



Growth potential of kenaf on coal tailings in South Africa

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DEDICATION

I dedicate this dissertation to my mother, Dorette Boshoff, whose own journey toward a master's degree was interrupted by unforeseen circumstances. From a young age, she instilled in me a deep respect for knowledge and a passion for learning. Her unwavering encouragement and the example she set have been the foundation of my academic pursuits. This work is a tribute to the love for research she cultivated in me and the legacy of perseverance she continues to embody.

Dankie Moeder

PREFACE

This dissertation is submitted in fulfilment of the requirements for the degree of Master of Science in Botany, with a focus on Plant Physiology. The research presented here was conducted between January, 2023 and May, 2025 under the supervision of Prof J.M. Berner at Faculty of Natural and Agricultural Science, North-West University.

The inspiration for this study stems from a deep interest in understanding how plants respond and adapt to environmental stressors, particularly on a physiological level. As global environmental challenges increase, the potential role of plants in remediation and ecosystem restoration has gained considerable attention. This research investigates the physiological performance of kenaf (*Hibiscus cannabinus*) when grown in coal tailings—an area where limited studies exist, especially within the South African context.

The research process has been both challenging and rewarding. It required not only theoretical understanding but also practical skills in plant cultivation, physiological measurement techniques, and data analysis. Working with specialized instruments such as the M-PEA fluorimeter and engaging with multi-disciplinary data has expanded my scientific perspective and reinforced the importance of collaboration in research.

Studying plants on such a detailed physiological level has deepened my awe for the intricacy of life. It is in these small, often overlooked processes that I am reminded of the greatness and love of God, who created such complexity for us to discover and steward. Each layer of understanding reveals more of His intentional design and care for creation.

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Lastly, I sincerely thank my family and friends for their unwavering support, patience, and belief in me during this journey.

ABSTRACT

Key terms: *Hibiscus cannabinus*, photosynthesis, chlorophyll a fluorescence, coal tailings, heavy metals.

The extraction and processing of mineral resources, particularly coal mining, leads to significant degrading of surrounding ecosystems, necessitating effective rehabilitation strategies. Coal tailings, the waste material from coal mining, are typically characterized by high heavy metal content and low pH, which pose severe challenges to plant growth. However, particular plant species exhibit physiological adaptations that enable survival under such stressors. Kenaf (*Hibiscus cannabinus*), an industrial crop known for its high fibre content and tolerance to metal-contaminated environments, presents potential for use in phytoremediation.

This study investigated the feasibility of cultivating kenaf on coal tailings and assessed its physiological performance and heavy metal absorption capacity. Kenaf was grown for two months in five soil treatments: control soil, two distinct raw coal tailing samples (Coal A and Coal B), and mixtures of each coal tailing with soil at a 1:2 ratio. Physiological responses were evaluated using an MPeA fluorimeter to measure prompt chlorophyll fluorescence and modulated 820 nm reflection, alongside chlorophyll content analysis. Upon harvest, plant and soil samples were analysed for heavy metal and rare earth element content.

Results indicated that kenaf maintained functional photochemical activity and chlorophyll synthesis across all treatments, except Coal B, which exhibited reduced PSII and PSI efficiency. Despite these reductions, physiological parameters remained within normal ranges, suggesting kenaf's capacity to tolerate and grow in harsh conditions. Moreover, kenaf demonstrated the ability to absorb heavy metals and rare earth elements from coal tailings. These findings support the potential use of kenaf for phytoremediation and mine rehabilitation in South Africa, highlighting its resilience and environmental value in metal-contaminated soils.

OPSOMMING

Sleuteltermes: *Hibiscus cannabinus*, fotosintese, chlorofil a fluoressensie, steenkoolresidu, swaarmetale.

Die ontginning en verwerking van minerale hulpbronne, veral steenkoolmynbou, lei tot ernstige agteruitgang van omliggende ekosisteme en vereis doeltreffende rehabilitasie strategieë. Steenkoolresidu's, die afvalmateriaal van steenkoolmynbou, word gekenmerk deur hoë swaarmetaalinhoud en lae pH-vlakke, wat groot uitdagings vir plantgroei inhou. Sekere plantspesies toon egter fisiologiese aanpassings wat hulle in staat stel om onder hierdie stresvolle toestande te oorleef. Kenaf (*Hibiscus cannabinus*), 'n industriële gewas wat bekend is vir sy hoë veselinhoud en toleransie teenoor metaalbesoedelde omgewings, toon potensiaal vir gebruik in mynbourehabilitasie.

Hierdie studie het die geskiktheid van kenaf vir groei op steenkoolresidu's ondersoek, asook sy fisiologiese reaksie en vermoë om swaar metale te absorbeer. Kenaf is tot begin blomstadium vir gekweek in vyf grondbehandelings: 'n kontrolegrond, twee rou steenkoolresidu-monsters (Steenkool A en Steenkool B), en mengsels van elk met grond in 'n 1:2-verhouding. Fisiologiese parameters is geëvalueer met behulp van 'n M-PEA fluorimeter om chlorofil fluoressensie en gemoduleerde 820 nm-refleksie te meet, tesame met 'n analise van chlorofil konsentrasie. Na afloop van die eksperiment is plant- en grondmonsters ontleed vir swaarmetaal- en skaars element inhoud.

Resultate het getoon dat kenaf funksionele fotochemiese aktiwiteit en chlorofilsintese kon handhaaf in al die behandelings, behalwe in Steenkool B, waar PSII- en PSI-doeltreffendheid verminder was. Ten spyte van hierdie afnames, het fisiologiese waardes binne normale grense gebly, wat dui op kenaf se vermoë om in strawwe substraat te oorleef. Verder het kenaf getoon dat dit in staat is om swaar metale en skaars elemente uit steenkoolresidu's te absorbeer.

Hierdie bevindings ondersteun die potensiaal vir die gebruik van kenaf in mynbourehabilitasie in Suid-Afrika, en beklemtoon sy doeltreffendheid en omgewingswaarde in metaalbesoedelde gronde.

LIST OF ABBREVIATIONS

ΔV_{IP}	I-P Phase
ABS	Absorption
AMD	Acid mine drainage
ANOVA	Analysis of variance
As	Arsenic
ATP	Adenosine triphosphate
B	Boron
CCM	Chlorophyll content meter
Cd	Cadmium
CFR	Chlorophyll fluorescence ratio
CO ₂	Carbon dioxide
Cr	Chromium
Cu	Copper
DF	Delayed fluorescence
ET	Electron transport
ETC	Electron transport chain
Fd	Ferredoxin
Fe	Iron
F _m	Maximum fluorescence
F _o	Initial fluorescence
F _v /F _m	Ratio of variable fluorescence to maximum fluorescence
GDP	Gross domestic product
Hg	Mercury
LED	light-emitting diode
LHC	Light-harvesting complex
MF	Modulated fluorescence

Mg	Magnesium
Mn	Manganese
Mo	Molybdenum
M-PEA	Multi-Function Plant Efficiency Analyzer
MR _{min}	Minimum modulated reflection
N	Nitrogen
NADP	Nicotinamide adenine dinucleotide phosphate
NADPH	Nicotinamide adenine dinucleotide phosphate (reduced form)
NFRPC	Natural fibre-reinforced polymer composites
Ni	Nickel
O ₂	Oxygen
P	Phosphorus
P ₆₈₀	Primary electron donor within Photosystem II
P ₇₀₀	Primary electron donor within Photosystem I
Pb	Lead
PC	Plastocyanin
PCR	Photosynthetic carbon reduction
PF	Prompt fluorescence
PI _{ABS}	Absolute performance index
PI _{Total}	Total performance index
PQ	Plastoquinone
PQH ₂	Plastoquinol
PSI	Photosystem I
PSII	Photosystem II
Q _A	Quinone A
RE	Reduction
ROS	Reactive oxygen species
Se	Selenium

TR	Trapping
Zn	Zinc
δ_{R0}	Flow of electrons past plastoquinone
φ_{E0}	Quantum yield of electron transport
φ_{R0}	Quantum yield for reduction of end electron acceptors at PSI
Ψ_{E0}	Initiation of electron transport

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CHAPTER 1 INTRODUCTION

Mine closure is a natural part of the life cycle of a mine, driven by the depletion of available ore and its long-term environmental, social, and economic impacts have been studied over the past few years. South Africa, as a developing nation, is highly dependent on the mining industries, which contribute significantly to its economy and employment sector (Hermanus et al., 2010; Ramaphosa, 2024). Coal mining in particular, is the cornerstone of South Africa's energy production, but it generates vast quantities of coal tailings—byproducts that pose severe environmental challenges, including contamination of water resources, land degradation, and erosion (Lei et al., 2016). These coal tailings are also known as refuse, waste piles, slate dumps and gob piles and contain high heavy metal content (Daniels et al., 2010). Given the scale of coal mining and the associated environmental degradation, sustainable rehabilitation strategies are urgently needed. Legislation mandates the inclusion of mine closure plans that promote ecological restoration, socio-economic development, and community well-being (Lei et al., 2016). However, the effective implementation of such plans remains a challenge.

1.1 Environmental impact of coal mining

The extraction and processing of mineral resources lead to damage of the surrounding environment (air and water pollution and land degradation) resulting in the need for the restoration of ecosystems and mine rehabilitation (Lei et al., 2016). For every 10,000 tons of open-cast coal mined, approximately 0.22 hectares of land are degraded (Lei et al., 2016). Coal tailings exacerbate this issue by contributing to water acidification, heavy metal contamination, spontaneous combustion, and loss of biodiversity (Cleophas et al., 2023). In a case study from Limpopo, 8000 ha of a village were lost due to mining activities (Shackleton, 2020). If left unmanaged, these tailings result in long-term environmental damage, necessitating reclamation efforts to restore soil quality, reduce contamination, and enable vegetation growth (Li et al., 2020). Reclamation of these coal tailings is essential to minimize the contamination and acidification of ground and surface water, erosion, spontaneous ignition, and damage to the surrounding land (Daniels et al., 2010). Legislation requires mines to ensure the maintenance of the quality of life of the surrounding communities, which is greatly affected by coal tailings (Hermanus et al., 2010). Thus, vegetation is vital to restore the ecological environment by reducing erosion and pollution, creating new habitats and improving the physical, chemical and biological properties of soil, and ensuring the safety and health of local communities (Li et al., 2020). Additionally, coal mining affects not only the environment, but also the socio-economic status of a country.

1.2 Socio-economic impact of coal mining

The mining industry plays a crucial role in South Africa's employment sector. In 2015 alone, it directly employed 457,698 people and contributed R286 billion to the national GDP (Ackermann et al., 2018). Additionally, the industry indirectly supported the livelihoods of 4.5 million individuals (Ackermann et al., 2018). Although the mining industry provides substantial economic and social benefits, mine closures can have a devastating impact, often resulting in widespread job losses within nearby communities (Ackermann et al., 2018; Hermanus et al., 2010). To mitigate these effects, legislation requires mining companies to provide sustainable economic alternatives as part of post-closure plans. However, implementing such initiatives at scale remains a pressing issue, particularly in rural communities heavily dependent on mining-related employment. Fortunately, phytoremediation through vegetation is a promising solution for both the environmental and socio-economic effects of coal mining.

