EFFECTS OF CHROMIUM (VI) OXIDE ON THE GROWTH AND ANATOMICAL STRUCTURE OF SORGHUM (*Sorghum bicolor*)

BY

Victoria Osebhahiemen EBADAN (Miss)

LSC2104143

DEPARTMENT OF PLANT BIOLOGY AND BIOTECHNOLOGY

FACULTY OF LIFE SCIENCES

UNIVERSITY OF BENIN

BENIN CITY

EDO STATE

NOVEMBER, 2025

EFFECTS OF CHROMIUM (VI) OXIDE ON THE GROWTH AND ANATOMICAL STRUCTURE OF SORGHUM (*Sorghum bicolor*)

BY

Victoria Osebhahiemen EBADAN (Miss)

LSC2104143

A PROJECT REPORT SUNMITTED TO THE DEPARTMENT OF PLANT BIOLOGY AND BIOTECHNOLOGY, IN THE FACULTY OF LIFE SCIENCES OF UNIVERSITY OF BENIN, BENIN CITY, IN PPARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCES (B.Sc.) DEGREE IN PLANT BIOLOGY AND BIOTECHNOLOGY

NOVEMBER, 2025

STUDENT'S THESIS

AUTHOR'S STATEMENT

I hereby grant the University of Benin, through the University of Benin Library, a non-exclusive, worldwide right to reproduce and distribute my thesis and abstract (hereinafter "the Work"), in whole or in part, through any media, in its present form or any translated version for preservation and accessibility, provided such translation does not alter its content. This grant is royalty-free, and I retain the right to publish the Work in its current or future versions elsewhere.

Warranties

I further affirm that:

1. I am the sole author of the Work and grant the University of Benin the right to make it available four (4) years after the award of my degree, in compliance with the University of Benin Senate regulations.
2. The Work does not contain confidential information requiring third-party consent for disclosure.
3. I have exercised due diligence to ensure that the Work is original and does not breach any Nigerian law or infringe upon any third party's copyright or other Intellectual Property Rights, to the best of my knowledge.
4. Where the Work includes copyrighted material not owned by me, I have obtained unrestricted permission from the copyright holder to grant this license to the University of Benin Library. Such third-party materials are clearly identified and acknowledged within the Work.
5. In the event of any copyright dispute concerning the Work, I agree to indemnify and hold harmless the University of Benin, its officers, employees, and agents from any liability arising from the material authorized under this agreement. 6. The University of Benin is

under no obligation whatsoever to take legal action on my behalf as the Depositor in the event of an intellectual property rights infringement or any other related dispute in the material deposited.

Victoria Osebhahiemen EBADAN	**Signature/Date**	**Email**
Author		

Prof. E. D. Vwioko	**Signature/Date**	**Email**
Supervisor		

Prof. B. Ikhajiagbe	**Signature/Date**	**Email**
Head of Department		

CERTIFICATION

This is to certify that this work was carried out Victoria Osebhahiemen EBADAN of the Department of Plant Biology and Biotechnology, in the Faculty of Life Sciences in the University of Benin, Benin City.

_____ _____

PROF. E. D. VWIOKO **DATE**

SUPERVISOR

_____ _____

PROF. B. IKHAJIAGBE **DATE**

HEAD OF DEPARTMENT

_____ _____

EXTERNAL EXAMINER **DATE**

DEDICATION

This project is dedicated first to God Almighty, whose grace, infinite love and mercy have brought me this far.

I am deeply grateful to my parents for their love and care. To Mrs. Fidelia Ebewele, Mr. Brown Ebewele, Mr. G. F. Okoyo, Mr. Jude Azobor and Mrs. Justina Aigberadion who supported my academic journey, I pray for God's unending blessings upon your lives.

I owe heartfelt heartfelt gratitude to Abasiofon T. Ita for his kindness, encouragement and steady reassurance.

ACKNOWLEDGEMENTS

I give all glory and honour to Almighty God for His grace, wisdom and strength throughout the course of this project report and my entire academic journey.

My sincere appreciation goes to my supervisor, Prof. D.E Vwioko for his invaluable patience and fatherly direction that brought this work to completion. I am equally grateful to all the lecturers and staff of the Department of Plant Biology and Biotechnology, University of Benin, for their dedication and the knowledge imparted during my years of study.

I deeply appreciate my parents, uncles and aunts for their care and generosity.

Words fail me to convey the depth of my gratitude to Abasiofon T. Ita and Toby O. Dan-Okoro whose constant kindness, love and encouragement helped me stay grounded until the end.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF PLATES

ABSTRACT

Soil contamination by heavy metals especially chromium (vi) oxide is a major environmental concern due to its toxicity and persistence in agricultural soils. This study investigated the effect of chromium (VI) oxide on the growth and anatomy of *Sorghum bicolor* to assess its tolerance and sensitivity. Sorghum seeds were subjected to five concentrations of chromium (VI) oxide (0, 30, 50, 70, and 100 ppm) in soil, under a completely randomized design (CRD) with four replicates per treatment. Growth parameters, including germination percentage, plant height, stem girth and number of leaves, were studied and recorded up to six weeks after planting (WAP). Anatomical analyses of root and stem transverse sections were performed to assess internal tissue responses.

Results showed a concentration specific decline in all growth parameters, with the control plants exhibiting the highest mean values for germination percentage (20.00%), plant height (59.50cm) and stem girth (0.84cm) and the least values for germination percentage (10.75%), plant height (36.00cm) and stem girth (0.76) recorded for plants grown in 100 ppm Cr^{6+} treatment. Vegetative growth parameters decreased with increasing chromium concentration, indicating growth suppression. Anatomical observations revealed darkening of the epiblema and loss of cortical cells in the root of plants as well as deposition of crystal-like substance is observed in the cortex of the stem of plants exposed to higher chromium levels. These alterations indicate that chromium induces oxidative stress and structural injury, disrupting normal cell function and secondary growth. The findings support that chromium (VI) exerts significant inhibitory effects on both morphological and anatomical development of *Pennisetum glaucum*, emphasizing the detrimental impact of chromium contamination on crop productivity.

Keywords: Chromium (VI) oxide, *Vigna unguiculata*, germination, vegetative growth, anatomy.

CHAPTER ONE

INTRODUCTION/LITRATURE REVIEW

1.1 CHROMIUM AS A HEAVY METAL

Heavy metals are naturally occurring elements with atomic densities greater than 5 g cm^{-3} and are found everywhere in the earth's crust, atmosphere, and water bodies (Anoliefo et al., 2006). They are released into the environment through both natural processes such as volcanic activity and weathering of rocks and anthropogenic activities including mining, electroplating, industrial waste disposal and agricultural practices. Among these metals, chromium (Cr) has gained considerable attention due to its wide industrial use and potential toxicity to living organisms. Chromium exists in multiple oxidation states ranging from Cr^{2+} to Cr^{6+}, but the trivalent [Cr(III)] and hexavalent [Cr(VI)] forms are the most stable and environmentally significant (Kotaś & Stasicka, 2000).

Cr(III) is relatively immobile and less toxic and in contrast, Cr(VI) compounds such as chromium(VI) oxide (CrO_3) which was the chromium source in this study, are highly soluble, reactive and capable of penetrating biological membranes. These properties make Cr(VI) a potent contaminant in soils and water bodies near industrial regions. Chromium(VI) is widely used in metal finishing, leather tanning, dye production and pigment formulation. In Nigeria, poor industrial waste management and inadequate treatment of effluents have led to the accumulation of chromium and other heavy metals in agricultural soils (Vwioko et al., 2006).

Once introduced into the environment, chromium undergoes complex chemical transformations. Depending on pH, redox potential and organic matter content, Cr(VI) can be reduced to Cr(III), while Cr(III) may be oxidized back to Cr(VI) under certain conditions. This cyclical transformation contributes to its persistence in the ecosystem (Panda & Choudhury, 2005). In soil systems, chromium interacts with clay minerals, humic substances and oxides of iron and

manganese, which influence its mobility and availability to plants. High concentrations may lead to bioaccumulation and toxicity, affecting both terrestrial and aquatic life forms.

In the Nigerian context, studies conducted by Prof. D.E Vwioko and colleagues at the University of Benin revealed significant levels of chromium in soil samples collected around automobile workshops, petroleum filling stations and dumpsites within Benin City (Vwioko et al., 2008). Such findings indicate that chromium contamination has local environmental implications that may extend to food crops grown in these areas. The toxicity of chromium in plants, therefore, poses not only an ecological hazard but also a potential risk to food safety and human health.

Chromium's dual nature as a necessary industrial element and a persistent environmental pollutant makes it an important focus of agricultural and environmental research. Understanding its behavior in soil and plant systems provides critical insight into how contamination affects plant physiology, crop productivity and ecosystem balance.

1.2 SOURCES OF CHROMIUM

Chromium enters the soil through both natural and man-made processes. Naturally, weathering of chromite-bearing rocks and volcanic activity release chromium compounds into the soil and water systems. However, in most agricultural regions, especially in developing countries such as Nigeria, anthropogenic inputs far exceed natural sources (Orhue & Uzu, 2009). Industrial effluents from metal plating, textile dyeing, tanneries and chemical manufacturing are major contributors. Additional sources include municipal sewage sludge, fly ash from coal combustion and the application of chromium-based fertilizers and pesticides (Shanker et al., 2005). Once released, chromium tends to bind strongly to soil particles, particularly in soils rich in clay and organic matter.

The accumulation of chromium in soil depends largely on soil pH, redox conditions, organic carbon content and the presence of other ions. Under oxidizing conditions and in alkaline soils,

Cr(VI) forms soluble chromate (CrO_4^{2-}) and dichromate ($Cr_2O_7^{2-}$) ions, which are highly mobile and bioavailable. In contrast, Cr(III) forms insoluble hydroxides and oxides, reducing its mobility and uptake by plants (Kotaś & Stasicka, 2000). The conversion between these two oxidation states is dynamic; microbial activity and organic matter can reduce toxic Cr(VI) to the less harmful Cr(III), while manganese oxides may catalyze the reverse oxidation, therefore maintaining chromium's persistence in the environment.

