Sci. Technol. Adva.*. **1**:64-71.

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. *J. Res. Pharma. Sci.***2**:01-06.

Iqbal,M.Z.,Saeeda,S. and Muhammed,S.(2001). Effects of chromium on an important

arid tree (*Caesalpinea pulcherima*) at Karachi city, *Pakistan. Ekol. Bratislava.***20**:414-22.

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 J.Exp.Biol.***31**:708-710.

Johnson, P., Loganathan, C., Krishnan, V., Sakayanathan, P., Raji, V., Vijayan, S., Sathishkumar, P., Murugeshan, K. and Palvannan, T. (2018). Plant extract as environmental friendly green catalyst for the reduction of hexavalent chromium in tannery effluent. *Environ. Technol.*. **39**:1376-1383.

Jot, S. and Subhadra, A.V. (2010). The effect of mercury on nitrate reductase activity in bean leaf segments (*Phaseolus vulgaris*) and its chelation by phytochelatin synthesis. *Life Sci. Medi. Res.*, LSMR-13.

Juneja, S. and Prakash, S. (2005). The chemical form of trivalent chromium in xylem sap of maize (*Zea mays* L.) *Chem. Speciat. Bioavailab*. **17**:161-169.

Kabata-Pendias, A. and Pendias, H.(2001). *Trace Elements in Soils and Plants*. (3rd Edn.) CRC. Press.

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*, New York.237-250.

Kahle,H.(1993). Response of roots of trees to heavy metals. *Environ.Exp.Bot*. **33**:99-119.

Kalpana, P., Balasubramanian, K., Kalaivani, R.A. (2018). Evaluation of heavy metals in selected medicinal plants and their corresponding soils collected from environmentally diverse locations of India. *Res.J.Pharmac. Technol*. **11**: 3489-3493.

Kamprath,E.J. and Foy,C.D. (1971). Lime-Fertilizer-plant interactions in acid soils. In: Olsan,R.A., Army,T.J., Hanway, J.J., Kilmer,V.J. (Ed.) *Fertilizer Technology and Use*. Soil Science Society of America, Madison 105--141.

Kanazawa,S.,Sano,S.,Koshiba,T. and Ushimaru,T.(2000). Changes in antioxidants in cucumber cotyledons during natural senescence: comparison with those during dark induced senescence. *Physiol.Plant*.**109**:211-216.

Kar, M. and Mishra, D. (1976). Catalase, peroxidase and polyphenoloxidase activities during rice leaf senescence. *Plant physiol*. **57**: 315-319.

Karthik, Chinnannan, V., Sri Ramkumar, A., Pugazhendhi, K., Gopalakrishnan and Indra Arulselvi, P. (2017). Biosorption and biotransformation of Chromium (VI) by novel cellulosic Microbium *Funkei* strain AR6. *J.Taiwan Insti. Chem,.Eng*. **70**:282-290.

Karthik, C., Oves, M., Thangabalu, R., Sharma, R., Santhosh, S. and Arulselvi, P.I. (2016). Cellulosimicrobium funkei- like enhances the growth of *Phaseolus vulgaris* by modulatoing oxidative damage under chromium (VI) toxicity. *J. Adv. Res*. **7**:839-850.

Khasim, S.M. (2002). *Botanical Microtechnique: Principles and Practice*. Capital publishing company, New Delhi.

Kikui,S.T.,Sasaki,M. and Maekawa (2005). Physiological and genetic analyses of aluminium tolerance in rice, focusing on root growth during germination.*J.Inorganic Biochem*.**99**:1837-1844.

Kim, Y.y., Yang, Y.Y., and Lee, Y. (2002). Pb and Cd uptake in rice roots. *Physiol.Plant.***116:**368-372.

Kneer,R. and Zenk,M.H. (1992). Phytochelatins protect plant enzymes from heavy

metal poisoning. *Phytochem*. **31**:2663-2667.

Kochian,L.V. and Shaff,J.E.(1991). Investigating the relationship between aluminium toxicity, root growth and root-generated ion currents. In: R.I.Wright,V.C.Baligar,R.P.Murrmann,(Eds.).*Plant-Soil Interactions at Low pH. Kluwer Academic*, Dordrecht,769-778.

Kochian,L.V.(1995). Cellular mechanisms of aluminium toxicity and resistance in plants. *Annu. Rev.Plant Physiol. Plant Mol. Biol*.**46**:237-60.

Kochian,L.V.,Pineros,M.A. and Hoekenga,O.A.(2005). The physiology, genetics and molecular biology of plant aluminium resistance and toxicity. *Plant and soil.* **2 74**:175-195.

Kollmeir,M.,Felle,H.H. and Horst,W.J.(2000). Genotypical differences in aluminium 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 aluminium. *Plant Physiol*.**122**:945-956.

Kuboto, H., Satoh, K., Yamada, T. and Maitani, T. (2000). Phytochelatin homologues induced in hairy roots of horse radish. *Phytochem*. **53**:239-245.

Kumar, P. and Chauhan, M.S. (2019). Adsorption of chromium(VI) from the synthetic aqueous solution using chemically modified dried water hyacinth roots. *J.Environ*. *Chem.Engin*. 7:103218.

Kundu,D.,Dey,S., and Raychaudhuri,S.S.(2018). Chromium(VI)-induced stress response in the plant *Plantago ovate* Forsk *invitro*.*Genes Environ*. **40**:1-13.

Kupper, H., Kupper, F. and Spiller, M. (1998). In situ detection of heavy metal substituted chlorophylls in water plants. *Photosynth. Res*. **58**:123-133.

Kupper, H., Lombi, E., Zhao, F.J. and McGrath, S.P. (2000). Cellular

compartmentation of cadmium and zinc in relation to other elements in the hyperaccumulator *Arabidopsis halleri*. *Plants.* **212**: 75-84.

Kupper, H., Mijovilovich, A., Meyerklaucke, W. and Kroneck, P.M.H. (2004). Tissue and age-dependent differences in the complexation of cadmium and zinc in the Cd/Zn hyperaccumulator *Thlaspi caerulescens* (Ganges ecotype) revealed by X-ray absorption spectroscopy. *Plant physiol*. **134**: 748-775.

Kuppuswamy,K.M., Jounalagadda,B. and arockiasamy, S.(2013). GC-MS analysis of chloroform extract of *Croton bonplandianum*. *Int. J. Pharma. Bio. Sci*. **4**:613-617.

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.

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. *Biol. Plant*. **35**:443-446.