1.3 Kenaf as a potential phytoremediation of coal tailings

Although coal tailings have various growth-limiting factors for vegetation, kenaf is adaptable to many soil types and conditions (Yu-xi et al., 2013). Kenaf has a vigorous root system that allows the plant to absorb harmful metals from the soil (Ayadi et al., 2017). One of the main concerns about non-food crops is their economic value. However, due to the increasing environmental issues, the development of new technologies and fibre-based products is growing (Ayadi et al., 2017). Kenaf is a valuable industrial crop, used for fibre production in various industries for example the paper, automotive products, food and medicine (Sim & Nyam, 2021). It is cultivated in various countries in South Africa, China, United States, southern Europe, Malaysia, Thailand, Bangladesh and Thailand (Afzal et al., 2022). In addition to relieving the pressure on wood production, kenaf is a promising option for the remediation of abandoned mines or mine tailings (Zhao et al., 2022).

1.4 Relevance of the study

1.4.1 Problem statement

Given that photosynthesis is a primary indicator of plant health and a critical determinant of growth under abiotic stress conditions (Ghosh et al., 2023), understanding how kenaf performs on coal tailings is essential. This study aims to address the knowledge gap by investigating kenaf's physiological responses to coal tailings, with the ultimate goal of determining its potential for mine rehabilitation in South Africa.

Coal mining in South Africa generates large volumes of tailings, which pose significant environmental and socio-economic challenges. These tailings are characterized by high concentrations of heavy metals, low pH, and nutrient deficiencies, which severely limit their suitability for revegetation (Xu et al., 2023). The lack of sustainable reclamation strategies for coal tailings has resulted in widespread land degradation, contamination of water resources, and long-term negative impacts on local communities (Daniels et al., 2010). Despite legislative requirements for mine rehabilitation, the development of effective and economically viable solutions remains inadequate (Hermanus et al., 2010).

Phytoremediation is a promising solution for the environmental and socio-economic issues about coal mining in South Africa (Cleophas et al., 2023). It entails the utilization of solar energy used by plants to extract toxic metals from polluted soils (Zhao et al., 2022). However, this method poses challenges of its own, including that cultivation of plants is time-consuming and that the absorbed heavy metals can harm humans when the plants are consumed (Roby et al., 2017). Thus, non-edible fast-growing plants with economic value show the most promise as candidates for phytoremediation (Cleophas et al., 2023).

Kenaf (*Hibiscus cannabinus*), a fast-growing, multipurpose fibre crop, demonstrates potential for cultivation on degraded and metal-polluted soils due to its tolerance to abiotic stresses and its economic value (Yu-xi et al., 2013). Its fibres are widely used in industries such as construction, paper, and automotive manufacturing, offering an opportunity to combine environmental restoration with economic benefits (Khandai et al., 2019; Monti & Alexopoulou, 2013). However, little is known about kenaf's physiological responses to coal tailings, particularly the effects of heavy metal stress on its photosynthetic and photochemical processes (Mareri et al., 2022).

The findings serve as a contribution to the development of sustainable solutions for mitigating the environmental and socio-economic impacts of coal mining.

1.4.2 Aim and Objectives

This study aimed to determine the growth potential of kenaf planted on coal tailings in South Africa. It primarily used physiological parameters to evaluate the kenaf's photochemical efficiency.

With the following objectives:

1. To evaluate the photochemical performance of kenaf by measuring chlorophyll a fluorescence, 820 nm reflection and chlorophyll content.
2. To determine the absorption and translocation of heavy metals in kenaf.

3. To study the effect of trace elements in coal tailings on photosynthesis in kenaf.

1.5 Thesis summary

Chapter 2: Literature Review

This chapter presents a comprehensive review of relevant literature, including theoretical frameworks and key concepts. It broadly includes the growth requirements of kenaf, the various effects of coal mining on the environment and economy, and plant physiology and phytoremediation mechanisms. Previous research and identified gaps are addressed and discussed.

Chapter 3: Methodology

This chapter details the research design, instruments, including M-PAE (Multi-Function Plant Efficiency Analyzer) and CCM (Chlorophyll Content Meter), physiological parameters used, and data collection and analysis methods.

Chapter 4: Results

This chapter clearly presents the research findings using tables, graphs, and statistical analyses. Key trends and patterns are highlighted, providing a factual basis for further discussion in Chapter 5.

Chapter 5: Discussion

This chapter interprets the results, relating them to the research question: Can *Hibiscus cannabinus* grow on coal tailings? It explores the implications of the findings, discusses limitations, and provides recommendations for future research to expand this study.

Chapter 6: Conclusion and Recommendations

The final chapter summarizes the key findings, which are that kenaf maintains good plant health while absorbing heavy metals and rare earth elements. Practical recommendations are offered, and suggestions for future research are outlined, emphasizing the broader impact of the study.

CHAPTER 2 LITERATURE REVIEW

This literature review explores the origin, morphology, and economic uses of kenaf (*Hibiscus cannabinus*), contextualizes its relevance within the South African coal mining landscape, and highlights its potential as a phytoremediator on coal tailings. Finally, it identifies key research gaps related to its physiological response to metal-polluted substrates.

2.1 Kenaf (*Hibiscus cannabinus*)

Hibiscus cannabinus is a dicotyledonous annual herbaceous plant that originated in Africa and India (LeMahieu et al., 1991; Monti & Alexopoulou, 2013). Kenaf is a fast growing, bast fibre plant that can grow up to six meters high in a period of six to eight months (Afzal et al., 2022; University of Kentucky, 2014) and can produce up to 30 t.ha⁻¹ of dry stem material (Ayadi et al., 2017). It is the third traditional crop in the world after bamboo and wood (Cleophas et al., 2023). Kenaf is also known for its importance in horticulture and economic value (Ayadi et al., 2017). Since it is a summer crop, it is commonly planted in spring and can be harvested after 3 to 4 months, at the beginning of the flowering stage (Department of Agriculture, 2012; Sim & Nyam, 2021). For kenaf to produce long stems, it should be planted in October to ensure longer day lengths of 12 hours (Department of Agriculture, 2012).

Taxonomy

Hibiscus cannabinus is commonly known as kenaf (South Africa), Gambo (West Africa), Til (North Africa), Ambari (Taiwan) and Mesta (India) (Ayadi et al., 2017), and forms part of the Malvaceae family (Sim & Nyam, 2021). Species in the genus, *Hibiscus*, can be classified under six sections, known as: *Azanza*, *Ketmia*, *Alyogen*, *Calyphyllia*, *Abelmoschus* and *Furcaria*. Kenaf is included in the group *Furcaria* that consists of medicinal, fibre and food crops (Ayadi et al., 2017). Kenaf is closely related to roselle (*Hibiscus sabdariffa* L.), hollyhock (*Althaea rosea* L.), cotton (*Gossypium hirsutum* L.) and okra (*Hibiscus esculentum* L.) (Monti & Alexopoulou, 2013). However, kenaf is more adaptable to different climates and soils than these and other fibre crops (Afzal et al., 2022).

2.1.1 Description of Kenaf

Stems

Kenaf is known for its fibrous stems and consists of three different fibres, namely pith (polygonal parenchyma cells), core (white part of the inside of the stem) and bast (outer part of the stem)

(Ayadi et al., 2017). The main fibre types in these stems are short fibres in the ligneous area and long fibres in the cortical layer (Monti & Alexopoulou, 2013). The fibre in the core of the stem is about 0.6 mm long, whereas the bast fibres in the outer bark reach lengths of 2.5 mm (Monti & Alexopoulou, 2013). The centre of the stem is made up of spongy tissue (Ayadi et al., 2017).

Leaves

Depending on the age and cultivar of kenaf, the leaf shape may differ. However, the main two types of leaves stay consistent – divided and simple (Ayadi et al., 2017). These leaves have serrated edges (Sim & Nyam, 2021). The leaves start out simple and as the plant matures more divided leaves are produced (Ayadi et al., 2017; Monti & Alexopoulou, 2013). Depending on the cultivar, it mainly varies between deeply and shallow lobed leaves (Mahbubul Islam, 2019). Kenaf's leaves can be confused with those of marijuana, or in the case of the entire leaves, cotton and okra (Monti & Alexopoulou, 2013). A nectar gland can be found on the mid-vein of the leaves and the seed capsule.

Roots

Kenaf has an effective root system, consisting of a long taproot with an extensive lateral root system, exceeding a depth of one meter (Ayadi et al., 2017; Monti & Alexopoulou, 2013). This root system makes kenaf adaptable in varying soil moisture conditions, since it can absorb deep soil water (Ayadi et al., 2017). This long taproot allows the plant to use residual nutrients from prior crops (Monti & Alexopoulou, 2013). This type of root system is ideal for phytoremediation.

2.1.2 Environmental requirements for growth on marginal lands

Climate

Kenaf prefers a warm tropical climate with daily temperatures being between 20°C and 30°C (University of Kentucky, 2014), with the optimum temperature of 15-27°C. Kenaf is sensitive to cooler temperatures and is frost-sensitive (Mahbubul Islam, 2019; University of Kentucky, 2014) with a minimum temperature of 10°C to prevent a drastic decline in seed production, fibre yield and biomass (Ayadi et al., 2017). In South Africa, this crop is mainly present in the province of KwaZulu-Natal, since it requires a tropical or subtropic climate (Cleophas et al., 2023; Department of Agriculture, 2012).

Soil

Kenaf is adaptable to many different soils, but performs best in deep, well-drained loamy soil (Department of Agriculture, 2012; LeMahieu et al., 1991). For optimal growth, high moisture levels are preferred, especially for its productive root structure (Ayadi et al., 2017). However, this root system is developed to acclimate to changes in soil moisture, which leads to kenaf being moderately tolerant to drought stress and soil salinity (Ayadi et al., 2017). In the case that the soil is too moist for too long, the growth may be limited (Afzal et al., 2022). Furthermore, kenaf prefers soil with a neutral pH (Department of Agriculture, 2012). However, overall, kenaf adapts to poor soil conditions, like low fertility, drought and pH variety, better than its related species (Ayadi et al., 2017; Mahbubul Islam, 2019).

2.1.3 The importance of natural fibre

The negative environmental impacts of synthetic fibre in various industries can be reduced by using natural fibre (Lee et al., 2021; Monti & Alexopoulou, 2013). Thus, non-wood lignocellulosic fibre sources are sought after since wood production has shown increased environmental concern (Ayadi et al., 2017). As a result, interest in additional possibilities for fibre sources is rising, especially fast-growing indigenous plants (Ayadi et al., 2017). However, the strength of natural fibre may pose a problem, and hybridization is suggested to introduce natural fibre to the relevant industries (Lee et al., 2021).