In the University of Benin and surrounding industrial areas, studies have shown that poor disposal of industrial waste contributes significantly to elevated chromium levels in surface soils. Vwioko et al. (2008) and Okolo et al. (2018) reported that soils collected near auto-mechanic workshops and dumpsites in Benin City contained detectable levels of chromium capable of inducing toxic responses in plants such as Amaranthus hybridus and Chromolaena odorata. Similar studies by Orhue and Uzu (2009) also demonstrated that heavy metals, including chromium, accumulate more readily in acidic soils due to higher solubility and increased availability to plant roots.

Once absorbed by plants, chromium accumulates primarily in the root tissues. This is because the Casparian strip in the endodermis restricts its movement to the xylem, thereby limiting translocation to the shoots (Shanker et al., 2005). However, at higher concentrations, chromium may overcome this barrier and be transported to shoots of plants. Within the plant, chromium binds with carboxyl and phosphate groups in the cell wall and vacuoles, disrupting ion balance and causing oxidative stress.

The extent of chromium accumulation in soils and plants is influenced by environmental conditions, soil chemistry and chromium speciation. Its persistence in contaminated soils poses a long-term threat to plant growth, food safety and ecological health. Understanding the mechanisms of chromium accumulation is therefore essential for developing effective remediation strategies and sustainable agricultural practices.

1.3 EFFECTS OF CHROMIUM ON PLANTS

Chromium is widely recognized as a non-essential and potentially phytotoxic element that adversely affects plant growth and metabolism. When present in elevated concentrations, chromium induces physiological and biochemical disruptions, beginning with seed germination. Chromium toxicity often inhibits germination by interfering with water uptake and enzymatic activities essential for embryo growth (Shanker et al., 2005). Once absorbed, chromium interferes with nutrient transport, photosynthesis and hormonal regulation, thereby reducing overall plant vigor.

The toxic effects of chromium on plants are largely dependent on its valence state. Cr(VI) compounds such as chromium (VI) oxide (CrO_3) are highly soluble and mobile in soil, making them more bioavailable and therefore more toxic than Cr(III) forms (Kotaś & Stasicka, 2000). Chromium stress leads to chlorosis and necrosis of leaves, stunted growth, and decreased biomass accumulation. This is primarily due to inhibition of chlorophyll synthesis and disruption of the photosynthetic apparatus, especially the thylakoid membranes (Vwioko et al., 2008).

At the cellular level, chromium induces oxidative stress through the excessive generation of reactive oxygen species (ROS) such as superoxide radicals and hydrogen peroxide. These molecules damage lipids, proteins, and nucleic acids, disrupting membrane structure and the activity of enzymes (Gill & Tuteja, 2011). Anatomical studies by Vwioko et al. (2017) showed that chromium exposure in Amaranthus hybridus and Chromolaena odorata caused disorganization of the vascular bundles, thickened epidermal walls and reduced diameter in xylem vessels. Such structural distortions are direct indicators of metal-induced stress in plant tissues.

Furthermore, chromium affects mineral nutrition by competing with essential elements such as iron, magnesium and phosphorus for uptake sites. This imbalance results in nutrient deficiencies and poor plant performance (Orhue & Uzu, 2009). Long-term chromium accumulation in

agricultural soils also reduces microbial activity and enzyme-mediated soil fertility, indirectly affecting crop productivity. These cumulative effects highlight the importance of monitoring chromium levels in the environment, especially in regions near industrial and waste disposal sites.

Table 1.1: Effects of Chromium on Plants

Symptoms/Effects	**References (from Researchers and Review Articles)**
Oxidative stress and membrane damage	Shanker et al. (2005)
Stunted growth and reduced leaf production	Ghani (2011); Panda and Choudhury (2005)
Reduced transpiration and photosynthetic rate	Panda and Choudhury (2005)
Chlorosis and reduction in chlorophyll content	Ghani (2011); Panda and Choudhury (2005)
Enzyme inhibition and altered metabolism	Shanker et al. (2005); Gill and Tuteja (2011)
Impaired nitrate and nutrient uptake	Gill and Tuteja (2011); Panda and Choudhury (2005)
Cell wall thickening and vascular distortion	Shanker et al. (2005); Panda and Choudhury (2005)
Cell death and tissue necrosis	Shanker et al. (2005)

1.4 INTRODUCTION TO SORGHUM BICOLOR

Sorghum bicolor (L.) Moench is a cereal crop belonging to the family Poaceae, widely cultivated in tropical and subtropical regions. It is valued for its remarkable drought tolerance and its ability to thrive in nutrient-poor soils where other cereals often fail. The crop ranks among the five most important cereals globally, coming fifth after wheat, rice, maize and barley (ICRISAT, 2021). Sorghum serves as a major source of food, livestock feed and industrial raw material for brewing and bioethanol production (FAO, 2020).

In Nigeria, *Sorghum bicolor* plays a vital role in food security, particularly in the northern and middle-belt regions where it forms a major part of the staple diet. Nigeria accounts for approximately 40% of Africa's total sorghum output, with production exceeding 6 million tonnes annually (FAOSTAT, 2022). *Sorghum bicolor* adapts well to sandy loam soils and it is resilient to drought and high temperatures, making it suitable for cultivation in areas with inconsistent rainfall patterns (Ogedegbe and Edeki, 2019; Department of Plant Biology and Biotechnology, UNIBEN, 2021).

However, increasing environmental pollution caused by industrial discharge, automobile emissions, and agricultural runoff has introduced heavy metals, including chromium into arable soils. Chromium is a non-essential element that becomes toxic to plants when present at elevated concentrations, interfering with seed germination, nutrient uptake and photosynthetic efficiency (Adewuyi et al., 2021). Exposure to chromium compounds such as chromium (VI) oxide (CrO_3) has been shown to induce oxidative stress, structural deformities and reduced biomass accumulation in several crops (Vwioko et al., 2008; Okolo et al., 2018).

Given the agricultural and nutritional significance of *Sorghum bicolor*, evaluating its physiological and anatomical responses to chromium exposure is critical. This study investigates the effects of chromium (VI) oxide on the growth and internal structure of *Sorghum bicolor*,

providing insight into its tolerance capacity and the potential risks posed by chromium contamination on sorghum productivity in Nigeria.

Table 1.2 below presents the estimated area under cultivation, total production, and productivity of *Sorghum bicolor* in major producing countries across the world as of 2022. The table highlights the global importance of the crop, showing that Nigeria ranks among the leading producers in both cultivation area and output, underscoring its role in food security and economic sustainability.

Table 1.2: Area, Production, and Productivity of Sorghum in the World (2022)

Country	**Area (ha)**	**Production (million tonnes)**	**Productivity (t ha^{-1})**
India	5,600,000	4.5	0.80
Nigeria	5,100,000	6.1	1.20
United States	2,100,000	9.5	4.50
Ethiopia	1,900,000	5.0	2.63
Sudan	7,000,000	5.3	0.76
China	550,000	3.9	7.10
Argentina	950,000	4.0	4.21
Others	21,900,000	14.7	0.67

Source: FAOSTAT (2022)

1.5 BOTANY OF *SORGHUM BICOLOR*

Sorghum bicolor is a C_4 photosynthetic plant, meaning it utilizes the Hatch–Slack pathway for efficient carbon fixation under high temperature and light conditions (Doggett, 1988). Its stem is solid and filled with pith, which helps in water retention during drought. The root system is adventitious and deep, aiding in moisture absorption from subsurface layers. The leaves are linear-lanceolate, with parallel venation and a waxy coating that minimizes water loss. The flowers are bisexual and borne on a terminal panicle, while the seeds are small and enclosed by glumes.

Sorghum exhibits significant genetic diversity with numerous varieties cultivated for food, forage, or industrial use. In Nigeria, both red and white grain types are common and their distribution is often influenced by local climatic conditions. The plant's morphological adaptability is one of its key advantages in diverse ecological zones (FAO, 2020).

1.6 TAXONOMY OF SORGHUM BICOLOR

Kingdom: Plantae

Division: Magnoliophyta

Class: Liliopsida

Order: Poales

Family: Poaceae

Genus: Sorghum

Species: *Sorghum bicolor* (L.) Moench

1.7 PLANTING TEMPERATURE

The optimal temperature for the growth of *Sorghum bicolor* ranges between 25°C and 35°C, although it can tolerate higher temperatures during dry seasons (FAO, 2020). It grows best in well-

drained loamy soils with a pH between 6.0 and 7.5. While this crop is adapted to tropical climates, its productivity declines in waterlogged or saline soils.

1.8 PESTS

Sorghum is affected by a variety of insect pests including stem borers (*Busseola fusca)*, shoot flies (*Atherigona soccata*), and aphids (*Melanaphis sacchari*). These pests cause damage to the stem, leaves and panicles, resulting in reduced grain yield and plant vigor. However, in this study, no pest infestation was observed during the experimental period.

1.9 BENEFITS OF *SORGHUM BICOLOR*

Sorghum is a multi-purpose crop of immense nutritional, industrial, and ecological value. It serves as a staple food in many parts of Africa and Asia, providing energy and protein for millions of people. The grains are rich in carbohydrates, moderate in protein and contain essential minerals such as iron, calcium and phosphorus. Sorghum is also gluten-free, making it suitable for people with celiac disease (FAO, 2020).

In addition to its food value, sorghum plays a significant role in livestock feeding and soil conservation. The crop residues are used as fodder, while its deep roots help in soil stabilization and erosion control. Industrially, sorghum is used in brewing, ethanol production and as a base material for bio-degradable plastics. The plant's resilience and versatility make it an economically valuable crop for both rural and commercial farming systems.

1.10 CULTIVATION AND PLANTING OF *SORGHUM BICOLOR*

Sorghum thrives in tropical and semi-arid regions where rainfall ranges from 400–1000 mm per year. The soil should be moderately fertile, well-drained, and preferably sandy-loam. Before planting, the soil should be cleared of weeds and ploughed to allow aeration. Sorghum seeds are sown directly in the field or in pots at depths of 2–3 cm. Germination usually occurs within 3–5 days, depending on soil moisture.

Weeding is essential during the early growth stages to reduce competition for nutrients and light. Fertilization may involve the use of nitrogen, phosphorus and potassium in appropriate ratios to enhance growth and yield. Sorghum can be intercropped with legumes to improve soil fertility and minimize pest infestation.

1.11 GROWING GUIDELINES AND PROBLEMS

Successful cultivation of *Sorghum bicolor* requires adherence to appropriate agronomic practices such as seed selection, planting depth, spacing and soil management. Highly viable seeds are preferred and spacing of 25–30 cm between plants and 60–75 cm between rows ensures adequate light penetration and airflow. Adequate weeding and pest control enhance productivity.