Lepp, N.W., (1981). *Effect of Heavy Metal Pollution on Plants*. Vol.2. Applied Science Publishers, London.

Li,J., Pu,L., Liao, Q., Zhu, M., Dai, X., Xu,Y., Zhang, L., Hua, M. and Jin, Y. (2015). How anthropogenic activities affect soil heavy metal concentration on a broad scale: a geochemistry survey in Yangtze River Delta, Eastern China. *Environ. Earth Sci.* **73**:1823-1835.

Liu, J., Duan, C.Q., Zhang, X.H., Zhu, Y.N. and Hu, C. (2009). Subcellular distribution of chromium in accumulating plant *Leersia hexandra* Swartz. *Plant Soil*. **322**: 187-195.

Liu, Z., He, X., Chen, W., Yuan, F., Yan, K. and Tao, D. (2009).Accumulation and

tolerance characteristics of cadmium in a potential hyperaccumulator-*Lonicera japonica* Thumb. *Hazardous materials*. **10**: 81-97.

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. *Z. Pijanzenerniiehr. Bodenkd*. **157**: 447-451.

Lowry,O.H.,Rosebrough,N.J.,Farr,A.L. and Randall,R.J.(1951). Protein measurement with Folin phenol reagent. *J Biol.Chem***. 193**:265-275.

Lozano-Grande,m.,Gorinstein,S.,Espitia-Rangel,E.,Davila-Ortiz,G. and Martinez-Ayala,A.l.(2018). Plant sources, extraction methods and uses of squalene. *Int.J.Agron*.**2018**:1-13.

Ma,Q.,Rengel,Z., and Kuo,J.(2002). Aluminium toxicity in Rye (*Secale cereale*) root growth and dynamics of cytoplasmic Ca2+ in intact root tips. *Ann.Bot*. **89**:241-244.

Maestri, E., Marmiroli, M., Visioli, G. and Marmiroli, N. (2010). Metal tolerance and hyperaccumulation: cost and trade offs between traits and environmental and experimental botany. **68**:1-13.

Mahmoud, M.S. and Samah.,M.A.(2017). Calcium alginate as an ecofriendly supporting material for Baker's yeast strain in chromium bioremediation. *HBRC J.* **13:**245-254.

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 Physiol*. **110**:1145-1150.

Malajczuk,N. and Dell,B.(1995). Heavy metals in Philipines soils affect growth and

establishment of trees. *ACIR-For.News Letter*.**18**:2-3.

Mallick, S., Sinam, G., Mishra, R.K. and Sinha, S. (2010). Interactive effects of Cr and Fe treatments on plants growth, nutrition and oxidative status in *Zea mays* L. *Ecotoxicol. Environ. Saf.* **73**:987-995.

Mamadou,S.D. and Savage, N. (2005). Nanoparticles and water quality. *J.Nano.Res*.**7**:325-330.

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. J.Plant Physiol*.**156**:666-671.

Mateos-Naranjo, E., Redondo-Gomez, S., Cambrolle, J., Luque, T. and Figueroa, M.C. (2008). Growth and photosynthetic responses to zinc stress of an invasive cordgrass, *Spartina densiflora*. *Plant Biol.* **10**: 754-762.

Mathys.W. (1977). The role of malate, oxalate, and mustard oil glucosides in the evolution of zinc- resistance in herbage plants. *Physiol. Plant*. **40**: 130-136.

Matsumoto,H.(2000). Cell biology of aluminium toxicity and tolerance in higher plants. *Int.Rev.Cytol*.**200**:1-46.

Matysik, J., Bhalu, B. and Mohanty,P. (2002). Molecular mechanisms of quenching of reactive oxygen species by proline under stress in plants. *Curr.Sci*. **82**:525-532.

Megha,M.A.,Unnma,U.,Rameshpathy,M.,Karikalan,K.,Vickram,S.,KumaR,S.V.,Sridharan,B.(2013). Formulation of nano-encapsulated poly herbal ointment for anti-inflammation. *Der Pharmacia Lettre*.**5**:164-170.

Meidner, H. and Mansfield, T.A. (1968). Physiology of Stomata. McGraw Hill, London.

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 dubis*. *Afr.J Agri.Res*. 7:591-596.

Memon, A.R., Aktoprakligll, D., Ozdemir, A. and Vertiii, A. (2001). Heavy metal accumulation and detoxification mechanisms in plants. *Turk. J. Bot*: 111-121.

Memon,A.R. and Schroder,P.(2009). Implications of metal accumulation mechanisms to phytoremediation. *Environ Sci Pollut R.es*.**16:**162-175.

Mercurio,I., Melai,P., Capano,,D., Ceraso, G., Carlini, L. and Bacci, M. (2017). GCMS analysis of morning glory seeds freely in commerce: Can they be considered "herbal highs"? *Egypt. J. Foren. Sci*. **7**:1-6.

Michalak, (2006). Phenolic compounds and their antioxidant activity in plants growing under heavy metal stress. *Polish. J. Environ. Stud*. **15**:523-530.

Migocka, M. and Klobus, G. (2007). The properties of Mn, Ni and Pb transport operating at plasma membranes of cucumber roots. *Physiol. Plant*. **129**:578-587.

Minissi, S. and Lombi, E.(1997). Heavy metal content and mutagenic activity, evaluated by *Vicia faba* micronucleus test, of Tiber river sediments. *Mutation Research/ Genetic mutagenesis*. **393**:17-21.

Mishra, A. and Choudhuri, M.A. (1999). Monitoring of phytotoxicity of lead and mercury from germination and early seedling growth induces two rice cultivars. *Water Air Soil Pollut.* **144**:339-346.

Mishra, B., Donald, L.M., Roy, M., Lanzirotti, A. and Myneni, S.C.B. (2020). Uptake and speciation of zinc in edible plants grown in smelter contaminated soils. *PLOS ONE* :**15**:1-21.

Micera,M., Botto., 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.

Mittler,R.(2002). Oxidative stress, antioxidants and stress tolerance. *Trends Plant Sci*.**7**:405-410.

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. *Fresnius Environ.Bull*.**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. *J.Plant Nutr*.**18**:815-822.

Moreno, F.N., Anderson, C.W.N., Robinson, B.H. and Stewart, R.B. (2004). Mercury analysis of plant and soil samples using the hybrid-generation AAS method. In: Currie, L.D.,Hanly,J.A.(Eds.). *Tools for Nutrient and Pollutant Management.* Occasional report no.17. Fertilizer and lime research centre, Palmerston north, New Zealand,.425-433.