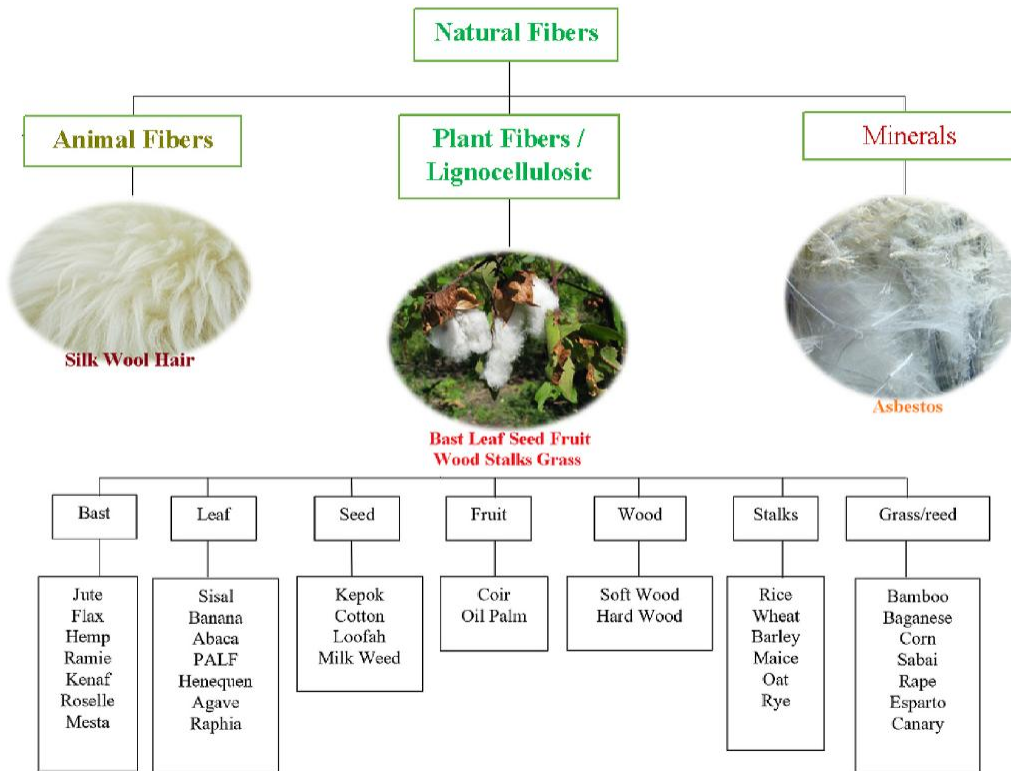


Figure 2.1: Flow diagram with the classification of natural fibres (Jawaid et al., 2022).

Hybrid composites are basically single fibre composites that is strengthened with other fibres, in this case natural fibres, giving it unique properties compared to single composites (Jawaid et al., 2022). These composites are known as natural fibre-reinforced polymer composites (NFRPCs). As a result of the natural fibre content of NFRPCs, it is renewable, recyclable, lightweight, has high specific strength and has lower manufacturing cost (Jawaid et al., 2022). However, in specific industries, NFRPCs can be challenging. Natural fibres have weak microbial resistance and are vulnerable to moisture absorption, negatively affecting long-term storage and mechanical properties of the material (Jawaid et al., 2022). Additionally, thermal stability of natural fibres can be a limiting factor. Fortunately, previous studies have shown that fibres from kenaf only start to decompose at temperatures of 247-455°C (Jawaid et al., 2022). In comparison with a few natural fibres, kenaf is primarily used in the automotive industry due to its high impact resistance (Lee et al., 2021). Previous studies showed that kenaf hybrid composites were able to absorb impact energies between 40 J and 135 J (Lee et al., 2021). These properties make kenaf an ideal candidate for cultivating in marginal or polluted soils, where traditional crops may not thrive, and where its biomass can offer post-remediation economic value.

2.1.4 Morphology of kenaf fibres

Physical properties

The stem consists of three main layers (Figure 2.2), the pith at the centre (sponge-like tissue), core (xylem) and bast (phloem) (Jawaid et al., 2022).

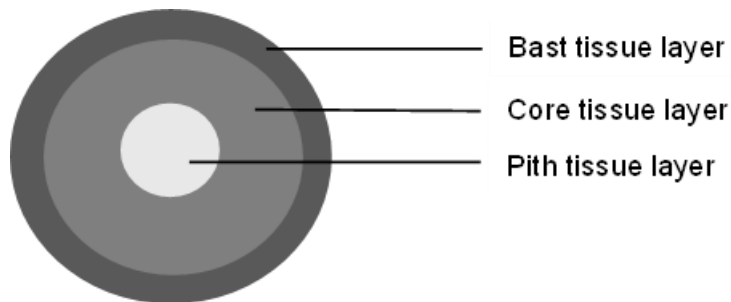


Figure 2.2: Different parts of kenaf stem (Jawaid et al., 2022; Lee et al., 2021).

Kenaf is known as the future lignocellulosic fibre crop, containing short and long fibres in the bark and core of the stem (H'ng et al., 2009; Monti & Alexopoulou, 2013). The core makes up 60-65% of the stem and the bast makes up 35-40% of the stem (Afzal et al., 2022). Short and porous fibres are found in the core, comparable to hardwood fibres. On the other hand, the bast is more like softwood fibres of trees (Afzal et al., 2022, University of Kentucky, 2014). Since the pith consists solely of parenchyma cells, kenaf fibres are primarily extracted from the core and bast (Ayadi et al., 2017; Jawaid et al., 2022). Ultimately, the bast is dense and fibrous, where the core is lighter and more wood-like (Figure 2.3) (H'ng et al., 2009).

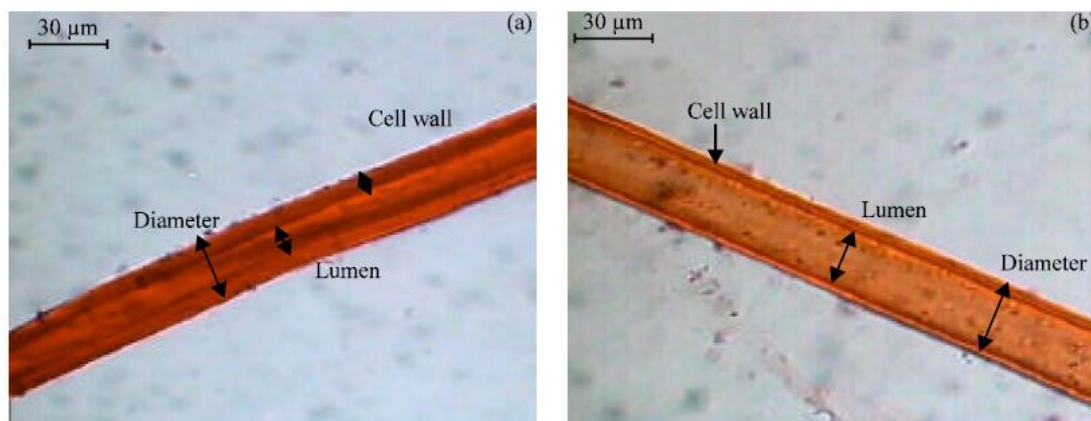


Figure 2.3: Microscopic images of bast (a) and core (b) under a 400X magnification (H'ng et al., 2009).

Since the bast and core of kenaf have different characteristics, they need to be separated (Lee et al., 2021). A method called microbial retting separates the bast and core from each other by abolishing the chemical bonds that connect them (Jawaid et al., 2022). Kenaf fibres can be processed into various forms, of which woven fabric shows the most strength among other natural fibres and is commonly used in the automotive industry (Lee et al., 2021). Research have shown

that the bast fibres produce higher quality pulp than the short fibres from the core (Ayadi et al., 2017; Monti & Alexopoulou, 2013).

Chemical properties

Kenaf are mainly made up of lignin (12-14%), cellulose (58-63%) and hemicellulose (21-24%), of which cellulose is the determining factor concerning the strength, application and production cost (Jawaid et al., 2022; Monti & Alexopoulou, 2013).

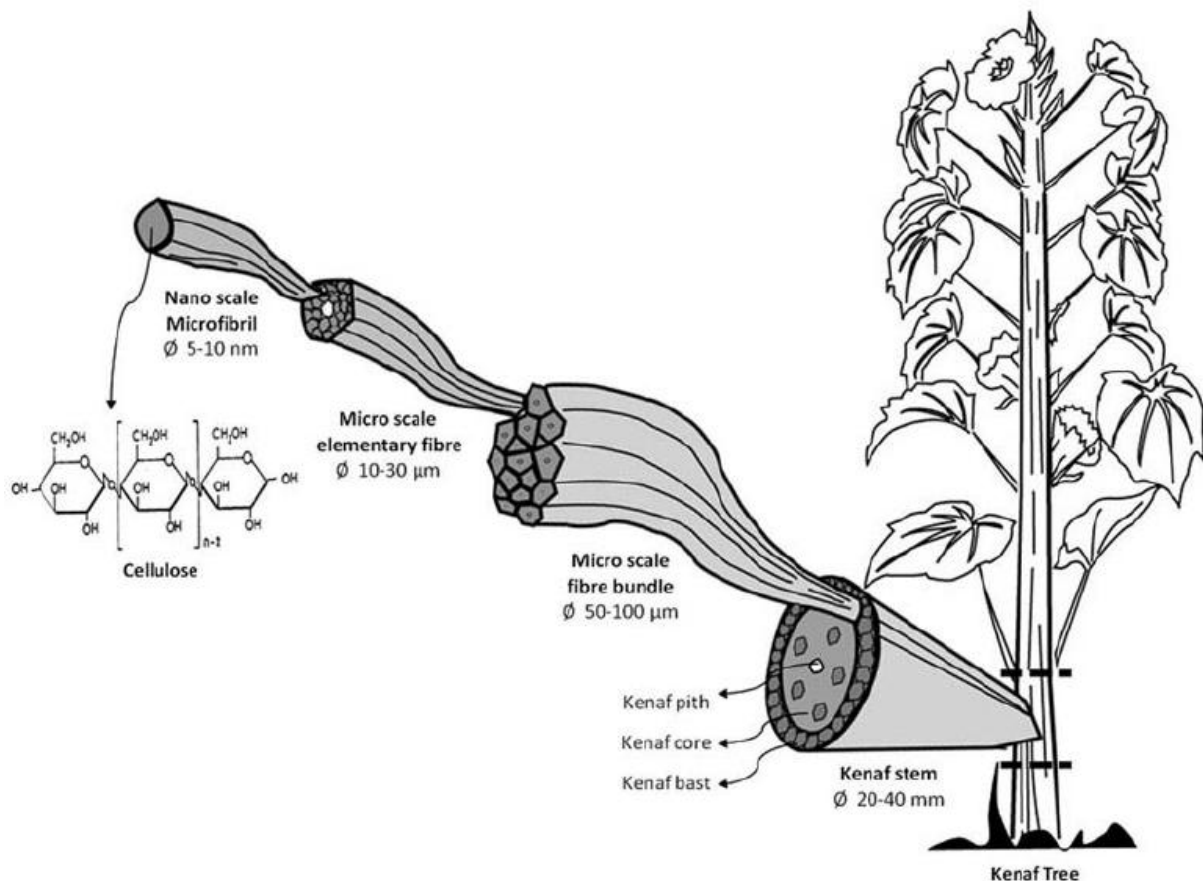


Figure 2.4: Summative image extracted from (Jawaid et al., 2022) illustrating the physical and chemical properties of kenaf fibres.

These physical- and chemical properties give kenaf the benefit of various industrial uses and thus increasing economic value. Furthermore, these morphological traits of kenaf increases its ability to absorb pollutants in the soil.