Common challenges in sorghum cultivation include weed competition, nutrient depletion and irregular rainfall. In some cases, water stress during flowering significantly reduces yield. Chromium contamination, as studied in this project, is another emerging problem that can hinder nutrient uptake and disrupt photosynthetic efficiency. Addressing these constraints through improved soil management and pollution control measures is vital for maintaining productivity.

1.12 JUSTIFICATION OF STUDY

Environmental pollution by heavy metals poses a significant challenge to sustainable agriculture, particularly in developing nations like Nigeria where industrial effluents are often discharged without adequate treatment. Chromium contamination is of particular concern due to its persistence and high toxicity. The University of Benin's environs, including nearby automobile and industrial areas, are known hotspots for chromium accumulation in soil (Vwioko et al., 2008). Investigating the physiological and anatomical effects of chromium on Sorghum bicolor is therefore relevant for understanding how such contamination may affect staple crop production and food safety.

Moreover, *Sorghum bicolor* is gaining increasing attention for its potential use in phytoremediation, which is the use of plants to extract and stabilize heavy metals from contaminated soils. By studying its response to chromium stress, this research contributes to both agricultural and environmental management goals. It provides data that can guide future remediation strategies and inform soil management practices in polluted areas.

1.13 AIM AND OBJECTIVES

The aim of this study is to investigate the effects of chromium (VI) oxide (CrO_3) on the growth and anatomical structure of Sorghum bicolor (L.) Moench, with emphasis on its influence on germination rate, plant height, stem girth, and leaf count.

Objectives:

The objectives of the study were:

i. To determine the effect of different concentrations of Cr^{6+} *Sorghum bicolor* germination.
ii. To assess the impact of chromium stress on growth parameters such as plant height, number of leaves and stem girth.
iii. To determine the anatomical structure of the stem and root cross sections of *Sorghum bicolor* grown in chromium treated soils.

CHAPTER TWO

MATERIALS AND METHODS

2.1 PLANT MATERIALS

The plant material used for this study was *Sorghum bicolor* (L.) Moench. The seeds were purchased from a farmer along Mission Road, Benin City, Edo State, Nigeria. The chemical material used for treatment was chromium (VI) oxide (CrO_3) purchased from a dealer, Pyrex-IG, along First East Circular Road, Benin City, Edo State, Nigeria.

2.2 STUDY AREA

The study was conducted in an open space beside the African Center for Mushroom Research and Technology (ACMRTI), University of Benin, Benin City, Edo State, Nigeria, with coordinate latitude 6.40288°N and longitude 5.60919°E.

2.3 COLLECTION AND PREPARATION OF SOIL

Topsoil was collected from a fallow land located beside the UNIBEN Microfinance Bank, Ugbowo Campus, Benin City, Edo State, using a spade. Care was taken to ensure that stones and debris were removed from soil.

Twenty (20) plastic bowls were used as experimental pots for this study. Each bowl was perforated at the base using a soldering iron to allow for adequate drainage of excess water. Using a weighing scale, 5kg of the top soil was measured into each bowl. The bowls were neatly arranged in rows and labelled with paper tape according to their respective treatment concentrations: 0 ppm Cr, 30 ppm Cr, 50 ppm Cr, 70 ppm Cr, and 100 ppm Cr with four replicates for each treatment, making a total of twenty pots.

2.4 EXPERIMENTAL DESIGN

The experiment was laid out in a completely randomized design (CRD) with five treatment levels of chromium (0, 30, 50, 70, and 100 ppm) and four replicates per treatment, making a total of 20 experimental units. Each treatment was randomly assigned to the experimental pots to minimize bias and ensure that differences in plant response could be attributed solely to the chromium concentrations.

2.5 PREPARATION OF CHROMIUM (VI) OXIDE TREATMENT

Chromium (VI) oxide (CrO_3) was used as the chromium source for this experiment. The following concentrations of 0 ppm, 30 ppm, 50 ppm, 70 ppm, and 100 ppm were prepared by dissolving the calculated amounts of CrO_3 in 3.6 L of deionized water for each treatment level.

2.6 CALCULATION OF CHROMIUM CONCENTRATIONS

A concentration of 1 ppm is equivalent to 1 mg of solute per litre (mg/L) of solution.

Since each treatment required 3.6 L of solution, the corresponding quantities of chromium were calculated as:

T_0 (Control): 0 ppm (deionized water only, with no chromium dissolved in it)

T_1: 30 ppm Cr (0.108g CrO_3 dissolved in 3.6L of deionized water)

T_2: 50 ppm Cr (0.18g CrO_3dissolved in 3.6L of deionized water)

T_3: 70 ppm Cr (0.252g CrO_3 dissolved in 3.6L of deionized water)

T_4: 100 ppm Cr (0.36g CrO_3 dissolved in 3.6L of deionized water)

2.7 SAFETY MEASURES

Chromium (VI) oxide (CrO_3) is highly toxic and carcinogenic. All handling was performed using protective gloves, lab coat, and nose masks. Treatment solutions were prepared in a laboratory

and any spills were cleaned immediately following standard laboratory safety protocols. Used materials and leftover solutions were disposed of properly.

2.8 VIABILITY TEST

Sorghum bicolor seeds were tested for viability using the simple water floatation technique. Seeds were placed in a bowl of clean water for 5-7 minutes. The seeds that sank to the bottom of the beaker were taken as viable and selected for planting.

2.9 PLANTING OF *SORGHUM BICOLOR* SEEDS

Twenty (20) viable seeds were planted per bowl at a depth of 2cm. Bowls were labelled by treatment (T_0–T_4) and arranged in the experimental area. Planting was done in the morning hours. After planting, the bowls were watered using the Cr^{6+} solution.

2.10 APPLICATION OF TREATMENTS

Immediately after planting, 200 mL of the appropriate treatment solution was applied to each bowl. Subsequent applications of the treatment solutions were carried out every four days to maintain consistent exposure levels. A total of four treatment applications were made within the 15-day experimental period.

2.11 GERMINATION

Seeds germinated after two days after planting. The emergence of seedlings was recorded in each pot, and a seed was considered germinated with the appearance of the radicle above the soil surface.

2.12 WEEDING

Manual weeding was carried out to remove unwanted plants that emerged within and around the experimental pots.

2.13 DATA COLLECTION

2.14 PARAMETER MEASURED

The following growth parameters were measured and recorded:

- Germination percentage (%)
- Plant height (cm)
- Number of leaves
- Stem girth (cm)

2.15 GERMINATION PERCENTAGE

Germination percentage was estimated using the formula given below;

$$\text{Germination Percentage} = \frac{\textit{No. of Germinated seeds}}{\textit{No. of seeds sown}} \times \frac{100}{1}$$

2.16 PLANT HEIGHT

Plant height was measured to assess the growth response of *Sorghum bicolor* to different Chromium (VI) oxide concentrations. Measurements were taken from the base of the stem at the soil surface to the top of the longest plant using a ruler. This was carried out hi-weekly.

2.17 STEM GIRTH

Stem girth was measured to evaluate the effect of Chromium (VI) oxide on the thickness of *Sorghum bicolor* seedlings. Measurements were taken using a piece of thread wound around the base of the stem and the length was determined on a ruler. This was carried out hi-weekly.

2.18 NUMBER OF LEAVES

Fully expanded leaves on each plants were counted to assess the effect of Chromium (VI) oxide on leaf development. This was carried out bi-weekly.

2.19 ANATOMICAL ANALYSIS OF PLANT STEM AND ROOT

Anatomical examination of the plant stem and root tissues was carried out in a Histopathology Laboratory located at Anatomy Department, Faculty of Basic Medical Sciences, University of Benin to observe the anatomical effects of chromium (vi) oxide treatments.

2.20 HARVESTING OF PLANT SAMPLES

One pot from each treatment was selected for harvesting the plant's stem and root. The excised plant parts were placed in labeled sample bottles containing 70% ethanol as a fixative, with labels indicating the corresponding treatment and plant part.

2.21 PREPARATION OF FIXATIVE

A 70% ethanol solution was prepared using distilled water and used as the fixative for the plant samples designated for anatomical analysis.

2.22 ANATOMICAL SECTIONING AND SLIDE PREPARATION

Stem and root samples were analyzed in the histopathology laboratory, and were processed for anatomical studies using standard histological techniques.

1. **Fixation:** Fresh stem and root segments (approximately 1–2 cm long) from each treatment were immediately placed in sample bottles containing 70% ethanol prepared with distilled water. This solution served as a fixative to prevent tissue degradation and preserve plant structure for subsequent analysis.
2. **Dehydration:** The fixed samples were passed through a graded ethanol series of 70%, 90%, and absolute ethanol, with each stage lasting 10 - 15 minutes, to ensure gradual removal of water from the tissues.

3. **Clearing:** The dehydrated samples were immersed in xylene for 30–45 minutes to replace the ethanol and make the tissues transparent, thereby facilitating proper infiltration of the embedding medium.

4. **Embedding:** The cleared samples were infiltrated and embedded in molten paraffin wax with a melting point of 56–58 °C. During embedding, the specimens were properly oriented in embedding molds containing melted wax and allowed to solidify at room temperature.

5. **Sectioning:** Thin transverse sections (8–15 µm) of the stem and root tissues were obtained from the paraffin blocks using a rotary microtome. The section ribbons were floated on warm water at 40 °C to flatten them and subsequently mounted on clean glass slides coated with Mayer's egg albumin, which served as an adhesive.

6. **De-waxing and Rehydration:** The mounted slides were deparaffinized by immersing them in xylene, after which they were rehydrated through a descending ethanol series (absolute, 90%, 70%, and 50%) and finally rinsed in distilled water.

7. **Staining:** The tissue sections were stained with safranin O to identify lignified cell walls and counterstained with fast green to visualize non-lignified tissues. Excess stain was carefully removed by gentle rinsing with ethanol.

8. **Dehydration and Clearing:** The stained sections were dehydrated through an ascending ethanol series and subsequently cleared in xylene to eliminate residual moisture, enhance tissue transparency, and prepare the samples for permanent mounting.

9. **Mounting:** Each section was mounted using a drop of DPX mountant, covered with a coverslip, and allowed to air-dry to obtain permanent microscopic slides.