Moreno, F.N., Anderson, C.W.N., Stewart, R.B. and Robinson, B.H. (2005). Mercury volatilization and phytoextraction from base metal mine tailings. *Environ.Pollut*. **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. *Environ. Exp. Bot*. **62**:78-85.

Morgan, J.F., Klucas, R.V., Grayer, R.J., Abian, J. and Becana, M. (1997). Complexes of iron with phenolic compound from soybean nodules and other legume tissues:prooxidant and antioxidant properties;*Free Radic.Biol.Med*. **22**:861.

Mossor-Pietraszewska,T.(2001). Effect of Aluminium on plant and growth metabolism. *Acta Biochim.Pol*.**48**:673-86.

Mossor-Pietraszewska,T.,Kwit,M., and Legiewicz ,M.(1997). The influence of aluminium ions on activity changes of some dehydrogenases and amino transferaces in yellow lupine. *Biol.Bull.Poznan*.**34**:47-48.

Mousavi,L. Salleh,R.M. and Murugaiyah,V. (2018). Phytochemical and bioactive compounds identification of *Ocimum tenuiflorum* leaves of methanol extract and its fraction with an anti-diabetic potential. *Int.J.Food Prop*. **21**:2390-2399.

Mukhopadhyay,N. and Aery,N.C. (2000). Effect of Cr (3) and Cr(4) on the growth and physiology of *Triticum aestivum* plants during early seedling growth. *Sec.Bot*. **55**:403-408.

Mukta,R.H., Khatun,M.R. and Huda,A.K.M.N. (2019). Calcium induces phytochelatin accumulation to cope with chromium toxicity in rice (*Oryza sativa* L.). *J.Plant Inter*. **14**:295-302.

Mysliwa-Kurdziel, B. and Strzalka, K. (2002). Influence of metals on biosynthesis of photosynthetic pigments. In: M.N.V. Prasad and K. Strazalka (Eds.). *Physiology and Biochemistry of Metal Toxicity and Tolerance in Plants*. Kluwer Academic Publishers. The Netherlands. 201-227.

Nag,P.,Paul,A.K. and Mukherji,S.(1980). Effects of mercury, copper and zinc on growth, cell division, GA induced amylase synthesis and membrane permeability of plant tissues. *Indian J.Exp.Biol*.**18**:822-827.

Nasim,S.A. and Dhir,B.(2010). Heavy metals alter the potency of medicinal plants. *Rev.Environ.Cont.Toxicol*. **203**: 139-149.

Nasiri,Y., Zehtab-Salmasi,S., Nasrullahzadeh,S., Najafi,N. and GhassemGolezani,K.

(2010). Effects of foliar application of micronutrients (Fe and Zn) on flower yield and essential oil of chamomile (*Matricaria chamomilla* L.) *J.Med.Plant Res*. **4**:1733-1737.

Nath,S., Chaudhury,J., Pahan,K. and Mandal,A.(1993). Mercury and organomercurial resistance in rhizobial strains. *Indian J.Exp.Biol*.**31**:50-53.

Navnidhi,C., Kour,R., Jaglan,S., Gupta,Y., Gat and Panghal. (2018). *Citrus medica*: Nutritional, phytochemical composition and health benefits- A review. *Food Funct*. **9**:1978-1992.

Noctor,G. and Foyer ,C.H. (1998). Ascorbate and glutathione: keeping active oxygen under control. *Annu.Rev.plant.Physiol.Plant Mole.Biol*.**49**:249-279.

Obata, H., Inoue, N., Imai, K. and Umebayashi, M. (1994). Cadmium tolerance of calli induced from roots of plants with difference in cadmium tolerance. *Soil Sci. Plant Nutr*. **40**:351-354.

Oliveira, H. (2012). Chromium as an environmental pollutant: insights on induced plant toxicity. *J. Bot*.

Olofossom, P., Hultqvist, M., Hellgren, L.I. and Holmdahl, R. (2014). Phytol: A chlorophyll component with anti-inflammatory and metabolic properties. In: Jacob, C., Kirsch, G., Slusarenko, A., Winyard, P. and Burkholz,T. (Eds.) *Recent Advances in Redox Active Plant and Microbial Products*. *Springer, Dordrecht*:345-359.

Orcutt,D.M. and Nilsen,E.T.(2000). *Physiology of Plants Under Stress: Soil and Biotic Factors*. *John Wiley & Sons, Inc.* New York.

Ozyigit,I.L., Vardar, F., Yasar, U., and Akinci, S. (2013). Long term effects of aluminium and cadmium on growth, leaf anatomy, and photosynthetic pigments of cotton. *Commun. Soil Sci. Plant Analyis*. **44**:3076-3091.

Palmer, C.M., Lou Guerinot, M.(2009). Facing the challenge of Cu, Fe and Zn homeostasis in plants. *Nat. Chem. Biol*. **5**:333-340.

Panda,S.K. and Choudhury,S.(2005). Chromium sress in plants. *Braz.J.Plant Physiol*.**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.(1998). Alteration of nitrate reductase activity by chromium ions in excised wheat leaves. *Ind.J.Angio.Biochem*.**2**:56-57.

Panda,S.K. and Patra,H.K.(2000). Does Cr.(III) produce oxidative damage in excised wheat leaves. *J.Plant Biol*.**27**:105-110.

Panda,S.K.(2003). Heavy metal phytotoxicity induces oxidative stress in *Taxithelium sp. Curr.Sci*.**84**:631-633.

Panda, S.K.(2007). Chromium- mediated oxidative stress and ultra structural changes in root cells of developing rice seedlings. *Plant physol*.**164**:1419-1428.

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. *Ann. Rev. Plant Biol*. **72**:463-95.

Parekh, D., Puranik, R.M. and Srivastva, H.S. (1990). Inhibition of chlorophyll biosynthesis by cadmium in greening maize leaf segments. *Biochem. Physiol. Pflanzen*. **186**:239-242.

Parmer,N.G., Vithalani,S.D., and Chanda, S.V.(2002). Alteration in growth and

peroxidase activity by heavy metals in *Phaseolus seedlings*. *Acta Physiol. Plant*. **24**:89-95.

Patra, M., Bhowmik, N., Bandopadhyay, B. and Sharma, A. (2004). Comparison of mercury, lead and arsenic with respect to genotoxic effects on plant systems and the development of genetic tolerance. *Environ. Experi. Bot.*. **52**:199-223.