2.1.5 Uses of kenaf

Kenaf has been used for livestock feed and fibre for centuries and is still expanding in its various applications (Ayadi et al., 2017). Fibre extracted from kenaf is more advanced than other natural

fibres because it is a fast-growing crop with low costs and adaptable in different growing conditions (Afzal et al., 2022). Kenaf shows various economic uses and value (Ayadi et al., 2017; Monti & Alexopoulou, 2013).

Industrial uses

The fibre industry pertaining to industrial materials is an excellent market for kenaf fibres, especially the long fibres from the bast layer of the plant (Ayadi et al., 2017). One of the biggest is the automotive industry, where kenaf is used to produce body material, frames, and car floors (Afzal et al., 2022). Kenaf is also used for building materials and furniture (Sim & Nyam, 2021). The bast fibre is used to reinforce thermoplastic composites and bioplastics (Ayadi et al., 2017; Sim & Nyam, 2021). Also, some companies use kenaf fibres to strengthen concrete materials (University of Kentucky, 2014). Although the seeds are seen as a by-product of other industrial uses, the seed oil can be used to produce products like paints, linoleum, varnishes and soap (Department of Agriculture, 2012). Furthermore, the interest in kenaf is rising in the energy sector for the production of bioethanol from the core layer of the plant (Ayadi et al., 2017). Methyl esters made from extracted seed oil of kenaf are also used as biodiesel (Sim & Nyam, 2021). Kenaf fibres are also very absorbent, adding to various uses (Department of Agriculture, 2012).

Pulp and textile uses

Kenaf is known as a future crop and a possible replacement or addition to non-wood pulp production (Ayadi et al., 2017). Raw pulp can be produced from kenaf for paper products like newspapers, packaging, and cardboard (Ayadi et al., 2017; Mahbubul Islam, 2019). Pulp made from kenaf fibre is comparable to products made from wood (Monti & Alexopoulou, 2013). Trials have been done to prove that different grade paper can be produced from kenaf and shows promise in terms of strength, ink absorption and print quality (Mahbubul Islam, 2019). Similarly, newspaper made from kenaf pulp had a better display than others and had a lower production cost (Mahbubul Islam, 2019). Additionally, kenaf is a source of fibre for the production of textiles like twine, rugs, and rope (Ayadi et al., 2017; University of Kentucky, 2014). Kenaf fibres have a lighter colour that requires less bleaching, making it more environmentally friendly (Cleophas et al., 2023; Mahbubul Islam, 2019).

Food and medicinal uses

The traditional use of kenaf is mainly for food and medicine (Monti & Alexopoulou, 2013). Current interests in Kenaf is not primarily for its medicinal value, although it has been a folk medicine in Africa for a long time (Sim & Nyam, 2021). Specifically, the leaves are used for treating fatigue,

anaemia, diabetes, cancer, liver disease and atherosclerosis (Mahbubul Islam, 2019; Sim & Nyam, 2021). Medicinal uses are underestimated, but pharmacological studies have proven that kenaf has antitumor, antiulcer, antibacterial, anti-inflammatory, antioxidant, antihyperlipidemia, antityrosinase and hepatoprotective properties (Department of Agriculture, 2012; Sim & Nyam, 2021). Its main chemical constituents include fatty acid, phenols and flavanoids (Monti & Alexopoulou, 2013; Sim & Nyam, 2021). Oil can also be extracted from kenaf seeds and contains various health benefits (Ayadi et al., 2017). In addition to medicine, leaves, flowers and seeds of kenaf have nutritional value and can be used as a whole or processed as a food additive, adding more economic value to the crop (Sim & Nyam, 2021). For instance, flour can be produced with the leaves and stems, as well as vegetable protein (Monti & Alexopoulou, 2013; Sim & Nyam, 2021). Leaves can be dried and used as a herb in dishes (Department of Agriculture, 2012).

Forage uses

One of the first uses of kenaf is for livestock feed, especially the leaves that are not used for fibre production (Ayadi et al., 2017; Mahbubul Islam, 2019). Previous studies show that, especially when kenaf is harvested at early maturity, the high protein content is a good feed for animals (Monti & Alexopoulou, 2013; Sim & Nyam, 2021). Due to the high protein content of kenaf, in addition to feed, the core layer of the plant is also used as an adsorbent for animal bedding (Ayadi et al., 2017; Mahbubul Islam, 2019). Lastly, kenaf loses its leaves after the first frost, which puts nitrogen, phosphate, calcium, magnesium and potassium back into the soil (Monti & Alexopoulou, 2013).

The various uses of kenaf to produce valuable products results in positive changes in the environment and socio-economic standing of the surrounding community (Cleophas et al., 2023). Beyond industrial fibre use, kenaf's adaptability and biomass yield make it a promising crop for phytoremediation and post-mining land recovery.

2.1.6 Remediation potential of kenaf

Phytoremediation is a method used to restore polluted soils by employing plants to absorb harmful environmental substances (Zhao et al., 2022). However, this approach can be challenging, as many commonly used phytoremediation plants are slow-growing (Yan et al., 2020). Additional limitations include edible crops, which may pose risks to human health if contaminated (Cleophas et al., 2023). Therefore, the ideal phytoremediation crop should be fast-growing, non-edible, and economically viable to optimize land use from both environmental and socio-economic perspectives.

Recently, there has been growing interest in using bast fibre plants for the phytoremediation of mining sites. These plants are advantageous because they are fast-growing, have short life cycles, and tolerate high concentrations of heavy metals in the soil (Cleophas et al., 2023). Moreover, fibre plants can be utilized to produce various industrial products without introducing harmful substances into the food chain, thus enhancing their economic value (Zhao et al., 2022). Additionally, their ability to generate large quantities of biomass makes them particularly suitable for phytoremediation (Cleophas et al., 2023).

Previous studies have demonstrated that kenaf can accumulate heavy metals such as chromium (Cr), lead (Pb), zinc (Zn), and cadmium (Cd), and exhibits greater tolerance to these pollutants compared to other fibre crop varieties (Zhao et al., 2022). Thus, kenaf has shown significant potential for remediating currently or previously mined soils (Cleophas et al., 2023).

2.2 Coal mining

Coal mining is a well-known, expanding industry in South Africa, due to its contribution to energy production. However, it leads to large quantities of waste, known as tailings. As a result, heavy metals accumulate in the soil, making it hazardous for the environment and human health, especially after mine closure (Yan et al., 2020).

2.2.1 Overview of the overall impact of coal mining in South Africa

The exploitation of coal results in waste dumps known as coal tailings (Gajić et al., 2018). These tailings are highly variable in density and fertility and contain a high quantity of non-degradable heavy metals (Yan et al., 2020). This negatively impacts plants, animals and the biological and chemical properties of the soil (Roby et al., 2017). The more pressing issue is the long-term effect of mine closure on the environment, which creates the need for remediation of the abandoned mining area (Strambo et al., 2019).

Table 2.1 Impact of coal mining on a specific area of the environment.

Category	Description	Key Pollutants/Impacts	Sources
Air Pollution	Fine particles such as coal fly ash from open mining can be carried by the wind, causing respiratory and skin irritations and potentially more serious health issues.	Irritation of throat, eyes, skin, nose; anemia, bronchitis, asthma, respiratory cancers	(Chu et al., 2023; Gajić et al., 2018)
Water Pollution	Coal mining affects water availability and quality. Primary concerns are acid mine drainage (AMD) and the spread of heavy metals. These pollutants severely affect human health.	Arsenic (As), Lead (Pb); acid mine drainage; increased mortality	(Kossoff et al., 2014; Mardonova & Han, 2023)
Land Degradation	Coal mining leads to geological and ecological disturbances, loss of biodiversity, and loss of arable land due to toxic tailings.	Tailings and fly ash occupy farmland, biodiversity loss and economic loss for farmers.	(Cleophas et al., 2023; Gajić et al., 2018; Mardonova & Han, 2023)
Soil Contamination	Accumulation of heavy metals in the soil affects plant growth and health, posing risks to the food chain. Some metals are essential in small amounts but toxic in excess.	Toxic levels of Fe, Ni, Cu, Mn; harmful levels of Pb, Cr, As, Hg; reduced plant performance and ecological imbalance	(Cleophas et al., 2023; Mardonova & Han, 2023)

Coal mining plays a significant role in job creation both globally and within South Africa (Mardonova & Han, 2023). Worldwide, the mining sector directly employs approximately 40 million individuals and indirectly affects the livelihoods of around 250 million people (Kossoff et al., 2014). In context, more than 400,000 individuals were directly employed by the mining industry in 2015, while an estimated 4.5 million people benefited indirectly. That same year, mining activities contributed R286 billion to South Africa's gross domestic product (Ackermann et al., 2018).

Several industries in South Africa benefit substantially from coal mining, particularly those in the transport, machinery, and retail sectors, as well as various ancillary businesses (Strambo et al., 2019). However, the industry also presents notable environmental and socio-economic challenges. These include destroying valuable wildlife, such as fish populations (Mardonova & Han, 2023), and the occupation of land that could otherwise be used for agricultural purposes (Strambo et al., 2019). Additionally, mine closures often result in significant job losses (Ackermann et al., 2018; Strambo et al., 2019). To address these consequences, developing

alternative, non-mining-related employment opportunities is critical for sustaining the livelihoods of affected communities (Hermanus et al., 2010; Strambo et al., 2019).

Repurposing previously mined land for agricultural use presents a viable and sustainable strategy to mitigate the negative environmental and socio-economic impacts of coal mining in South Africa. Implementing vegetation and other land rehabilitation techniques can improve ecological conditions through remediation while simultaneously generating employment opportunities for local communities (Daniels et al., 2010; Gajić et al., 2018; Zhao et al., 2022).

2.2.2 Remediation of contaminated soil due to mining activities with vegetation

Several remediation approaches have been developed for addressing heavy metal-contaminated soils, including physico-chemical and mechanical methods (Yan et al., 2020). However, phytoremediation has emerged as a more favourable alternative, leveraging the natural ability of plants to extract pollutants from contaminated soil (Cleophas et al., 2023; Yan et al., 2020; Zhao et al., 2022). This method is not only cost-effective but also widely accepted by local communities (Cleophas et al., 2023). Additionally, it addresses multiple environmental issues, such as soil erosion, the dispersion of toxic metals into the broader environment, nutrient deficiencies, and the risk of spontaneous combustion (Gajić et al., 2018; Roby et al., 2017; Yan et al., 2020).

Despite its advantages, the presence of heavy metals in soil significantly limits plant growth, making it difficult to identify a single plant species capable of effectively addressing all remediation needs (Yan et al., 2020; Zhao et al., 2022). Moreover, since plants absorb toxic heavy metals from the soil (posing potential health risks to humans), non-edible crops are considered a safer and more appropriate choice for phytoremediation (Cleophas et al., 2023). Edible crops often lack the extensive root systems required for efficient pollutant extraction (Roby et al., 2017).

In this context, bast fibre crops such as kenaf represent promising candidates for phytoremediation. Kenaf is a fast-growing industrial crop that is highly adaptable to various soil types and possesses a dense root system conducive to pollutant uptake (Cleophas et al., 2023). Nonetheless, one of the primary challenges in remediating coal tailings is that certain soil conditions can inhibit photosynthesis, leading to reduced plant growth or even plant mortality (Cleophas et al., 2023).