10. **Microscopic Observation:** The prepared slides were examined and photographed under a light microscope using x4, ×10 and ×40 objective lenses for the anatomical assessment of stem and root tissues.

2.23 STATISTICAL ANALYSIS

The data obtained from the experiment were subjected to statistical analysis using excel and organized into mean ± standard deviation

CHAPTER THREE

RESULTS

The study investigates the effect of Chromium (vi) Oxide on the growth and anatomical structure of *Sorghum bicolor* in pot experiments. The results obtained from this study are shown in Table 3.1 – Table 3.4 and Plate 3.1 – 3.10 respectively.

The parameters studied include germination percentage, plant height, stem girth and number of leaves at different chromium concentrations (0 ppm, 30 ppm, 50 ppm, 70 ppm and 100ppm). Anatomical observations of the stem and root sections were also conducted to assess internal structural changes caused by chromium stress.

Table 3.1 shows the result obtained for seed germination of *Sorghum bicolor* in soils treated with chromium (vi) oxide. The highest percentage germination (20.00%) was observed in 0 ppm 15 days after planting (DAP), while least percentage germination (10.75%) was observed in 100 ppm 15 DAP. The results revealed that chromium (vi) oxide has a concentration-specific effect on the germination of *Sorghum bicolor;* also the mean value for 30 ppm, 50 ppm and 70 ppm was lower than control (0 ppm).

Table 3.1: Percent germination (%) of seeds of *Sorghum bicolor* sown in different concentration of chromium (vi) oxide (control (0 ppm), 30 ppm, 50 ppm, 70 ppm and 100ppm) treated soils.

	Percent germination (%)				
Conc. in soil	**3 DAP**	**6 DAP**	**9 DAP**	**12 DAP**	**15 DAP**
0 ppm	**12.50 ± 0.65**	**16.75 ± 0.48**	**19.00 ± 0.41**	**19.75 ± 0.25**	**20.00 ± 0.00**
30 ppm	**13.75 ± 0.48**	**15.50 ± 0.87**	**17.00 ± 0.58**	**18.25 ± 0.48**	**18.75 ± 0.48**
50 ppm	**12.50 ± 1.04**	**14.25 ± 0.48**	**15.25 ± 0.48**	**16.50 ± 0.65**	**17.25 ± 0.48**
70 ppm	**10.25 ± 0.85**	**11.00 ± 0.71**	**15.25 ± 0.48**	**12.75 ± 0.63**	**17.25 ± 0.48**
100 ppm	**10.50 ± 1.04**	**11.25 ± 0.85**	**12.50 ± 1.04**	**12.75 ± 1.03**	**10.75 ± 0.48**

DAP = Days after planting, Figure = mean ± S.D.

Table 3.2 shows plant height values obtained for *Sorghum bicolor* plants grown in different concentration of chromium (vi) oxide treated soils six weeks after planting. The highest mean plant height (59.50cm) was recorded in 0 ppm six weeks after planting, while the least mean plant height (36.00cm) was observed in 100 ppm six weeks after planting. In all, the control performed better than the plants grown in treated chromium soils.

Table 3.2: Plant height values obtained for *Sorghum bicolor* plants grown in different concentration of chromium (vi) oxide treated soils six weeks after planting.

	Plant height (cm)		
Conc. in soil	**2 WAP**	**4 WAP**	**6 WAP**
0 ppm	**21.50 ± 1.13**	**40.50 ± 1.32**	**59.50 ± 1.29**
30 ppm	**16.80 ± 1.63**	**39.00 ± 1.00**	**47.75 ± 3.42**
50 ppm	**16.80 ± 1.63**	**35.00 ± 2.29**	**41.50 ± 2.17**
70 ppm	**16.70 ± 4.11**	**33.25 ± 1.97**	**37.25 ± 1.92**
100 ppm	**16.30 ± 3.08**	**32.00 ± 2.38**	**36.00 ± 2.45**

WAP = Weeks after planting, Figure = mean ± S.D.

Table 3.3 shows stem girth values obtained for *Sorghum bicolor* plants grown in different concentration of chromium (vi) oxide treated soils six weeks after planting. The highest mean stem girth (0.84cm) was recorded in 0 ppm six weeks after planting, while the least mean stem girth (0.76cm) was recorded in 100 ppm six weeks after planting. In all, the control performed better than the plants grown in treated chromium soils.

Table 3.3: Stem girth values obtained for *Sorghum bicolor* plants grown in different concentration of chromium (vi) oxide treated soils six weeks after planting.

	Stem girth (cm)		
Conc. in soil	**2 WAP**	**4 WAP**	**6 WAP**
0	**0.80 ± 0.01**	**0.82 ± 0.01**	**0.84 ± 0.01**
30	**0.79 ± 0.01**	**0.80 ± 0.01**	**0.81 ± 0.01**
50	**0.78 ± 0.01**	**0.78 ± 0.01**	**0.78 ± 0.01**
70	**0.76 ± 0.01**	**0.76 ± 0.01**	**0.77 ± 0.01**
100	**0.75 ± 0.01**	**0.74 ± 0.01**	**0.76 ± 0.01**

WAP = Weeks after planting, Figure = mean ± S.D.

Table 3.4 shows number of leaves produced per plant of *Sorghum bicolor* grown in different concentration of chromium (vi) oxide treated soils six weeks after planting (WAP). The highest mean value for number of leaves produced per plant (12.00) was recorded in 0 ppm six weeks after planting, while the least mean value for number of leaves produced per plant (8.50) was observed in 100 ppm six weeks after planting. In all, the control produced more leaves than any other plants grown in treated chromium soils.

Table 3.4: Number of leaves produced per plant of *Sorghum bicolor* grown in different concentration of chromium (vi) oxide treated soils six weeks after planting.

	Number of leaves		
Conc. in soil	**2 WAP**	**4 WAP**	**6 WAP**
0 ppm	**4.50 ± 0.29**	**9.25 ± 0.48**	**12.00 ± 0.41**
30 ppm	**4.25 ± 0.25**	**8.75 ± 0.48**	**10.75 ± 0.63**
50 ppm	**4.00 ± 0.41**	**8.00 ± 0.41**	**10.00 ± 0.41**
70 ppm	**3.75 ± 0.25**	**7.50 ± 0.29**	**9.25 ± 0.48**
100 ppm	**3.50 ± 0.29**	**7.00 ± 0.41**	**8.50 ± 0.29**

WAP = Weeks after planting, Figure = mean ± S.D.

Plates 3.1 to 3.5 show different magnifications of the transverse section of the root of *Sorghum bicolor* plant grown in varying concentrations of chromium (VI) oxide treated soils (0 ppm, 30 ppm, 50 ppm, 70 ppm and 100ppm). In the root transverse sections of treated *Sorghum bicolor* plants studied, the epiblema layer and cortical layer showed some observable changes with increasing chromium concentration (Plates 3.1 to 3.5). Darkening of the epiblema layer and loss of some cortical cells was observed in higher concentrations, the cortex maintained its shape, with no significant change in the vascular bundles.

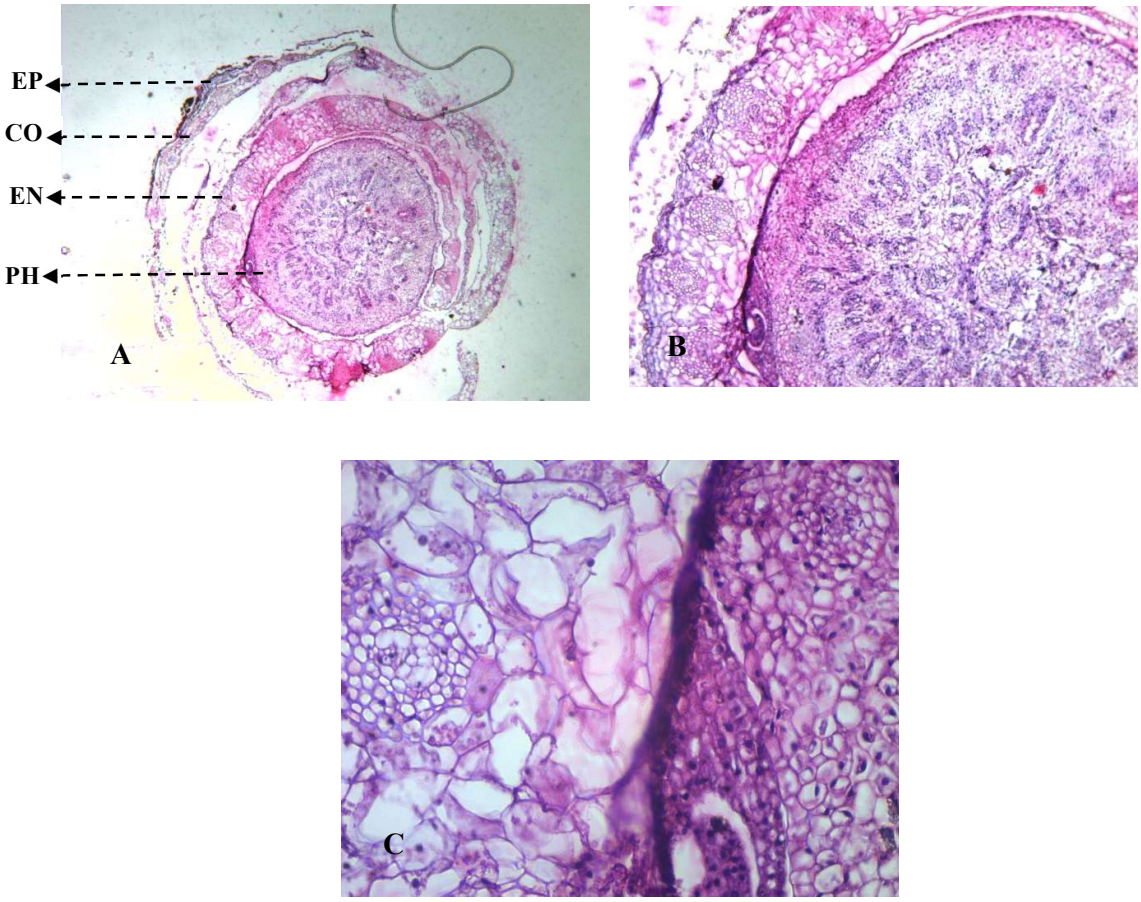

Plate 3.1: Different magnifications of the transverse section of the root of *Sorghum bicolor* plant grown in control (0 ppm) soil. (A = x40, B = x100, C = x400). **Keys:** EP: Epiblema, CO: Cortex, EN: Endodermis, PH: Phloem