Pavan,M.A. and Bingham.(1982). Toxicity of aluminium to coffee seedlings grown in nutrient solution. *Soil Sci.Soc.Am.J*. **46**:993-997.

Pawar,R.S. and Toppo, F.A. (2012). Plants that heal wounds:A review. *Herba polonica.*58.

Peraita, J.R., Gardea-Torresdey, J.L., Tiemann, K.J., Gomez, E., Arteaga, S., Rascon, E. and Parsons, J.G. (2001). Uptake and effects of five heavy metals on seed germination and plant growth in alfalfa (*Medicago sativa* L.). *Bulletin of Environ. Contamin. Toxicol*. **66**:727-734.

Perfus-Barbeoch, L., Leonhardt, N., Vavasseur, A. and Forestier, C. (2002). Heavy metal toxicity:cadmium permeates through calcium channels and disturbs the plant water status. *The Plant J.* **32**:539-548.

Phrompittayarat, W., Jetiyanon,K., Wittaya Areekul, S. and Putaloun,W. (2011). Influence of seasons, different plant parts, and plant growth stages on Saponin quantity and distribution in *Bacopa monnieri*. Songklanakarin *J.Sci.Technol*.**33**:193-199.

Pilon-Smits,E.(2005). Phytoremediation. *Annu. Rev. Plant. Biol*.**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 Al^{3+} induced an ion channels. *Plant physiol*. **125**:292-305.

Pinto,M.E.A.,Araujo,S.G.,Morais,M.I.Sa,N.P.,Lima,C.M.,Rosa,C.A.,Siqueria,E.P.,Johann,S. and Lima,L.A.R.S.(2017). Antifungal and antioxidant activity of fatty acid methyl esters from vegetable oils. *An. Acad. Bras Cienc*.**89**:1671-1681.

Poschenrieder,C., Gunse,B.,Corrales,I. and Barcelo,J.(2008). A glance into aluminium toxicity and resistance in plants. *Sci.Total Environm*. **400**:356-368.

Prado, C., Ponce, S.C., Pagano, E. Prado, F.E., Roas, M. (2016). Differential physiological responses of two *Salvinia* species to hexavalent chromium at a glance. *Aquat. Toxicol.* **175**:213-221.

Prakash,V.(2017). Terpenoids as source of anti-inflammatory compounds. *Asian J.Pharm.Clin.Res*.**10**:68-76.

Prasad,M.N.V.(1997). Trace metals.In:M.N.V.Prasad (Ed.). *Plant Ecophysiology*. *John Wiley & 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 muscarum*. FEMS *Microbiol. Lett*. **82**:95-100.

Pulford,I.D., Watson,C. and McGregor,S.D. (2001). Uptake of chromium by trees: prospects for phytoremediation. *Environ. Geochem. Health*. **23**: 307-311.

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. *Ecotoxicol. Environ. Saf.* **114**: 179-189.

Qureshi,J.A., Thurman,D.A., Hardwick,K. and Collin, H.A. (1995). Uptake and accumulation of zinc, lead and copper in zinc and lead tolerant *Anthoxanthum odoratum* L. *New phytol*.**100**:429-434.

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. *Environ.Toxicol.Chem*. **28**:189-196.

Rai,V., and Mehrotra,S.(2008). Chromium induced changes in ultramorphology and secondary metabolites of *Phyllanthus amarus* schum and Thonn-A hepatoprotective plant. *Environ. Monitor.Asses*. **147**:307-15.

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 tenuiiflorm* L. *Plant Sci.***167**:1159-1169.

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 Sci*. **180**:169-181.

Raskin, I. and Ensley, B.D. (2000). Phytoremediation of toxic metals using plants to clean up the environment. *John Wiley & sons. Inc.* New York.

Rates,S.M.K.(2001). *Plant sources as drugs.Toxicon*..**39**:603-613.

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. *Stress Physiol.Biochem*.**6**:4-12.

Rauser, W.E. (1987). Changes in glutathione content of maize seedlings exposed to cadmium. *Plant Sci*. **51:**171-175.

Rauser, W.E. (1999). Structure and function of metal chelators produced by plants: the case of organic acids, amino acids, phytin and metallothioneins. *Cell Biochem. Biophys*. **31**:19-48.

Rauser,W.E. (1990). Phytochelatins. *Annu. Rev. Biochem*. **59**:61-86.

Reddy, G.N. and Prasad, M.N.V.(1990).Heavy metal binding protein/peptides: Occurrence, structure, synthesis and functions-A review. *Environ. Exp. Bot*. **30**:251-264.

Redondo-Gomez,S., Andrades-Moreno, L., Mateos-Naranjo, E., Parra, R., Burgos, J,V. and Aroca, R. (2011). Synergic effect of salinity and zinc stress on growth and photosynthetic responses of the cordgrass, *Spartina densiflora*. *J.Environ.Bot*. **62**:5521-5530.

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, Florida. 105-118.

Reichman, S.M. (2002). The responses of plants to metal toxicity: A review forusing on copper, manganese and zinc. *Australian Minerals and Energy Environment Foundation.*. 1-54.

Reichman, S.M., Asher, C.J., Mulligan, D.R. and Menzies, N.W. (2001). Seedling responses of three Australian tree species to toxic concentrations of zinc in solution culture. *Plant and soil*. **235**: 151-158.

Rengel, Z. (1996). Uptake of aluminium by plant cells. *New phytologist*. **134**:389-409.

Rosolem, C.A., Sacramento, L.V.S., and Oliveira, D.M.T. (2005). Kinetics of zinc uptake and anatomy of roots and leaves of coffee trees as affected by zinc nutrition. *J.Plant Nutri*. **28**:2101-2112.

Ross,H.B.(1994). On the use of mosses (*Hylocomium splendens* and *Pleurozium*

schreberi) for estimating atmospheric trace metal deposition. *Water Air Soil Pollut*. **50**:63-76.

Rout,G.R.,Sanghamitra,S. and Das,P.(1997). Different chromium tolerance among eight mung bean cultivars grown in nutrient culture.*J.Plant Nutri*.**20**:341-347.

Roy,S.j., Baruah, P.S., Lahikar, L., Gurung, L., Saikia,D. and Tanti, B. (2019). Phytochemical analysis and antioxidant activities of *Homolomena aromatic* Schott. *J.Pharmaco.Phytochem.***8**: 1379-1385.

Rugh, C.L., Gragson, G.M., Meagher, R.B. and Merkel, S.A. (1998). Toxic mercury reduction and remediation using transgenic plants with a modified bacterial gene. *Hortscience*. **33**:618-621.