2.2.3 Limiting factors of coal tailings on growth of plants

Plants are regularly used for phytoremediation and rehabilitation purposes to reclaim current and/or old mines and mine tailings. However, these tailings are heterogeneous in terms of physical, mineralogical and chemical properties, which makes remediation attempts challenging

(Daniels et al., 2010; Gajić et al., 2018). Furthermore, it has variable pH, nutrient deficiencies, high soluble salt content and heavy metals (Gajić et al., 2018).

Table 2.2: Impact of coal mining on specific areas of the environment

Limiting Factor	Description	Impacts	Potential Solutions	References
High Heavy Metal Content	Coal tailings contain high levels of heavy metals, particularly Zn, Pb, As, and Cu. Other metals include Hg, Cd, B, Co, Mn, Ni, Se, Mo, and Cr.	Disrupts photosynthesis and inhibits plant growth.	Use of heavy metal-tolerant and hyperaccumulator plant species for phytoremediation.	(Cleophas et al., 2023; Dooge et al., 2007; Gajić et al., 2018; Kossoff et al., 2014; Mardonova & Han, 2023)
Soil Acidification	Coal tailings often have high sulfate content, leading to acid mine drainage (AMD). Soil pH is highly variable and can change rapidly.	Low pH inhibits plant growth and nutrient availability.	Cultivation of acid-tolerant plant species.	(Daniels et al., 2010; Dooge et al., 2007; Neina, 2019)
Low Fertility	Coal tailings lack essential nutrients, particularly nitrogen (N) and phosphorus (P).	Poor plant growth and establishment without external fertilization.	Use of nitrogen-fixing plants such as legumes to naturally improve soil fertility.	(Daniels et al., 2010; Gajić et al., 2018)
Particle Size / Water Retention	Heterogeneous particle size leads to variable moisture retention. Larger particles reduce water-holding capacity due to faster percolation. Poor soil structure overall.	Difficulty maintaining adequate soil moisture levels for plant growth.	Select plant species adapted to variable moisture conditions; possible soil amendments to improve structure.	(Daniels et al., 2010; Gajić et al., 2018; Kossoff et al., 2014)

Despite these limitations (Table 2.2), existing literature indicates that kenaf can tolerate adverse conditions and grow in contaminated soils. However, to develop effective and sustainable remediation strategies, it remains essential to investigate the physiological responses and absorption mechanisms of plants under such unfavourable environmental conditions (Gajić et al., 2018; Yan et al., 2020).

2.3 Plant physiological studies

Photosynthesis is a fundamental process for plant survival and overall health. It occurs within the chloroplasts, specifically in the thylakoid membranes, where a series of light-dependent chemical

reactions take place (Ördög, 2011). During this process, solar energy is harnessed to drive the electron transport chain, ultimately producing energy-rich molecules essential for plant growth and function (Hopkins & Huner, 2009). Understanding plant physiological processes, particularly photosynthesis, is crucial in selecting appropriate species for the remediation and restoration of abandoned, closed, or active mining sites (Gajić et al., 2018; Roy et al., 2024). Since photosynthetic activity reflects a plant's stress response, resilience, productivity, and adaptability, it is a valuable indicator of success in mine rehabilitation efforts.

2.3.1 Relation between the environment, physiology, and growth

There are certain environmental factors that affect the physiological performance of plants, which in turn has an influence on the growth and yield (Roy et al., 2024). Figure 2.5 illustrates how the environmental factors, plant physiology and plant growth correlate.

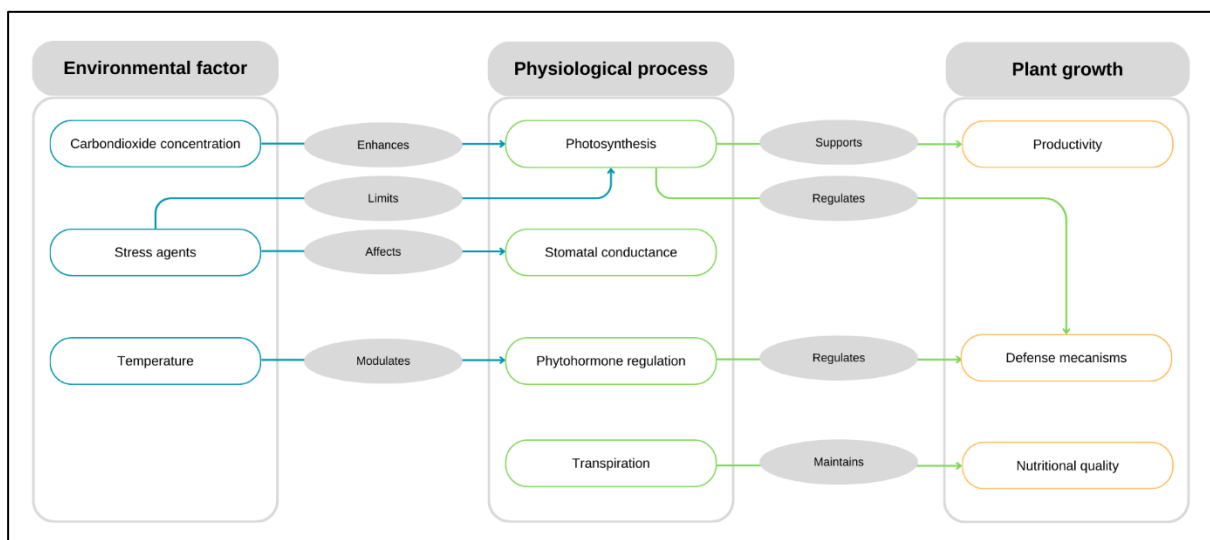


Figure 2.5: A diagram illustrating the effects of CO₂ concentration, stress agents and temperature on photosynthesis, stomatal conductance, phytohormone regulation, and transpiration, followed by the effects on the productivity, defence mechanisms, and nutritional quality of a plant, adapted from (Roy et al., 2024).

In this study, it is essential to evaluate the photosynthetic efficiency of the plants since yield and growth of plants are dependent on the process of photosynthesis (Bussotti et al., 2020a). Plant's survival is dependent on the synthesis of energy molecules like ATP and NADPH (Ghosh et al., 2023). These molecules are then used for fixing CO₂ into energy-storing molecules like sugars (Ghosh et al., 2023), which are essential for the growth of plants (Hopkins & Huner, 2009).

2.3.2 Distinction between photochemical and photosynthetic efficiency

The process where light is absorbed and converted to energy storage molecules is known as the photochemical efficiency of the plant (Krause, G.H. & Weis, 1991). This refers to the light-dependent phase of photosynthesis. On the other hand, the photosynthetic efficiency of a plant takes the light-dependent and light-independent phases into account. This includes the Calvin cycle, where Rubisco plays an essential role in photosynthesis as well as photorespiration (Ghosh, et al., 2023). An ideal photorespiration is achieved by maintaining a CO₂ concentration gradient and ensuring the necessary substrates for the photosynthetic carbon reduction (PCR) cycle (Hopkins & Huner, 2009).

This process of light being absorbed by plants and used for photochemical processes to produce essential molecules for the survival of the plant is dependent on each other and is influenced by environmental factors (Roy et al., 2024). Therefore, it is important to evaluate these interactions to determine the plants' growth outcome. This can be executed with chlorophyll fluorescence.

2.3.3 Chlorophyll a fluorescence as a method to monitor plant physiological efficiency

One of the most widely used methods to evaluate the photochemical vitality of plants, especially under stressful conditions, is chlorophyll a fluorescence (Bussotti et al., 2020a; Krause, G.H. & Weis, 1991; Singh et al., 2022). Chlorophyll a fluorescence can be divided into three types of fluorescence, namely prompt fluorescence (PF), modulated 820nm reflection (MF), and delayed fluorescence (DF) (Kalaji et al., 2017). This study focuses on the data retrieved with the Multi-Function Plant Efficiency Analyzer (M-PEA) fluorimeter. This technique utilizes chlorophyll fluorescence parameters, both modulated and prompt fluorescence, in vivo (Salvatori, et al., 2014). Specifically, to study the physiological response of plants to stresses by evaluating the rate of electron transport during photosynthesis (Guidi et al., 2019; Samson et al., 1999). As a result, this method can be used in various fields such as research, crop production, ecosystem monitoring and phenotyping (Salvatori et al., 2014; Singh et al., 2022).

Prompt fluorescence

Prompt fluorescence is measured after dark-adapted plants are exposed to a short (1 s) pulse of red light (650 nm) (Bussotti et al., 2020b). Then, the OJIP curve, also known as the fluorescence curve, is produced when dark-adapted plants experience fluorescence induction (Salvatori et al., 2014). This curve is polyphasic and produces four main steps, O (0.03 ms), J (2 ms), I (30 ms), and P (300 ms), where O is also F₀ and P is F_m (Figure 2.6) (Bussotti et al., 2020b; Salvatori et al., 2014).

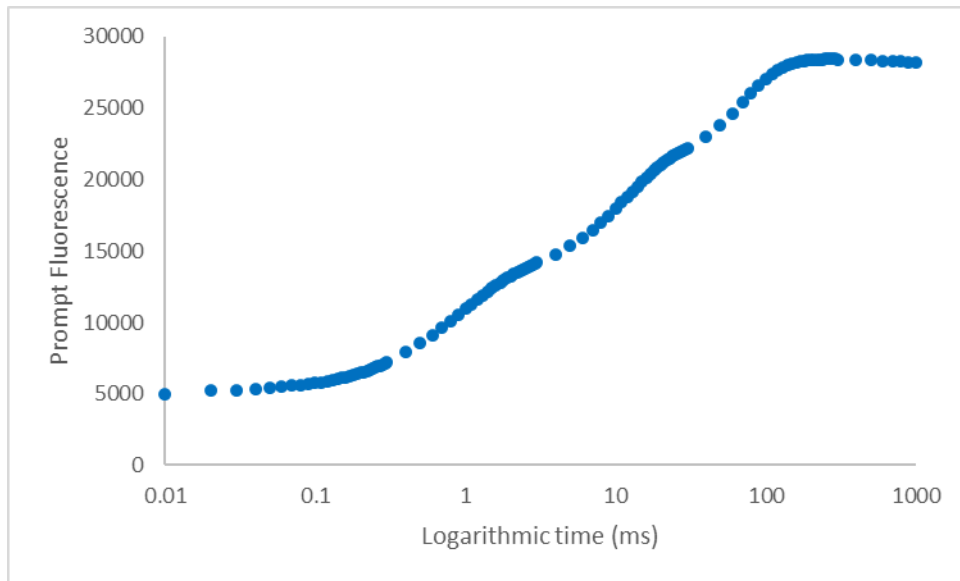


Figure 2.6: The OJIP transient derived from data gathered with the M-PEA, depicting the prompt fluorescence after dark adaptation over logarithmic time (ms). This transient translates into PSII function.

The OJIP fluorescence transient, obtained from dark-adapted leaves, can be analysed using the JIP-test, which provides insight into energy absorption (ABS), trapping (TR), electron transport (ET), and reduction (RE) (Bussotti et al., 2020b).