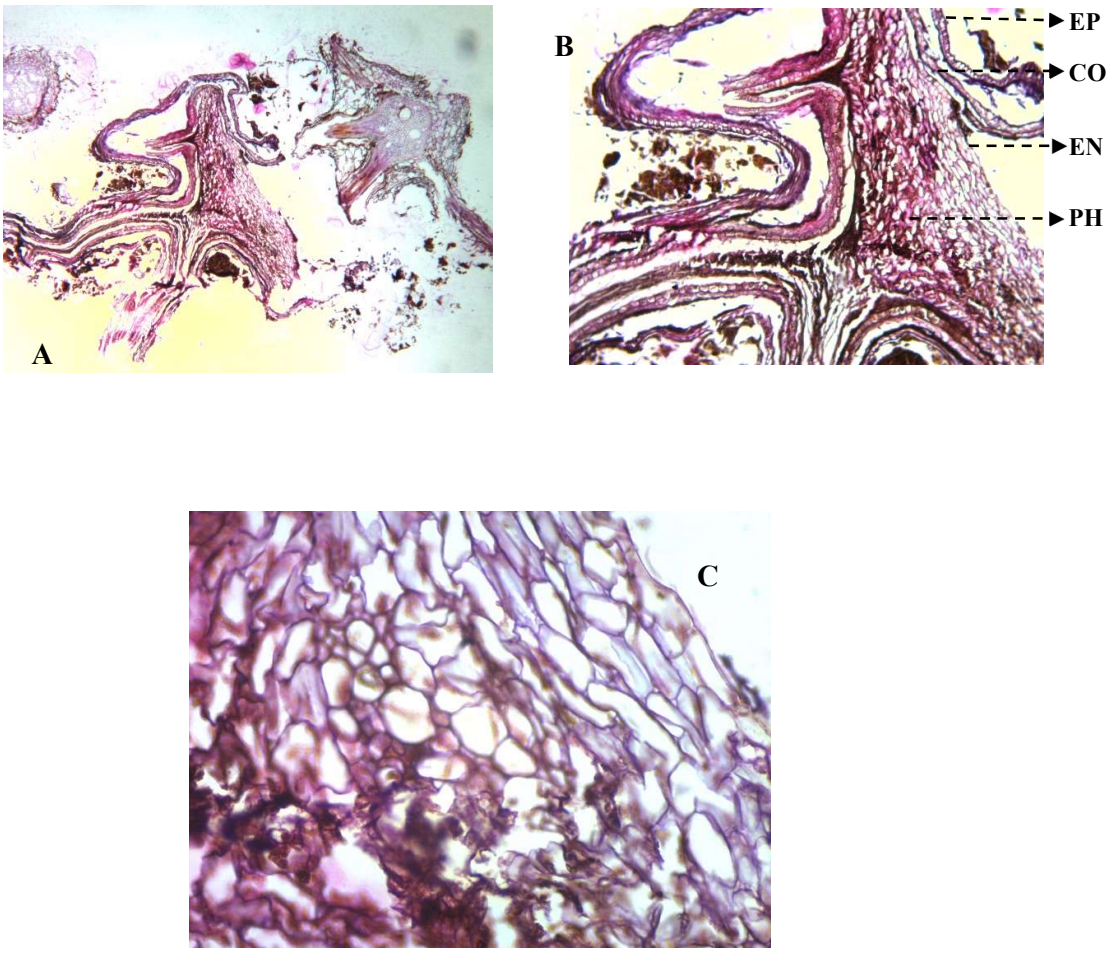

Plate 3.2: Different magnifications of the transverse section of the root of *Sorghum bicolor* plant grown in 30 ppm Cr soil. (A = x40, B = x100, C = x400). **Keys:** EP: Epiblema, CO: Cortex, EN: Endodermis, PH: Phloem

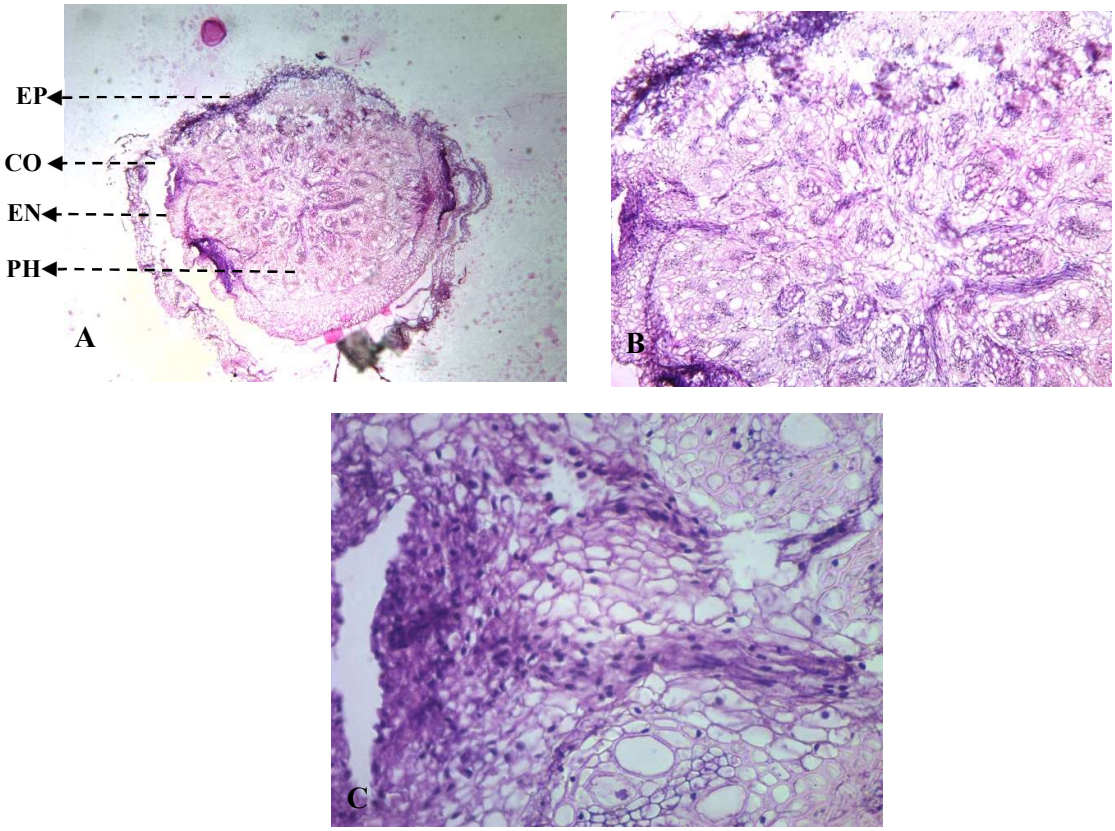

Plate 3.3: Different magnifications of the transverse section of the root of *Sorghum bicolor* plant grown in 50 ppm Cr soil. (A = x40, B = x100, C = x400). **Keys:** EP: Epiblema, CO: Cortex, EN: Endodermis, PH: Phloem

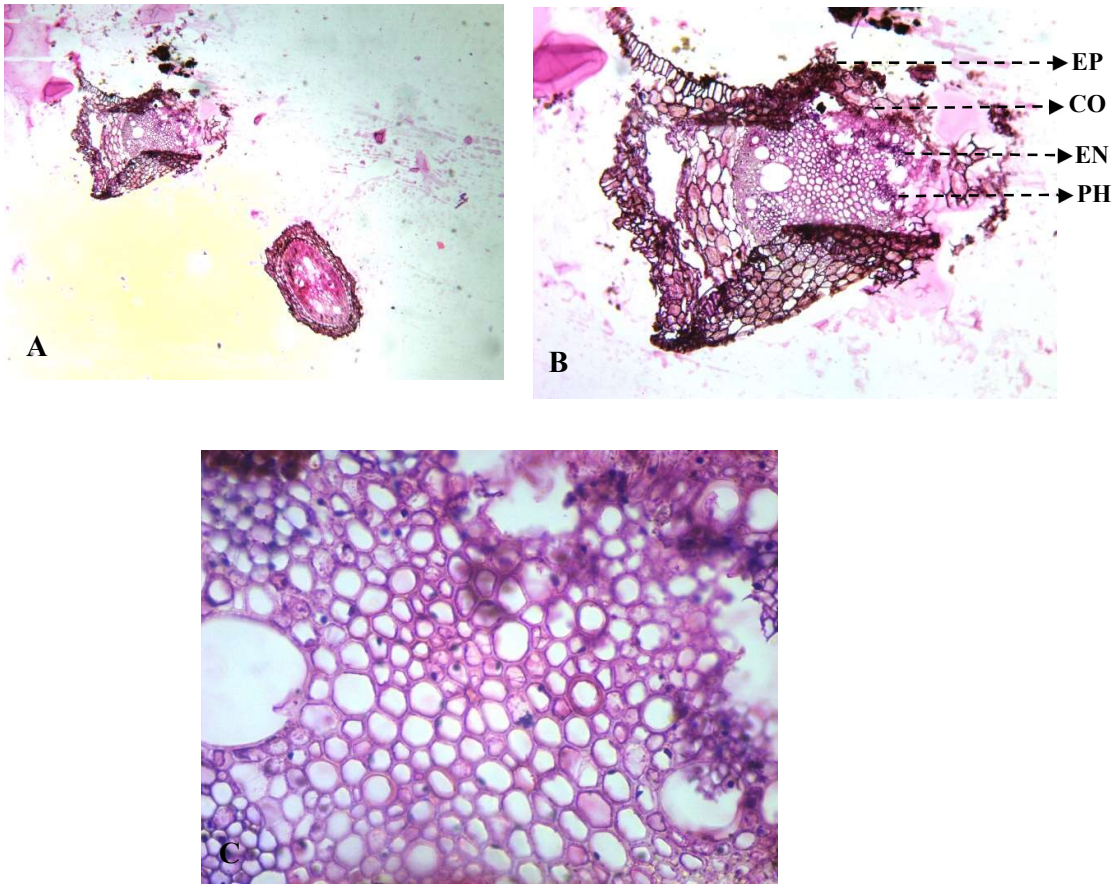

Plate 3.4: Different magnifications of the transverse section of the root of *Sorghum bicolor* plant grown in 70 ppm Cr soil. (A = x40, B = x100, C = x400). **Keys:** EP: Epiblema, CO: Cortex, EN: Endodermis, PH: Phloem