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. *J.Exp.Botany*. **44**: 437-446.

Ryan, P.R., Skerrett, M., Findlay, G.P., Delhaize, E. and Tyerman, S.D. (2002). Aluminium activates an anion channel in the apical cells of wheat roots. *PNAS*. **94**:6547-52.

Ryu,K.R.,Choi,J.Y.,Chung,S. and Kim,D.H.(2011). Anti scratching behavioural effect of the essential oil and phytol isolated from *Artemisia princeps* Pamp. in mice.*Planta Med.***77**:22-26.

Saha,S.,Mandal,B.,Hazra,G.C.,Dey,A.,Chakroborty,B.,Adhikari,B,Mukhopadhyay,S. K. and Sadhukhan,R. (2015). Can agronomic biofortification of zinc be benign for iron in cereals? *J.Cereal Sci*. **65**:186-191.

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. J.Ethanopharmacol*. **71**:275-280.

Saleem,M. (2009). Lupeol, a novel anti-inflammatory and anti cancer dietary triterpene. *Cancer Letters*. **285:**109-115.

Salt, D.E., Smith, R.D. and Raskin, I. (1998). Phytoremediation. *Annu. Rev. Plant Physiol.Plant Mol. Biol.* **49**:643-668.

Salt,D.E.,Blaylock,M.,Kumar,N.P.B.A.,dushenkov,V.,Ensley,B.D.,Chet,I. and Raskin,I.(1995). Phytoremediation: A novel strategy for the removal of toxic metals from the environment using plants. *Biotechnol*.**13**:468-474.

Samantary,S. and Das,P.(1997). Accumulation and distribution of chromium, nickel and iron in the mung bean plant. *Fresnius Envir.Bull*.**6**:633-641.

Samantary,S.,Rout,G.R. and Das,P.(1996). A study of soil plant and root shoot relationship in rice (*Oryza sativa* L.cv.Pathan) grown on chromiferous mine soil. *Proc.Natl.Acad.Sci.*USA.**66**:349-357.

Sampananish, P., Pongsapich, W., Khaodhiar, S. and Khan, E. (2006). Chromium removal from soil by phytoremediation with weed plant species in Thailand. *Water, Air, and Soil Pollution*:*Focus*. 191-206.

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) *Inoranic Plant Nutrition*. .**15B**:563-596.

Santos,C. 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 invivo and *invitro* models. *Neurosci J. Article* ID 949452.

Saradhi,A. and Saradhi,P.P.(1991). Proline accumulation under heavy metal stress. *Plant Physiol*. **138**:554-558.

Saravanan, J., Shariff, W.R., Joshi, N,H., Varatharajan, R., Joshi, V.G. and Karigar. (2010). Preliminary pharmacognostical and phytochemical studies of leaves of *Hemigraphis colorata*. *Res.J.Pharmaco.Phyto*. **2**:15-17.

Sasaki, T., Yamamoto, Y., Ezaki, B., katsuhara, M. and Ahnis, J. (2004). A wheat gene encoding an aluminium activated malate transporter. *Plant J*. **57**:645-53.

Savitha,B.C., Thimmaraju,R., Bhagyalakshmi,N. and Ravishankar, G.A. (2006). Different biotic and abiotic elicitors influence betalain production in hairy root cultures of *Beta vulgaris* in shakeflask and bioreactor. *Pro.Biochem*. **41**:50-60.

Scheller, H.V., Huang, B., Hatch, E. and Goldsbrough, P.B. (1987). Phytochelatin synthesis and glutathione levels in response to heavy metals in tomato cells. *Plant Physiol*. **85**: 1031-1035.

Sermakkani,N. and Thangapandian,V.(2011).GC-MS analysis of Cassia italic leaf methanol extract. *Asian J.Pharm. clini. Res.***5**:90-94.

Setia, R.C. and Bala, R. (1994). Anatomical changes in root and stem of wheat (*Triticum aestivum* L.) in responses to different heavy metals. *Phytomorph*. **44**:95-104.

Shahid, M., Austruy, A., Echevarria, G., Arshad, M., Sanaullah, M., Aslam, M., Nadeem, M., Nasim, W. and Dumat, C. (2014). EDTA-enhanced phytoremediation of heavy metals: A review. *Soil Sediment Contam.Int.J*. **23**:389-416.

Shahid, M., Shamshad, S., Rafiq, M., 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.

Shahkarami, S., Goudarzi, F., Amiri-Ardekanl, E. and Dehghani, S. (2021). Heavy metals contamination in two species of medicinal plants in the Iranian market. *Int. J. Environ. Anal. Chem.*. 1-10.

Shakoor,M.B., Ali,S., Hameed,A., Farid, M., Hussain,S., Yasmeen,T., Najeeb, U., Bharwana, S.A., and Abbasi, G.H. (2014). Citric acid improves lead(Pb) phytoextraction in *Brassica napus* L. by mitigating Pb-induced morphological and biochemical damages. *Ecotoxicol. Environ. Saf.* **109**:38-47.

Shalini,S., and Sampathkumar,P.(2012). Phytochemical screening and anti microbial activity of plant extracts for disease management. *Int.J.Curr. Sci*.**1**:209-218.

Shankar, B.S. (2019). A critical assay of heavy metal pollution index for the groundwaters of Peenya industrial area, Bangalore, India. *Environ. Monit. Assess*.191:1-14

Shanker, A.K., Sudhagar, R., Pathmanabhan, G. (2003). Growth, Phytochelatin SH and antioxidative response of sunflower as affected by chromium speciation. 2nd International Congress of Plant Physoiology on Sustainable Plant Productivity Under Changing Environment. New Delhi, India.

Shanker,A.K., Cervantes,C., Loza-Tavera,H. and Avudainayagam,S. (2005). Chromium toxicity in plants. *Environ.Intl*. **31**:739-753.

Shanker,A.K., Djnaguiraman,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 radiate* (L.)R.Wilczek.cv CO roots. *Plant Sci.***166:**1035-1043.

Sharma,P.,Kumar,A.,Bhardwaj,R.(2016). Plant steroidal hormone epibrassinolide regulate-Heavy metal stress in *Oryza sativa* L. by modulating antioxidant defense expression.*Environ.Exp.Bot*.**122**:1-9.

Shaw, B.P. and Rout, N.P.(1998). Age-dependent responses of *Phaseolus aureus* Roxb. To inorganic salts of mercury and cadmium. *Acta Physiol. Plant*. **20**:85-90.