Table 2.3: Definitions and roles of parameters used in the JIP-test of chlorophyll *a* fluorescence (Hopkins & Huner, 2009).

Parameter		Description	Role in Photosynthesis
ABS	Absorption	Light energy is captured by pigments	Starts the light reaction
TR	Trapping	Energy is transferred to the reaction center of chlorophyll	Excites electrons for transport
ET	Electron Transport	Electrons move through ETC, generating ATP	Powers ATP synthesis
RE	Reduction	Electrons reduce NADP^+ to NADPH	Stores energy for the Calvin cycle

Thus, prompt fluorescence is used to determine the redox efficiency of photosystem II (PSII) and electron transport to the end electron acceptors (Salvatori et al., 2014).

Modulated 820 nm reflection

The signal measured at 820 nm provides information about the transportation of electrons from the plastoquinone (PQ) pool to the end electron acceptors of photosystem I (PSI), emphasizing the oxidation and re-reduction of PSI (Salvatori et al., 2014). The modulated 820 nm reflection curve (Figure 2.7) can be derived from this signal.

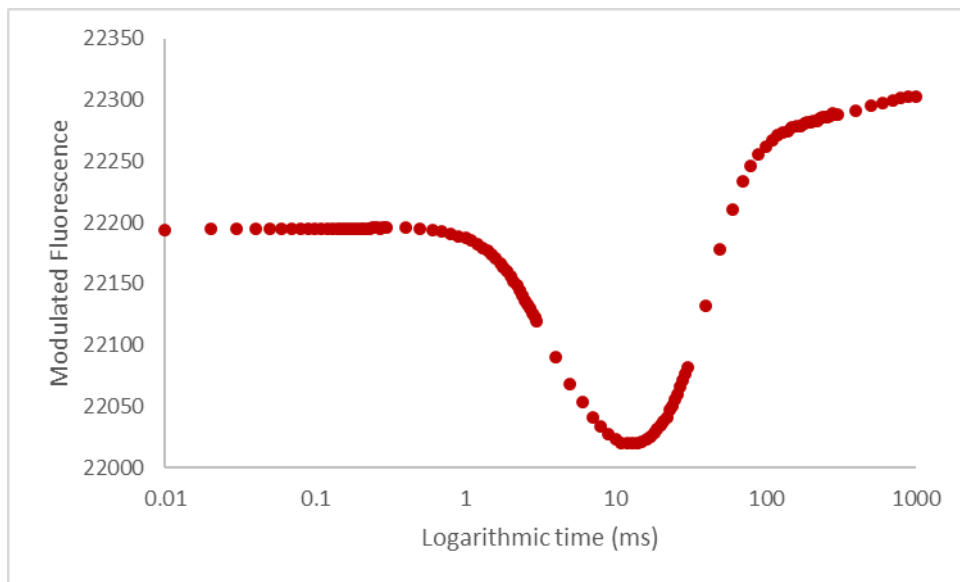


Figure 2.7: The modulated 820 nm reflection curve derived from data gathered with the M-PEA, depicting the modulated fluorescence after dark adaption over logarithmic time (ms). This curve gives important information about PSI function.

The modulated 820 nm reflection curve consists of a fast phase (oxidation) and a slow phase (re-reduction) and can be correlated with the OJIP transient.

Table 2.4: Fast phase and slow phase of the modulated 820 nm reflection curve, its time scale, and key components (Hopkins & Huner, 2009).

Phase	Time Scale	Primary Event	Key Components	Interpretation
Fast phase	1–10 ms	Oxidation of P_{700} and PC	P_{700}^+ , PC^+	PSI activation, electron loss
Slow phase	100 ms – seconds	Reduction of P_{700}^+ and PC^+	e^- from PSII	Replenishing electrons from PSII

As the curve reaches the end of the fast phase (minimum), the PQ pool is reduced and can be compared to the J-I step of the OJIP curve (Kalaji et al., 2017). Similarly, the rise in the modulated signal (slow phase) represents the second reduction (re-reduction) of plastocyanin (PC^+) and the

reaction centre in PSI (P_{700}^+) and is comparable to the I-P step of the OJIP curve (Kalaji et al., 2017).

Chlorophyll content

Chlorophyll plays a critical role in photosynthesis since it forms the antenna structures in the light-harvesting complexes (LHC) in the photosystems, which is responsible for the absorption of sunlight energy (Hopkins & Huner, 2009; Ördög, 2011). Therefore, the amount of chlorophyll present affects the photochemical efficiency of plants (Gao et al., 2014). Various methods can measure chlorophyll concentration in plants, but in this study, the focus will be on measurements with CCM-300 chlorophyll meter. With this method, the total chlorophyll content is measured in mg.m^{-2} (Opti-Sciences Inc., 1999). Furthermore, it is based on fluorescence, whereby the emission ratio at 735 nm and 700 nm are measured and calculated to give the relative chlorophyll concentration (Opti-Sciences Inc., 1999). The fluorescence at the P-step of the OJIP curve (F_p) is directly proportional to the total chlorophyll concentration in the plant (Gao et al., 2014). Since the CCM-300 takes fluorescence emissions into account, it gives an indication of the amount to active chlorophyll pigments in the plants (Hopkins & Huner, 2009; Opti-Sciences Inc., 1999). Thus, correlations and observations can be made by comparing the OJIP curve with the chlorophyll content.

2.3.4 The electron transport chain – photochemical phase and thermal phase parameters

The electron transport chain (Figure 2.8) is the first step in photosynthesis, where protein complexes form a chain of chemical reactions by using water and sunlight to produce energy molecules, NADPH and ATP (Hopkins & Huner, 2009). The main protein complexes involved in this process are photosystem II (PSII), cytochrome b_6f , photosystem I (PSI) and ATP synthase (Ördög, 2011).

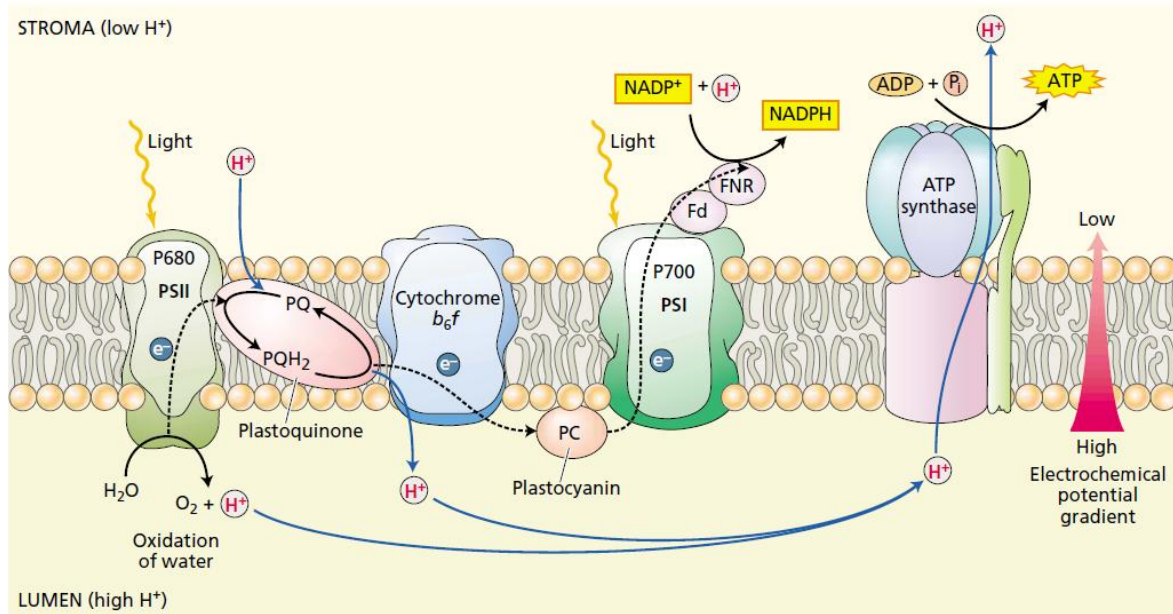


Figure 2.8: The electron transport chain (ETC) taking place in the thylakoid membranes of plants (Taiz et al., 2023).

During the ETC, H_2O is oxidized in PSII and produces O_2 on the lumen side, where photons are absorbed and through an excited reaction center (P_{680}), an electron is released (Ördög, 2011). Plastoquinone (PQ) is then reduced to form plastoquinol (PQH_2). Following this, PQH_2 is oxidized by cytochrome b_6f complex to transport electrons to PSI with a transporter, plastocyanin (PC) (Hopkins & Huner, 2009). During the oxidation of PQH_2 , H^+ molecules are transferred through the membrane and a proton gradient is formed in the lumen. When the electrons reach PSI, NADP^+ is reduced to form NADPH by means of ferredoxin (Fd) (Ördög, 2011). Lastly, ATP synthesis is caused by the proton gradient that is pumped through ATP synthase from the lumen to the stroma (Hopkins & Huner, 2009; Ördög, 2011).

The ETC correlates with the OJIP curve (Figure 2.6) and modulated 820 nm reflection curve (Figure 2.7) and can be drawn on the same graph to give an overall view of the photochemical efficiency of PSII and PSI respectively (Figure 2.9) (Hopkins & Huner, 2009; R. J. Strasser).