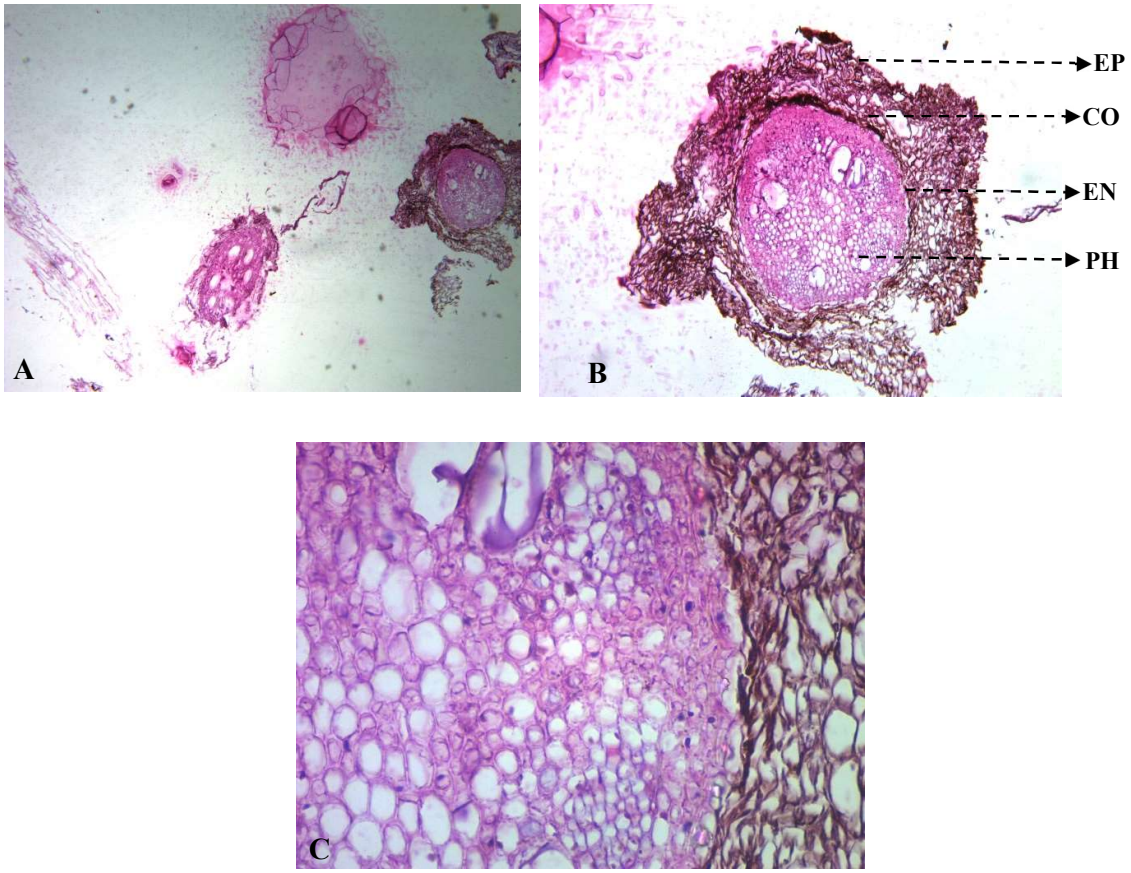

Plate 3.5: Different magnifications of the transverse section of the root of *Sorghum bicolor* plant grown in 100 ppm Cr soil. (A = x40, B = x100, C = x400). **Keys:** EP: Epiblema, CO: Cortex, EN: Endodermis, PH: Phloem

Plates 3.6 to 3.10 show different magnifications of the transverse sections of the stem of *Sorghum bicolor* plants grown in different concentrations of chromium (VI) oxide treated soils (0 ppm, 30 ppm, 50 ppm, 70 ppm and 100ppm). The stem of *Sorghum bicolor* showed some observable changes with higher treatment concentrations. The epidermis layer appears to have no significant change across all treatments (0 ppm-100 ppm), The vascular bundles show no significant change but loss of cells and deposition of crystal-like substance is observed in the cortex.

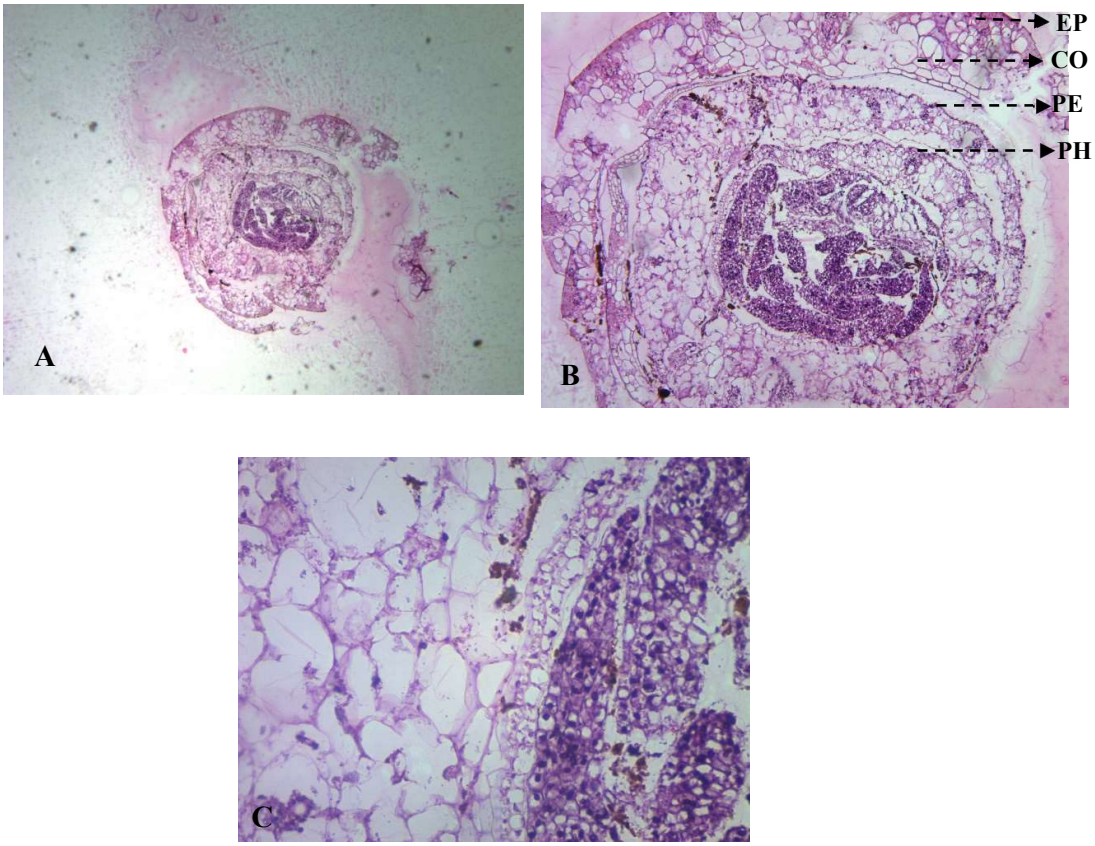

Plate 3.6: Different magnifications of the transverse section of the stem of *Sorghum bicolor* plant grown in control (0 ppm) Cr soil. (A = x40, B = x100, C = x400). **Keys:** EP: Epidermis, CO: Cortex, PE: Pericycle, PH: Phloem

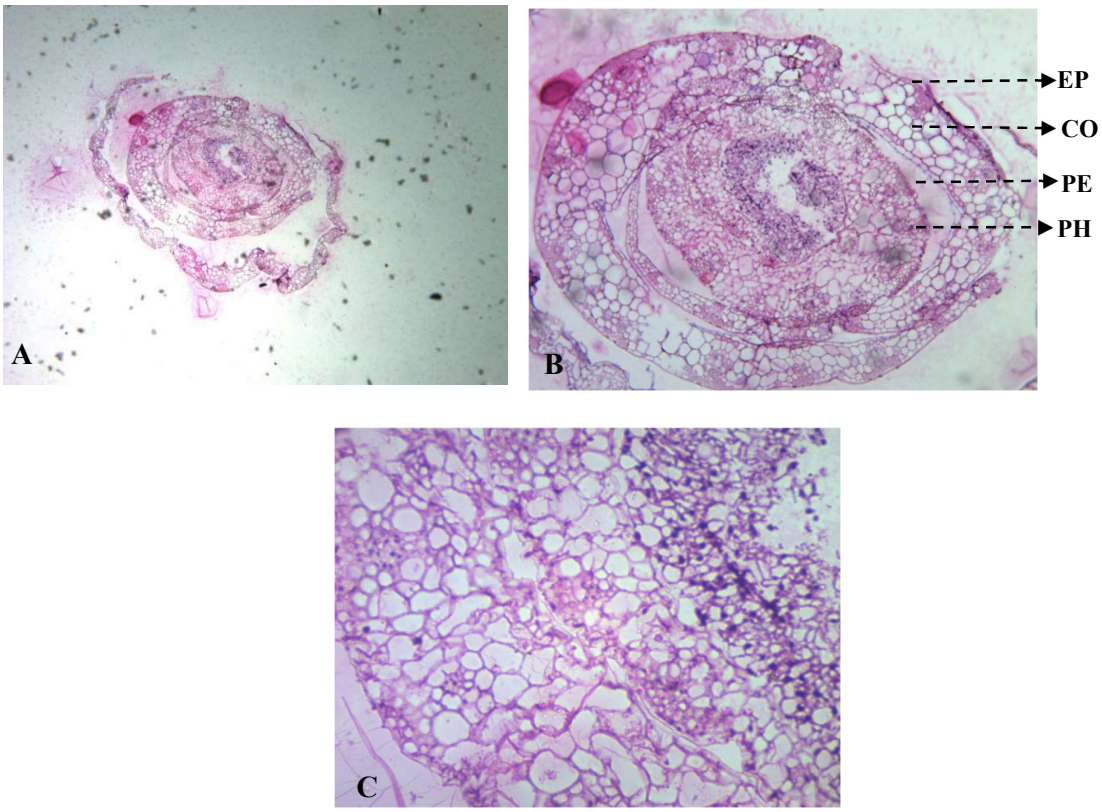

Plate 3.7: Different magnifications of the transverse section of the stem of *Sorghum bicolor* plant grown in 30 ppm Cr soil. (A = x40, B = x100, C = x400). **Keys:** EP: Epidermis, CO: Cortex, PE: Pericycle, PH: Phloem