Shaw,B.P. (1995). Effect of mercury and cadmium on the activities of antioxidative enzymes in the seedlings of *Phaseolus aureus*. *Biol.Plant*. **37**:587-596.

Sheoran, I.S., Aggarwal, H. and Singh, R. (1990). Effect of cadmium and nickel on invivo carbon dioxide exchange rate of pigeon pea. *Plant soil*. **129**:243-249.

Shetty,S.,Udupa,S. and Udupa,L.(2007). Evaluation of antioxidant and wound healing effects of alcoholic and aqueous extract of *Ocimum sanctum* Linn in rats. *Adv. Access Publ*. **5**:95-101.

Shokuhi,K.H. and Ghanati,F.(2007). Aluminium induced growth inhibition and changes in cell wall components of tobacco cells. *J.Sci.Teacher Train Univ*.**7:**855-864.

Shukla, O., Dubey, S. and Rai, U. (2007). Preferential accumulation of cadmium and chromium: toxicity in *Bacopa monnieri* L. under mixed metal treatments. *Bull. Environ. Contam. Toxicol*. **78**:252-257.

Siedlecka, A. and Krupa, Z. (2002). Functions of enzymes in heavy metal treated plants. In: M.N.V.Prasad and M.N.V. Kazimierz, S.K. Strzalka (Eds.) *Physiology and Biochemistry of Metal Toxicity and Tolerance in Plants*. Kluwer Academic,Dordrecht. Netherlands. 314-317.

Silja, V.P., Varma, K.S. and Mohanan, K.V. (2008). Ethnomedicinal plant knowledge of the mullu kuruma tribe of wayanad district kerala. *Indian J. Trad. Know*. **7**:604-612.

Silva,S.(2012). Aluminium toxicity targets in plants. *J.Bot*.**12**:1-8.

Silvio,D.F., Anna,C., Rosanna,T., Micale,L., Maestra,V., Savarino, P., Zentilin,E.,

Marabotto,M. and Deborah,M.P. (2016). Reduction of hexavalent chromium by fasted and fed human gastric fluid. I. Chemical reduction and mitigation of mutagenicity. *Toxicol. App. Pharm*. **306**:113-119.

Simon,L.,Smalley,T.J.,Benton Jones Jnr,J. and Lasseigne,F.T.(1994). Aluminium toxicity in tomato.I. Growth and mineral nutrition. *J.Plant Nutr*.**17**:293-306.

Singh,A.K. (2001). Effect of trivalent and hexavalent chromium on spinach (*Spinaceia oleracea* L.) *Environ.Ecol*. **19**:807-810.

Singh, H.P., Mahajan, P., Kaur, S., Batishi, D.R. and Kohli, R.K. (2013). Chromium toxicity and tolerance in plants. *Environ. Chem. Lett*. **11**:229-254.

Singh, S., Seaxena, R., Pandey, K., Bhatt, K. and Singh, S. (2004). Response of antioxidant in sunflower (*Helianthus annuus* L.) grown on different amendments of tannery sludge: its metal accumulation potential. *Chemosphere*. **57**: 1663-1673.

Singh,S., Prasad,S.M. and Singh,V.P. (2020). Additional calcium and sulfur manages hexavalent chromium in *Solanum melongena* L. seedlings by involving nitric oxide. *J.Haz.Mat*. **398**:122607.

Singh,D. and Aeri,V.(2013). Phytochemical and pharmacological potential of *Acanthus ilicifolius*. *J.Pharm. Bioallied Sci*.**5**:17-20.

Singh,V.P.(2005). *Toxic Metals and Environmental Issues*. *Sarup and Sons*, New Delhi.

Singla-pareek, S.L., Yadav, S.K., Pareek, A., Reddy, M.K. and Sopory, S.K. (2006). Transgenic tobacco overexpressing glyoxalase pathway enzymes grow and set viable seeds in zinc-spiked soils. *Plant Physiol*. **140**: 613-623.

Sinha, S. (1999). Accumulation of Cu, Cd, Cr, Mn and Pb from artificially contaminated soil by *Bacopa monnieri. Environ.Monit. Assess*. **57**:253-264.

Sinha, S., Bhatt, K., Pandey, K., singh, S. and Saxena, R. (2003). Interactive metal accumulating and its toxic effects under repeated exposure in submerged plant Najas indica. Chem. Bull. Environ. *Contam. Toxicol*. **70**:696-704.

Skinner,K., Wright,N. and Porter-Goff,E. (2007). Mercury uptake and accumulation by four species of aquatic plants. *Environ.Poll*. **145**:234-237.

Solanki,R. and Dhankhar, R. (2011). Biochemical changes and adaptive strategies of plants under heavy metal stress. *Bio*. **66**:195-204.

Srivastava, N.K. and Srivastava, A.K. (2010). Influence of some heavy metals on growth, alkaloid content and composition in *Catharanthus roseus* L.*Ind.J.Pharm.Sci*. **72**:775.

Stefanov, K.L., Popova,I., Kamburova,E., Pancheva., Kimenov,G., Kuleva,L., and Popv,S. (1993). Lipid and sterol changes in *Zea mays* caused by lead ions. *Phytochem*. **33**:47-51.

Steffens, J.C. (1990). The heavy metal binding peptides of plants. *Annu. Rev. Plant Physiol. Plant Mol.* Biol. **41**:533-575.

Stohs, S.J. and Bagchi, D. (1995). Oxidative mechanisms in the toxicity of metal ions. *Free radicals. Biol. Med*. **18**: 321-336.

Subramoniam, A., Evans,D.A., Rajasekharan,S. and Nair, G.S.(2001). Effect of *Hemigraphis colorata* (Blume) H.G.Hallier leaf on wound healing and inflammation in mice. *Int.J. Pharmacol*..**33**:283-285.

Suntar,I.,Akkol,E.K.,Nahar,L. and Sarker,S.D.(2012). Wound healing and antioxidant properties: do they coexist in plants ? *Free Rad.Antiox*.**2**:1-7.

Suszcynsky, E.M. and Shann, J.R. (1995). Phytotoxicity and accumulation of mercury in tobacco subjected to different exposure routes. *Environ. Toxicol. Chem*. **14**: 61-67.

Taiz, L. and Zeiger, E. (2002). *Plant Physiology*: (IIIrd Ed.) Sinauer Associates, *Inc. Publishers*, Sunderland, Massachussetts.

Taiz, L., Zeiger, E., Moller, I.M., and Murphy, A. (2015). *Plant Physiology and Development*. (VIth Ed.) Sinauer Associates, *Inc. Publishers*, Sunderland, Massachussetts.