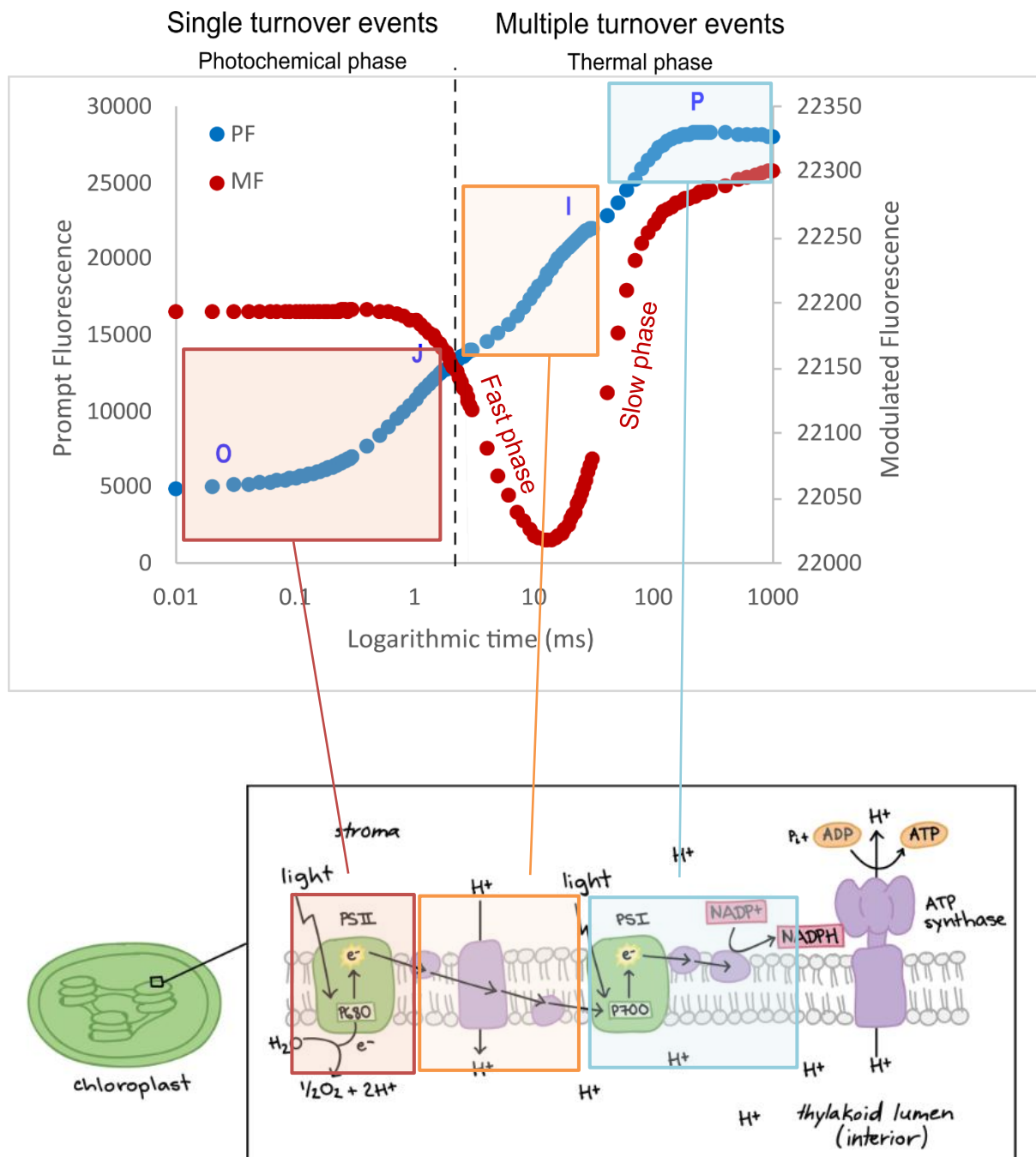


Figure 2.9: Diagram depicting the correlation between the OJIP curve, modulated 820 nm reflection curve and the electron transport chain by using images extracted from <https://11nq.com/KhanAcademy>.

Many parameters derived from these curves can be used to define the photochemical efficiency and performance of the plant; however, in this study, the ETC is divided into two phases, photochemical phase and thermal phase, along with its parameters (Strasser et al., 2004)

Photochemical phase

The photochemical phase focuses on PSII function, where sunlight energy is absorbed and trapped to split water and release an electron, also known as the single turnover events (Strasser et al., 2004). The key parameters to evaluate this phase include F_v/F_m , PI_{ABS} , Ψ_{Eo} , and ϕ_{Eo} (Bussotti et al., 2020b). These parameters are only derived from the OJIP curve. Ultimately, these parameters signify the capacity of the plant to absorb and trap sunlight energy (Hopkins & Huner, 2009). F_v/F_m is a robust parameter that correlates strongly with Ψ_{Eo} , and ϕ_{Eo} , which is proportional to the rest of the ETC and PSI, where PI_{ABS} relates only to the photochemical phase (Bussotti et al., 2020b). Since F_v/F_m considers the first few steps of the ETC, it is a very common parameter to use to define the overall efficiency of PSII (Bussotti et al., 2020b; Strasser et al., 2004).

Thermal phase

The parameters representing this phase are derived from the OJIP curve and modulated 820 nm reflection curve. The OJIP-derived parameters include ΔV_{IP} , δ_{Ro} , and ϕ_{Ro} , where ΔV_{IP} represents the I-P phase of the OJIP curve (Bussotti et al., 2020b; Zivcak et al., 2015). This parameter is similar to F_v/F_m , in the sense that it is a good parameter to use for the overall thermal phase of the ETC. These three parameters define the flow of electrons from PQ to the end-electron acceptors in PSI (Bussotti et al., 2020b). Although the thermal phase is mainly linked to PSI, it is highly dependent on PSII. Thus, it still forms part of the OJIP parameters. However, when considering the parameters derived from the modulated 820 nm reflection curve, only PSI function is defined (Kalaji et al., 2017). These parameters include MR_o/MR_{min} , and the slopes of the fast- and slow phase respectively (Schansker et al., 2014). The oxidation and re-reduction of PSI is evaluated with these parameters and forms part of the thermal phase (Zivcak et al., 2015). The fast phase of the modulated 820 nm reflection curve indicates an increase in oxidized states of P_{700} and PC, and the slow phase shows the re-reduction of these components (Salvatori et al., 2014). Also, the fast phase occurs during the J-I phase of the OJIP curve, where the slow phase occurs during the I-P phase (Salvatori et al., 2014; Zivcak et al., 2015).

Photochemical and thermal phase

There are also parameters that are used to define the overall efficiency of photosynthesis. In this case, the PI_{Total} and chlorophyll content are discussed. PI_{Total} provides an integrated measure of PSII performance, encompassing absorption, trapping, and electron transport capacity (Bussotti et al., 2020b). Chlorophyll content is also directly proportional to PSII and PSI function, since both photosystems contain chlorophyll to absorb and retain sunlight energy (Gao et al., 2014; Ördög, 2011). It is important to evaluate both PSII and PSI functions to be able to make accurate observations since they have different functions. PSII absorbs red light to split water and release an electron, where PSI absorbs far-red light and reduces $NADP^+$ to NADPH (Ördög, 2011).

Therefore, the photosynthetic function of a plant can be defined by the singular parameter of PI_{Total} , or two separate parameters, namely F_v/F_m and ΔV_{IP} (Bussotti et al., 2020b).

2.3.5 Mechanisms in plants to detoxify heavy metal polluted soils

As discussed earlier, heavy metals have detrimental effects on plant growth. Various plants have developed mechanisms to extract heavy metals and other pollutants from soil (Cleophas et al., 2023). These mechanisms can be classified broadly under phytoextraction, phytovolatilisation, phytostabilization, and rhizodegradation as illustrated in Figure 2.10 (Cleophas et al., 2023; Yan et al., 2020).

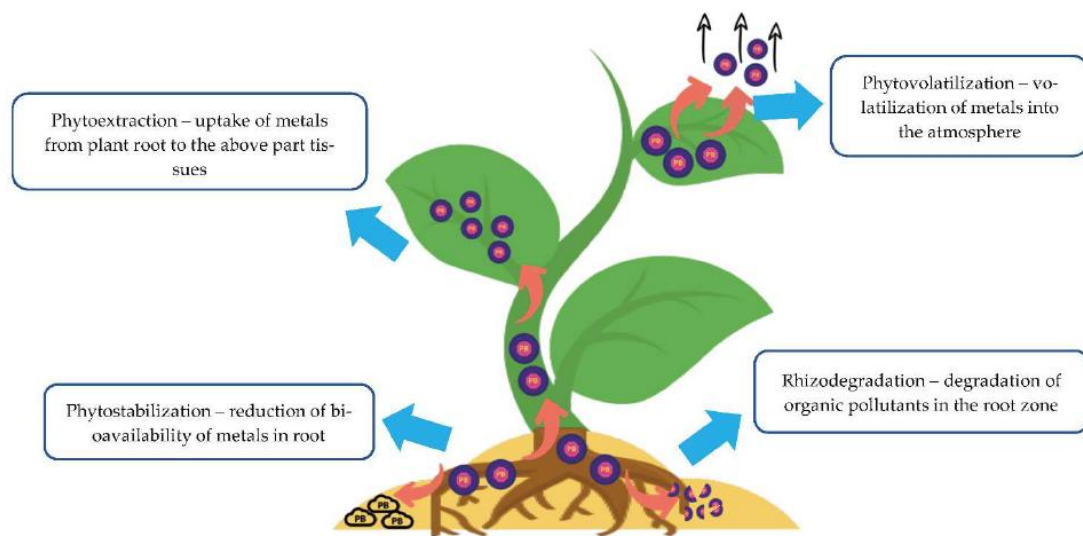


Figure 2.10: Schematic diagram illustrating the different mechanisms of plants in the process of phytoremediation (Cleophas et al., 2023).

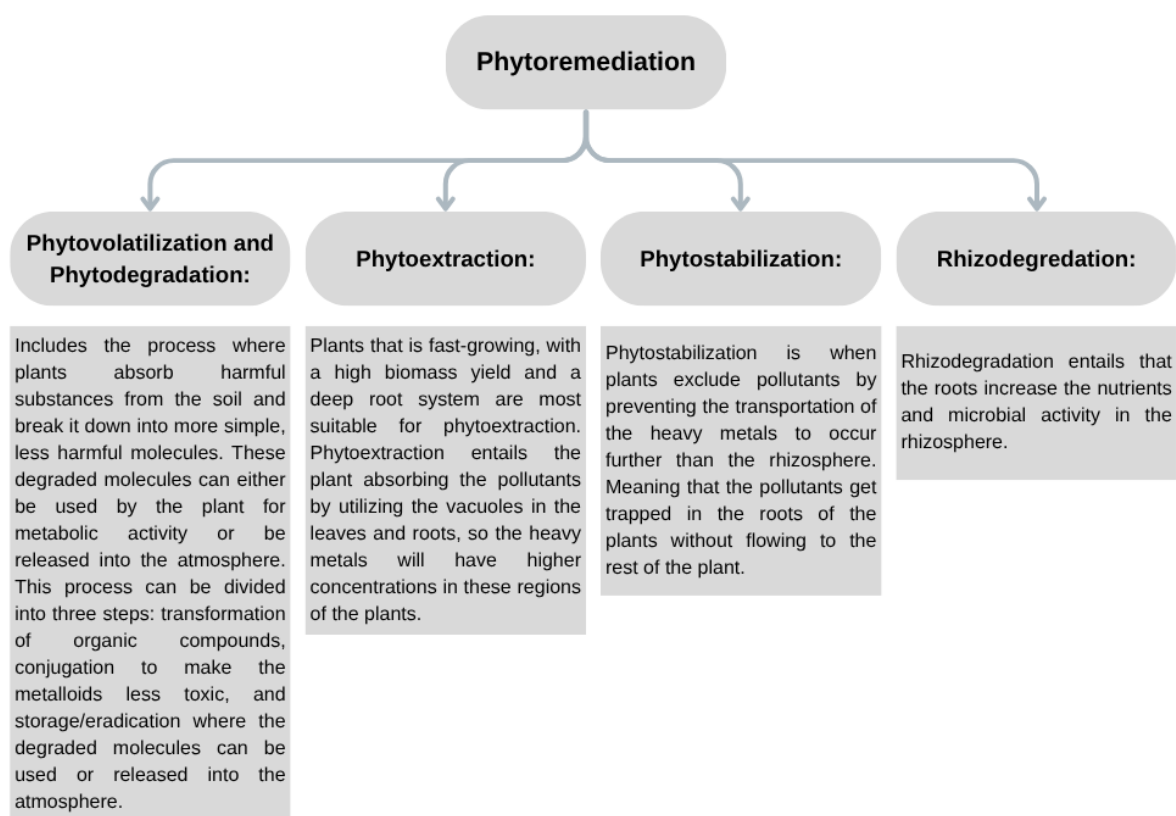


Figure 2.11: A flow diagram defining the mechanisms of phytoremediation (Gajić et al., 2018; Yan et al., 2020).