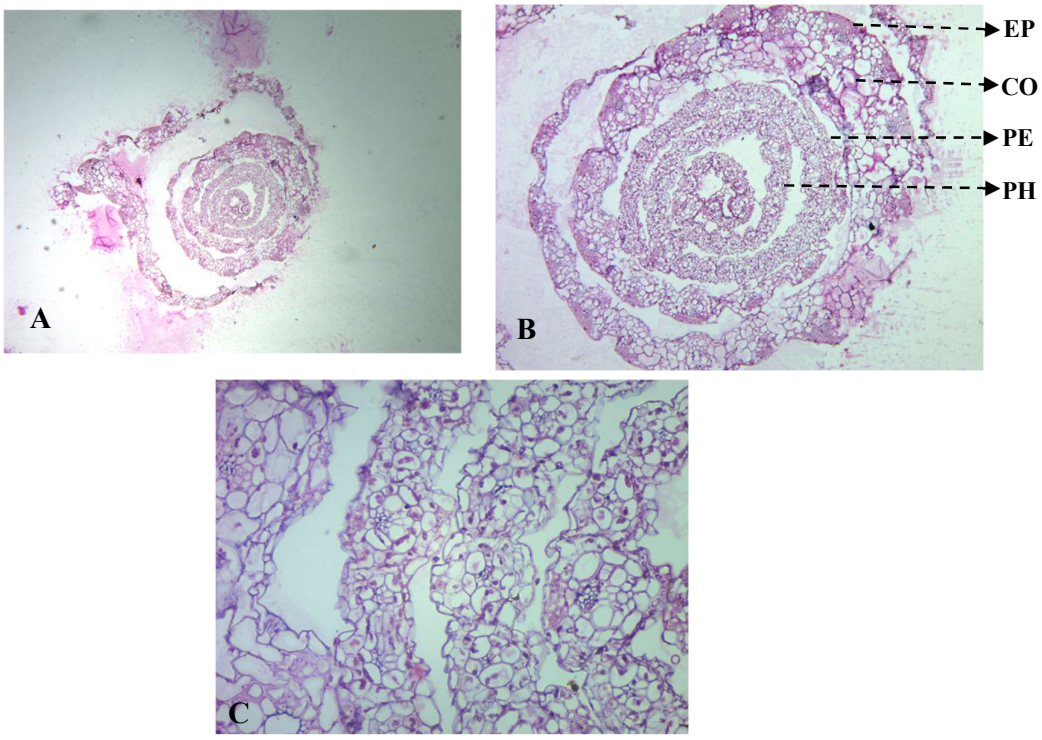

Plate 3.8: Different magnifications of the transverse section of the stem of *Sorghum bicolor* plant grown in 50 ppm Cr soil. (A = x40, B = x100, C = x400). **Keys:** EP: Epidermis, CO: Cortex, PE: Pericycle, PH: Phloem

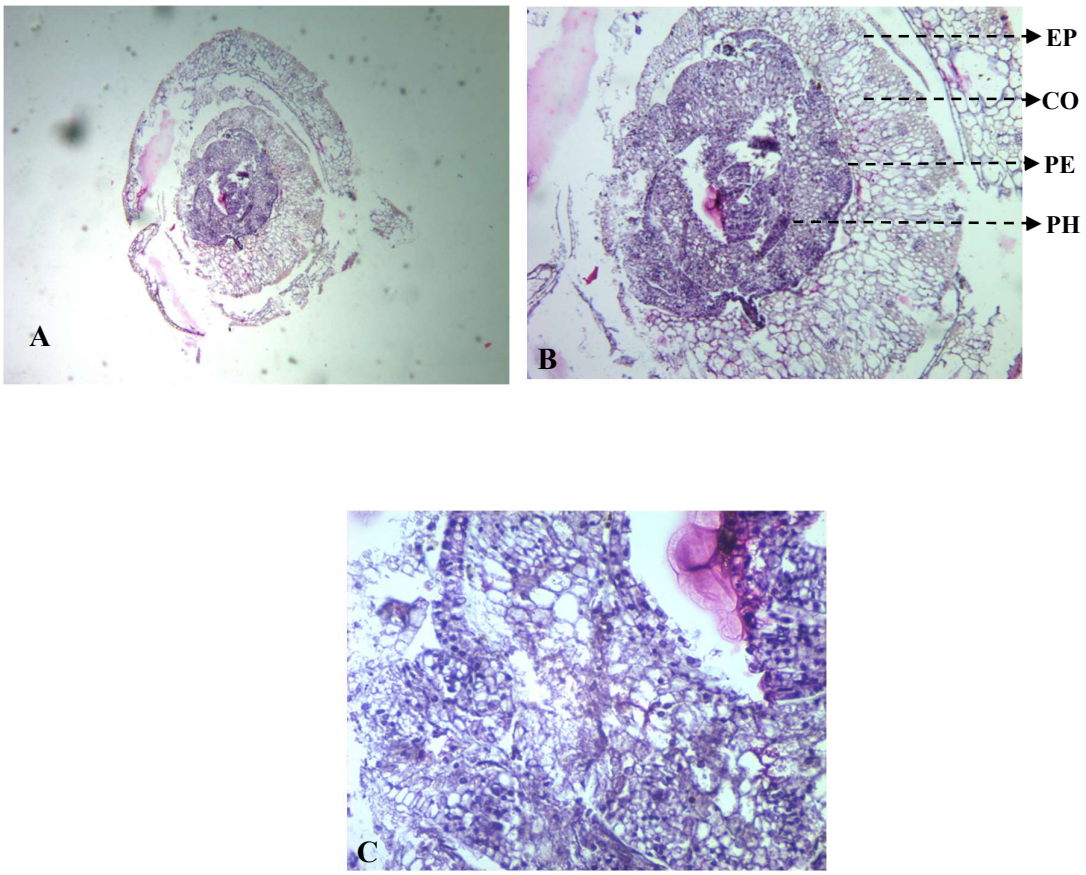

Plate 3.9: Different magnifications of the transverse section of the stem of *Sorghum bicolor* plant grown in 70 ppm Cr soil. (A = x40, B = x100, C = x400). **Keys:** EP: Epidermis, CO: Cortex, PE: Pericycle, PH: Phloem

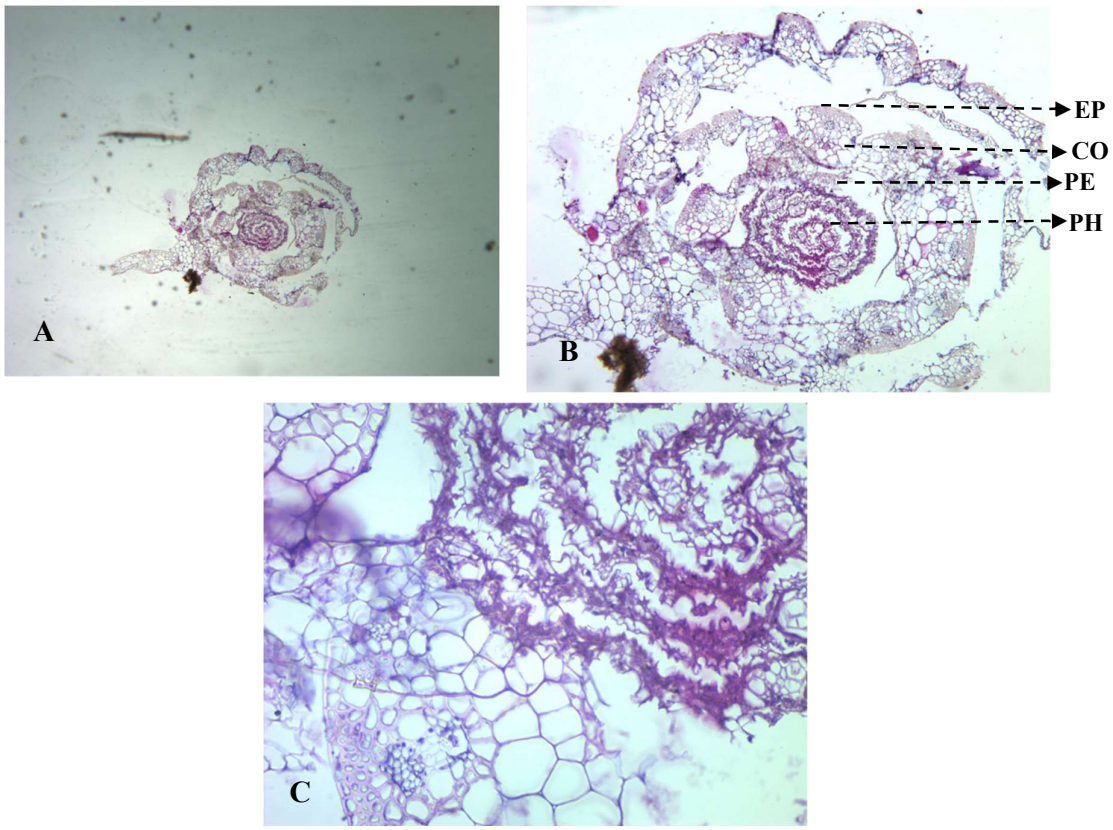

Plate 3.10: Different magnifications of the transverse section of the stem of *Sorghum bicolor* plant grown in 100 ppm Cr soil. (A = x40, B = x100, C = x400). **Keys:** EP: Epidermis, CO: Cortex, PE: Pericycle, PH: Phloem

CHAPTER FOUR

DISCUSSION AND CONCLUSION

4.1 DISCUSSION

Soil pollution arising from heavy metal build up in the environment leads ecosystem concerns because the interdependence of living organisms is affected in the food chain and imbalance may become the order of the day. Chromium, one of the heavy metals that accompany fertilisers, is likely to buildup in agricultural soils through regular application of fertilizers. Chromium toxicity often inhibits germination by interfering with water uptake and enzymatic activities essential for embryo growth (Shanker et al., 2005). At the cellular level, chromium induces oxidative stress through the excessive generation of reactive oxygen species (ROS) such as superoxide radicals and hydrogen peroxide. These molecules damage lipids, proteins, and nucleic acids, disrupting membrane structure and the activity of enzymes (Gill & Tuteja, 2011). Anatomical studies by Vwioko et al. (2017) showed that chromium exposure in Amaranthus hybridus and Chromolaena odorata caused disorganization of the vascular bundles, thickened epidermal walls and reduced diameter in xylem vessels. Such structural distortions are direct indicators of metal-induced stress in plant tissues.