Tamboli,F.A., Rangari,V.D., Killedar,S.G., Jadhar,S.U., Ghatage,t.S. and Kore,V.P. (2018). Comparative phytochemical evaluation of natural micropropagated plants of *Bacopa monnieri* (L.) and Marmara. *Pharm. J*. **22**:66-73.

Taylor,G.J.(1988). The physiology of aluminium tolerance; in Metals Ions in Biological Systems(H.Sigel .(Ed.). *Aluminium and its role in Biology*. *Marcel-Dekker*, New York.**24**:165-198.

Taylor,G.J.(1995). Overcoming barriers to understanding the cellular basis of aluminium resistance. *Plant and Soil*.**171**: 89-103.

Tennstedt,D. Peisker,C., Bottcher, A.,Trampczynska. And Clemens, S. (2009). Phytochelatin synthesis is essential for the detoxification of excess zinc and contributes significantly to the accumulation of zinc. *Plant Physiol*. **149**:938-948.

Thilagavathi,T., Arvindganth,R., Vidhya,D. and Dhivya,R. (2015). Preliminary phytochemical screening of different solvent mediated medicinal plant extracts evaluated. *Int.Res.J.Pharm*.**6**:246-248.

Tice,K.R., Parker,D.R. and DeMason,D.A. (1992). Operationally defined apoplastic

and symplastic aluminium fractions in root tips of aluminium intoxicated wheat. *Plant Physiol*. **100**:309-318.

Tripathi, A.K., Tripathi,S. and Tripathi,S. (1999). Changes in some physiological and biochemical characters in *Albezia lebbeck* as bioindicators of heavy metal toxicity. *J.Environ.Biol*.**20**:93-98.

Tukendorf, A. and Rauser, W.E. (1990). Changes in glutathione and phytochelation in roots of maize seedlings exposed to cadmium. *Plant Sci*. **70**:155-166.

Turner, A.P. (1994). The responses of plants to heavy metals. In: S.M. Ross(Ed.). *Toxic Metals in Soil-Plant Systems*. *John Wiley & Sons Ltd*., 154-187.

Usman, K., Al-Ghouti, M.A., Abu-Dieyeh, M.H. (2019). The assessment of cadmium, chromium, copper and nickel tolerance and bioaccumulation by shrub plant *Tetraena qataranse*. *Sci.Rep*. **9**:1-11.

Ukwubile,C.A., Ahmed,A., Katsayal,U.A., Ya,U.J. and Mejida,S.(2019). GCMS analysis of bioactive compounds from *Melastomastrum capitatum*(Vahl) Fern leaf methanol extract:an anticancer plant.*Scientific African*.**3**:e0059.

Ur-Rehman, K., hamayun, M., Afsal Khan, S., Iqbal, A. and Hussain, A. (2019). Heavy metal analysis of locally available anticancer medicinal plants. *Biosci. Biotechnol. Res. Asia*. **16:**105-111.

Usha,G. and Nazarine, F. (2003). Bioactivity of methyl palmitate obtained from a Mangrove plant *Salvadora persica* L. (http: // www. freepatentsonline.com / 6638546.html)

Usha,M.,Venugopoj,V.K. and Saraswathi,P.(2002). Cadmium content of plants as affected by soil application of cadmium and farmyard manure. *J.Trop.Agric*.**40**:70-80.

Vaculk, M., Konlechnor, C., Langer, I., Adlassnig, W., Puschenreiter, M., Lux, A. and Hauser, M. (2012). Root anatomy and element distribution vary between two *Salix caprea* isolates with different Cd accumulation capacities. *Environ. Pollut*. **163**:117-126.

Vajpayee,P.,Sharma,S.C.,Tripathi,R.D.,Rai,U.N. and Yunus,M. (1999). Bioaccumulation of chromium and toxicity to photosynthetic pigments, nitrate reductase activity and protein content of *Nelumbo nucifera* Gaertin. *Chemosphere*. **39**:2159-2169.

Vajpayee,P.,Tripathi,R.D.,Rai,U.N.,Ali,M.B. and Singh,S.N. (2000). Chromium (VI) accumulation reduces chlorophyll biosynthesis, nitrate reductase activity and protein content in *Nymphaea alba* L.*Chemosphere*.**41**:1075-1082.

Vallee,B.L. and Ulmer,D.D.(1972). Biochemical effects of mercury, cadmium and lead. *Annu.Rev.Biochem*.**41**:91-128.

Van Assche, F. and Clijsters, H. (1990). Effect of metals on enzyme activity in plants. *Plant Cell Environ*. **13**:195-206.

Van Steveninick, R,F,M., Van Steveninck, M.E., Fernando, D.R., Horst, W.J., and Marschner, H. (1987). Deposition of zinc phytate in globular bodies in roots of *Deschampsia caespitosa* ecotypes: a detoxification mechanism? *J. Plant Physiol*. **131**:247-257.

Vareda, J.P., Valenta, A.J.M. and Duraes, L. (2019). Assessment of heavy metal pollution from anthropogenic activities and remediation strategies: A review. *J. Environ. Manag*. **246**:101-118.

Vazquez, M.D., Poschenrider, 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 Physiol*. **199**: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.* Z. *Naturforsch*. **60**:252-259.

Venkataraman, B., Samuel,L.A., Pardhasaradhi,M., NarashimhaRao,B., Nagavamskrishna,A., Sudhakar,M. and Radhakrishnan,T.M. (2012). Antibacterial,antioxidant activity and GCMS analysis of *Eupatorium odoratum*. *Asian J. Pharm Clin Res*.**5**:99-106.

Verkleji, J.A.C. and Schat, H. (1990). Mechanisms of metal tolerance in higher plants. In:A.J. Shaw(Ed.) *Heavy Metal Tolerance in plants*: *Evolutionary Aspects*. CRC Press, Florida. 179-193.

Vyas, J. and Puranik, R.M. (1993). Inhibition of nitrate reductase activity by mercury in bean leaf segments. *Indian Journal of Plant Physiol*. **36**:57-60.

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. *Int. J. Mol. Sci*. **21**:728.

Wang, M.H. and Bradshaw, A.W. (1982). A comparison of the toxicity of heavy metals, using root elongation of rye grass, *Lolium perenne. New Phytol*. **91**:255-261.

Wang,Y.n. Wang, H.X. Shen, Z.J., Zhao, L.L., Clarke, S.R., Sun, J.H. Du, Y.Y. and Shi, G.L. (2009). Methyl palmitate, an acaricidal compound occurring in green walnut husks. *J. Econo. Entomo*. **102**: 196-202.

Wani,W., Masoodi, K.Z., Zaid, A., Wani, S.H.,Shah,F., Meena, V.S., Wani, S.A. Mosa, K.A.(2018). Engineering plants for heavy metal stress tolerance. *Rendiconti Lincei. Scienze Fisiche e Naturali.***29**:709-723.

Webb,E.C.(1992.Enzyme nomenclature, recommendations of vthenomenclature Committee of the International Union of Biochemistry and Molecular Biology. NewYork,NY,USA: Academic Press.

Wheeler, D.M. and Power, I.L. (1995). Comparison of plant uptake and plant toxicity of various ions in wheat. *Plant Soil*. 172:167-173.

White, P.J. and Broadley, M.R. (2009). Biofortification of crops with seven mineral elements often lacking in human diets- iron, zinc, copper, calcium, magnesium, selenium and iodine. *New phytol*. **182**: 49-84.

Wilkins, (1978). The measurement of tolerance to edaphic factors by means of root growth. *New Phytol*. **80**:623-633.

Willekens,H.,Chamnongpol,S.,Davey,M.,Schaudner,M.,Langebartels,C.,VanMontagu ,M.,Inze,D. and Van Camp,W.(1997). Catalase is a sink for H2O2 and is indispensible for stress defence in C3 plants. *EMBO J*.**16**:4806-4816.

Winkel-Shirley, B. (2002). Biosynthesis of flavonoids and effects of stress. *Curr.Opin.Plant Biol*. **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 phytol*. **91**:255-261.

Woolhouse,H.W. (1983). Toxicity and tolerance in the response of plants to metal. In:O.L.Lange,P.S.Nobel,C.B. Osmond and H.Ziegler (Eds.). Encyclopedia of plant physiology (New seies) Vol.12C Physiological plant Ecology III. *Springer-Verlag*,New York.245-302.

Xiong,Z.T.(1999). Lead accumulation and tolerance in *Brassica chinensis* L. grown in sand and liquid culture. *Toxicol. Environ.Chem***. 69**:9-18.

Yadav,M., Chatterji,S., Gupta,S.K. and Watal,G. (2014). Preliminary phytochemical screening of six medicinal plants used in traditional medicine. *Int.J.Pharm.Pharm.Sci*.**6**:529542.

Yadav,S.,Sukla,O.P. and Rai,U.N.(2005). Chromium pollution and bioremediation. *Environ.News Arch*.**11**:1-4.

Yan, L., Riaz, M., Liu, J., Yu,M. and Cuncang, J. (2021). The aluminium tolerance and detoxification mechanisms in plants; recent advances and prospects. *Criti.Rev. Environ. Sci.Techno*. 1-38.

Yazaki,K., Arimura,G. and Ohnishi,T. (2017). Hidden terpenoids in plants: their biosynthesis, localization and ecological roles. *Plant cell Physiol*. **58**:1615-1621.

Yoon, J., Cao, X., Zhou, Q. and Ma, L.Q. (2006). Accumulation of Pb, Cu and Zn in native plants growing on a contaminated florida site. *Sci. Total Environ*. **368**:456-464.

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.*J.Pharm.Pharm.Sci*.**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.

Zelekew, O.A., Fufa, P.A., Sabir, F.K., Andoshe,D.M., Gultom,N.S., Abdellah, H., Kuo, D., Chen, X. and Devulapalli, G.K.(2022). Chromium ion accumulations from aqueous solution by the *Eichorinia crassipes* plant and reusing in the synthesis of Cr-Doped ZnO photocatalyst. *J.Nano*. 2022:4943844.

Zelko, I., Lux, A. and Czibula, K. (2008). Difference in the root structure of hyperaccumulator *Thlaspi caerulescens* and non hyperaccumulator *Thlaspi arvense*. *Int. J. Environ. Poll.*. **33**: 123-132.

Zenkin, F.K. and Munzuroglu, O. (2005). Effect of some heavy metals on content of chlorophyll, proline and some antioxidant chemicals in bean (*Phaseolus vulgaris* L.) seedlings. *Acta Biol. Cracov. Ser. Bot*. **47**:157-164.

Zhang, F.Q., Wang, Y.S., Lou, Z.P. and Dong, J.D. (2007). Effect of heavy metal stress on antioxidant enzymes and lipid peroxidation in leaves and roots of two mangrove plant seedlings (*Kandelia candel* and *Bruguiera gymnorrhiza*). *Chemosphere.* **67**:44-50.

Zhang, G.P., Fukami, H. and Sekimoto, M. (2002). Influence of cadmium on mineral concentration and yield components in wheat genotypes differing in Cd tolerance at seedling stage. *Field Crops Res*. **40**:1-7.

Zhang, W.H. and Tyerman, S.D. (1999). Inhibition of water channels by $HgCl_2$ in intact wheat root cells. *Plant Physiol*. **120**:849-857.

Zhang,j.,Wu,Y. and Zhao,X.(2014). Chemopreventive effect of flavonoids from Ougan(*Citrus reticulate* cv.suavissima) fruit against cancer cell proliferation and migration.*J.Funct.Foods*.**10**:511-519.

Zhang,X.,Zhang,F.Q.,Xia,Y., Wang,G.P. and Shen,Z.G.(2010). Excess copper induces production of hydrogen peroxide in the leaf of *Elsholtzia haichowensis* through apoplastic and symplastic Cu,Zn-superoxide dismutase. *J.Hazard.Mater*.**178**:834-843.

Zhou,Z.S., Guo,K., Elbaz,A.A. and Yang,Z.M. (2009). Salicylic acid alleviates mercury toxicity by preventing oxidative stress in roots of *Medicago sativa*. *Environ.Exp.Bot*. **65**:27-34.

Zimmels, Y. Kirzhner, F. and Roitman, S. (2004). Use of naturally growing aquatic plants for waste water purification. *Water Environ. Research Found*. **76**:220-230.

Zou, J.H., Wang, W.S., Jiang, W. and Liu, D.H. (2006). Effect of hexavalent chromium (VI) on root growth and cell division in root tip cells of *Amaranthus viridis* L. *Pak. J. Bot.* **38**:673-681.

Zurayk,R., Sukkariyah,B., Baalbaki, R. and Ghanem,A.(2001). Chromium phytoaccumulation from solution by selected hydrophytes. *Int.J. Phytorem.* **3**:335-350.