This study evaluated how different concentrations of Cr^{6+} affected Sorghum bicolor in terms of germination, growth, stem girth, leaf production, and anatomical integrity of root and stem sections.

The germination results showed that the control plants had consistently higher germination percentages compared to chromium-treated plants. Seeds exposed to 100 ppm exhibited the lowest germination percent, whereas the control recorded comparatively higher germination 15 days after planting (DAP). Similar observations were reported by Prakash et al. (2016) and Oliveira et al. (2015), who noted delayed but eventual recovery of germination in chromium-treated cowpea,

attributed to physiological adjustments and detoxification processes within the seed embryo. The early inhibition observed in this study is consistent with chromium-induced suppression of hydrolytic enzymes necessary for seed germination, while the subsequent improvement in germination percentage over time may indicate the partial neutralization of toxicity.

Vegetative growth parameters, including plant height, stem girth, and number of leaves, were lower in chromium-treated plants compared to the control, indicating the suppressive effect of chromium on growth. The significant difference in plant height across treatments reflects chromium interference with cell elongation, photosynthesis, and water balance. Chromium toxicity is known to inhibit cell division and elongation by disturbing hormonal regulation, especially auxins and gibberellins, and by disrupting photosynthetic pigment synthesis (Shah et al., 2022; Ejaz et al., 2023). The reduced growth observed in this experiment corresponds with reports by Anitha et al., (2015), who documented decreased shoot and root biomass in *Vigna unguiculata* under Cr (VI) exposure. Similarly, Oliveira et al. (2015) and Kiran and Prasad (2020) found that chromium exposure led to shorter shoots and smaller leaves, primarily due to reduced chlorophyll synthesis and impaired stomatal regulation. However, the absence of a uniform decline in growth with increasing concentration in this study may be attributed to the complex interactions between metal speciation, soil composition, and plant defense mechanisms.

The anatomical examination of roots and stems provided additional evidence of chromium-induced structural modification. In the roots of treated plants, there was deposition of crystal-like substances in the cortex particularly at higher concentrations. Histological disruptions like this are common under heavy metal stress, where chromium ions accumulate in the apoplast, leading to deformations of the cellular components. The stems of chromium-treated plants displayed irregular cortical cell arrangement. The deposition of the crystal-like structures in the cortex, as observed microscopically, suggests abnormal lignification or deposition of stress-related compounds as a defense mechanism. These anatomical responses mirror findings by Oliveira et

al. (2015), who observed similar histopathological alterations in plant stems and roots under chromium exposure. The increase in lignified tissues is an adaptive response to immobilize chromium ions, thereby restricting their movement to metabolically active regions (Ghuge et al., 2023).

The overall correlation between morphological and anatomical results confirms that chromium (VI) oxide impairs plant function at multiple organizational levels. The reduction in plant height, stem girth, and number of leaves corresponds with the disruption of internal tissue organization, which together diminish photosynthetic efficiency and translocation of nutrients.

Although the present study provides valuable insights into the responses of *Sorghum bicolor* to chromium (VI) oxide, certain limitations exist. Chromium accumulation within plant tissues was not quantified, making it difficult to establish direct relationships between internal chromium concentration and observed symptoms. Additionally, biochemical parameters such as chlorophyll content and antioxidant enzyme activity were not assessed, limiting the mechanistic depth of interpretation. Despite these constraints, the study highlights chromium's dual role as a phytotoxic and potential selective agent for stress adaptation.

4.2 CONCLUSION

The exposure of *Sorghum bicolor* to chromium (VI) oxide caused delayed germination, reduced vegetative growth, and marked histological alterations in root and stem tissues. These effects collectively demonstrate that chromium (VI) oxide is phytotoxic to sorghum, affecting both external morphology and internal anatomy. Nevertheless, the species exhibited limited tolerance, possibly through structural reinforcement and stress adaptation mechanisms.

In summary, chromium (VI) oxide stress disrupts the physiological and anatomical integrity of *Sorghum bicolor* but also triggers adaptive modifications that may contribute to partial survival under contaminated conditions.

REFERENCES

Adewuyi, G. O., Oloyede, O. I., and Akinola, M. O. (2021). Phytotoxic effects of chromium on seed germination and early seedling growth of selected cereal crops. *Journal of Environmental and Agricultural Studies*, **9**(2): 45–56.

Anitha, C. T., Subin, M. P., Sidhimol, P. D., and Krishnapriya, N. P. (2012). Retardation of toxic effects of chromium in *Vigna unguiculata* (L.) Walp by the application of Pseudomonas fluorescens. *Nature Environment and Pollution Technology*, **11**(2), 187–192.

Anoliefo, G. O., Ikhajiagbe, B., Okonofua, B. O., and Wekhe, S. N. (2006). Metal contamination in soils and plants growing near automobile workshops in Benin City, Nigeria. *African Journal of Environmental Science and Technology*, **10**(3): 143–152.

Department of Plant Biology and Biotechnology, University of Benin. (2021). Crop Production Manual: Sorghum bicolor Cultivation Guide. Benin City: University of Benin Press.

Doggett, H. (1988). Sorghum (2nd Edition). Longman Scientific and Technical, London.

Ejaz, U., Shujaul, M. K., Noreen, K., Zeeshan, A., Sadia, J., Zarrin, F. R., Linda, H. L., Heesup, Han., and Antonio, R. (2023). Detoxifying the heavy metals: a multipronged study of tolerance strategies against heavy metals toxicity in plants. *Frontiers in Plant Science, 14*

FAO. (2020). Sorghum and Millets in Human Nutrition. Food and Agriculture Organization of the United Nations, Rome.

FAOSTAT. (2022). Statistical Database for Global Sorghum Production. Food and Agriculture Organization, Rome.

Ghani, A. (2011). Effect of chromium toxicity on growth, chlorophyll and some mineral nutrients of Brassica juncea L. *Emirates Journal of Food and Agriculture*, **23**(4): 301–307.

Ghuge, S. A., Mehta, A., and Hossain, K**.** (2023). Heavy metal stress in plants: Molecular mechanisms and phytoremediation approaches. *Frontiers in Plant Science, 14*.

Gill, S. S., and Tuteja, N. (2011). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiology and Biochemistry*, **48**(12): 909–930.

ICRISAT. (2021). Sorghum: A Global Crop for the Future. International Crops Research Institute for the Semi-Arid Tropics, Hyderabad, India.

Kiran, B. R., and Prasad, T. N. (2020). Structural alterations and oxidative stress in chromium-treated plants. *Journal of Plant Physiology*, 251, 153213.

Kotaś, J., and Stasicka, Z. (2000). Chromium occurrence in the environment and methods of its speciation. *Environmental Pollution*, **107**(3): 263–283.

Ogedegbe, S. A., and Edeki, S. A. (2019). Agronomic performance and drought tolerance of Sorghum bicolor (L.) Moench in southern Nigeria. *Nigerian Journal of Botany*, **32**(1): 112–120.

Okolo, C. C., Vwioko, D. E., and Osagie, O. I. (2018). Heavy metal accumulation and physiological effects in Amaranthus hybridus grown on contaminated soils in Benin City, Nigeria. *Journal of Applied Sciences and Environmental Management*, **22**(5): 689–695.

Oliveira, M. L. J., Nascimento, C. W. A., Accioly, A. M. A., and Santos, A. C. Q. (2015). Chromium in soil organic matter and cowpea after four consecutive annual applications of composted tannery sludge. *Revista Brasileira de Ciência do Solo, **39***(2): 552–562.

Orhue, E. R., and Uzu, F. O. (2009). Heavy metal accumulation and mobility in soils of automobile workshops in Benin City, Nigeria. *Nigerian Journal of Soil and Environmental Research*, **9**(1): 24–31.

Panda, S. K., and Choudhury, S. (2005). Chromium stress in plants. *Brazilian Journal of Plant Physiology*, **17**(1): 95–102.

Prakash, K., Ravimycin, T., & Thamizhiniyan, P. (2016). Phytotoxic effect of chromium on cowpea (*Vigna unguiculata* L.). *International Journal of Advance Research and Innovative Ideas in Education, **2***(3), 1388–1394.

Saud, S., Khan, S., Fahad, S., and Chen, Y. (2022). Chromium toxicity in plants: Physiological, biochemical, and molecular mechanisms of tolerance. *Plants*, **11**(3), 310–322.

Shah, S., Depeng, W., Shah, F., Talha, J., Mariusz, J., Nader, R., A., and Rehab, Y., G. (2022). The impact of chromium ion stress on plant growth, developmental physiology, and molecular regulation. *Frontiers in Plant Science*, 13.

Shanker, A. K., Cervantes, C., Loza-Tavera, H., and Avudainayagam, S. (2005). Chromium toxicity in plants. *Environment International*, **31**(5): 739–753.

Srivastava, S., Dubey, S., Mishra, A., and Dwivedi, S. (2021). Chromium toxicity and its detoxification in plants: A review. *Environmental and Experimental Botany*, 190, 104589.

Vwioko, D. E., Anoliefo, G. O., and Fashemi, D. S. (2006). Metal contamination of Amaranthus hybridus L. grown in chromium-polluted soil amended with cow dung manure. *African Journal of Environmental Pollution and Health*, **5**(2): 46–54.

Vwioko, D. E., Anoliefo, G. O., and Uko, I. (2008). Effect of chromium on growth and chlorophyll content of Amaranthus hybridus L. *Nigerian Journal of Botany*, **21**(2): 261–267.

Vwioko, D. E., Osagie, O. I., and Okolo, C. C. (2017). Anatomical alterations in plants exposed to heavy metal stress: A study of Amaranthus hybridus and Chromolaena odorata. *Plant Research Journal*, **10**(1): 55–63.

Wakeel, A., and Xu, M. (2020). Chromium-induced morphological, physiological, and biochemical responses in plants: An overview. Environmental Science and Pollution Research, 27, 20530–20546.