Furthermore, these four mechanisms can also be classified under the plants' primary methods of defence against toxic metals, namely avoidance and tolerance (Yan et al., 2020). Avoidance is when plants limit the amount of heavy metals that it absorbs, by utilizing the exclusion barriers from the roots to the shoot of the plant (Gajić et al., 2018). This results in high concentrations of heavy metals in the root systems of plants (Gajić et al., 2018). Also, the roots cause a change in the pH of the rhizosphere, preventing the plant from absorbing the heavy metals present in the soil (Yan et al., 2020). Tolerance can be seen as the second line of defence. If the plant couldn't avoid the heavy metals, it has mechanisms that make it tolerant. This includes chelation, whereby the plant reduces the amount of free metal ions, making it less toxic for the plant (Gajić et al., 2018). This allows the pollutants to be stored in areas of the plant where they are less harmful. In the case where neither avoidance nor tolerance can take place, the production of reactive oxygen species (ROS) is triggered, which leads to severe physiological damage (Yan et al., 2020). As a result, the plant will activate the ROS-scavenging machinery as a defence mechanism.

2.3.6 Effects of trace elements on photosynthesis

The most common trace elements found in coal tailings include lead (Pb), mercury (Hg), nickel (Ni), arsenic (As), chromium (Cr), selenium (Se), zinc (Zn), copper (Cu), cobalt (Co), iron (Fe), manganese (Mn), sodium (Na), cadmium (Cd), and magnesium (Mg) (Zhao et al., 2022). For this study, these elements can roughly be classified under toxic and non-toxic elements. These trace elements can be classified as “toxic” if the absorption of the element causes a decline in the physiological performance of the plant, whereas the “non-toxic” elements have no adverse effects on the plant. Each one of these trace elements has an impact on the physiological processes of plants according to Table 2.5

Table 2.5: Trace elements found in coal and coal tailings and the impact or role they have on the physiological processes of plants (Cleophas et al., 2023; Gajić et al., 2018; Saleem et al., 2020; Yan et al., 2020; Zhao et al., 2022).

Physiological effect:	Trace element:									
	As	Pb	Hg	Co	Mn	Cd	Cu	Zn	Ni	Fe
Contribute to plant growth and photochemical processes					X		X	X	X	X
No known biological role in plants	X	X	X			X				
Limit biomass and yield	X	X	X			X				
Decrease chlorophyll synthesis	X	X	X	> 50 mg/kg	<10 mg/kg and >500 mg/kg	X	>20 mg/kg	< 10-20 mg/kg	> 40 mg/kg	> 500 mg/kg

Some trace elements, including Mn, Cu, Zn, Ni, and Fe, contribute to plant growth, (with the exception of specific concentration thresholds), while others, like As, Pb, Hg, and Cd, are growth-limiting factors. The majority of the toxic trace elements specifically have a limiting effect on chlorophyll synthesis in plants, which in turn affects photosynthetic efficiency.

2.4 Growth potential of kenaf on coal tailings in South Africa

Phytoremediation has gained increasing attention due to its cost-effectiveness and environmental sustainability, as it harnesses the natural ability of plants to absorb pollutants from the soil. Fast-growing species with substantial biomass are considered ideal for this purpose. As a result, kenaf (*Hibiscus cannabinus*) has been identified as a potential candidate for mine site remediation (Saleem et al., 2020). Previous studies have explored the use of kenaf as a hyperaccumulator of heavy metals (Saleem et al., 2020). However, the presence of heavy metals in soil can inhibit plant growth, posing significant challenges to plant establishment and development (Cleophas et al., 2023; Roby et al., 2017).

Despite these challenges, kenaf demonstrates high adaptability to a range of environmental conditions and soil types (Monti & Alexopoulou, 2013). Research indicates that kenaf exhibits greater tolerance to heavy metals compared to related species, which have shown adverse responses under similar conditions (Zhao et al., 2022). During these studies, kenaf was treated with various heavy metals to examine its response to the treatments. Additional studies have investigated the use of soil amendments to enhance kenaf growth on contaminated mine sites, with a focus on biomass production (Roby et al., 2017). On the other hand, although research shows kenaf's potential to absorb pollutants, root harvesting can be challenging due to its fine, dense root system. Also, since phytoremediation is a slow process, the short life cycle of kenaf can slow the process even further because of interrupted plant and harvesting seasons.

Existing research on kenaf mainly focuses on ecological aspects, including the biomass production and growth measurements (Cleophas et al., 2023). Furthermore, the phytochemistry and pharmacological value of kenaf was researched, with the focus on the medicinal constituents and uses (Sim & Nyam, 2021). Yet, there remains a notable gap in studies examining its physiological responses under phytoremediation conditions. Moreover, most research has been conducted in European and Asian contexts, with limited studies focused on South African conditions (Monti & Alexopoulou, 2013). Trials have predominantly been carried out in Cambodia, India, Pakistan, China, Indonesia, Thailand, Cuba, Vietnam, and Brazil (Cleophas et al., 2023). In South Africa, several plant species have been evaluated for mine waste phytoremediation, yet kenaf remains unexamined mainly in this context (Gajić et al., 2018). Consequently, existing literature recommends further investigation into kenaf's growth potential and phytoremediation capacity on South African mine tailings (Cleophas et al., 2023; Monti & Alexopoulou, 2013).

2.5 Possible research questions and hypothesis

Numerous studies have explored mine restoration through vegetation establishment or revegetation; however, these investigations have predominantly emphasized the biological, physical, and chemical characteristics of soils and plants (Xu et al., 2023). This focus has resulted in a gap in the literature concerning the physiological responses of plants grown on mine tailings. Given the ongoing and increasing issue of coal tailings in South Africa, along with the growing interest in kenaf (*Hibiscus cannabinus*), this study seeks to address several key questions: What is the physiological response of kenaf planted on coal tailings in the South African context? Can kenaf maintain functional photosynthetic activity under such conditions? Is kenaf capable of absorbing pollutants present in coal tailings?

To address these questions, the study will concentrate on the physiological performance of kenaf when cultivated in coal tailing substrates. Based on current literature, kenaf grown in coal tailings is anticipated to demonstrate reduced, yet functionally viable, photosynthetic efficiency, as assessed through specific physiological parameters outlined in Section 2.3.3.

CHAPTER 3 MATERIALS AND METHODS

3.1 Plant cultivation and experimental layout

This study was conducted as a pot experiment in the greenhouse at Northwest University Potchefstroom Campus with conditions listed in Table 3.1. Five *Hibiscus cannabinus* seeds were planted in each 18 cm diameter pot, which were placed in a Latin square for randomization of the experiment (Figure 3.1). The seedlings were not thinned to a particular number to maximize plant material for heavy metal analysis. To further enhance experimental rigour, six repetitions of the experiment were conducted sequentially over time, ensuring temporal randomisation. Each repetition was subsequently planted four weeks after the previous one, resulting in a total of six pots per treatment.

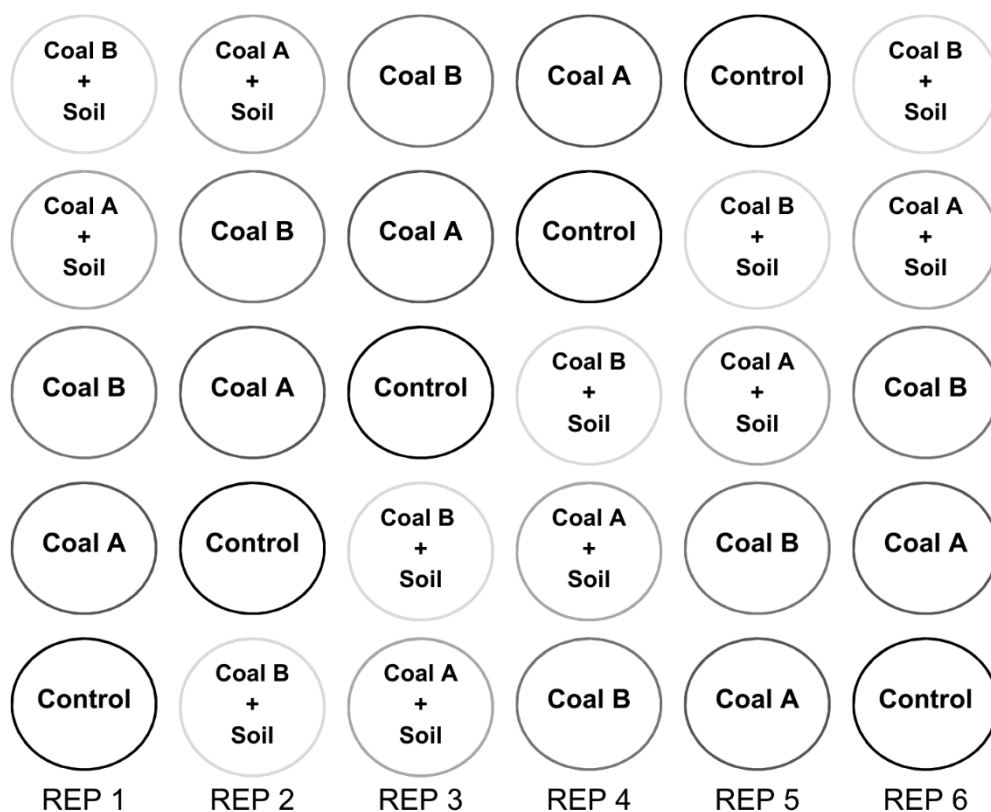


Figure 3.1: Latin square layout of the pots in the greenhouse.

The five soil treatments included sandy-loam soil (Control), raw coal tailings from two different mining sites (Coal A and Coal B) and coal tailings mixed with the sandy-loam soil in a 1:2 ratio (Coal A + Soil and Coal B + Soil). Each repetition was conducted over a period of eight weeks, starting on the day of seed sowing. Furthermore, these repetitions were carried out individually rather than concurrently to account for potential time-related variability. Plants were watered every

other day and a water-soluble fertilizer, Multifeed Classic, was applied weekly. This was applied according to the recommended concentration of the product.

Table 3.1: Environmental conditions of the Northwest University greenhouse during the months the experiment was conducted.

	Relative humidity (%)	Minimum Temperature (°C)	Maximum Temperature (°C)	CO ₂ concentration (PPM)	Daylength (hours)
August	46.34	10.20	34.40	470.76	16
September	38.22	10.90	40.40	458.81	16
October	38.50	12.30	43.00	457.87	16
November	42.44	16.4	39.20	457.74	16

3.2 Physiological measurements

When the first two true leaves appeared, roughly after three weeks, physiological measurements were taken weekly until the completion of each repetition. Each repetition was conducted over eight weeks. Photochemical readings were taken with a Multi-Function Plant Efficiency Analyzer (M-PEA) fluorimeter (Hansatech Instruments, King's Lynn, Norfolk, UK) to retrieve information about the prompt chlorophyll a fluorescence and modulated 820 nm reflection. This device uses a far-red modulated 820 nm LED, which is optically filtered to measure the P_{700} absorbance. The activity of P_{700} is then recorded with a fast-response PIN photodiode and a 16-bit AD converter. With these measurements, the plant gets exposed to a beam of light with an intensity of up to $1000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ by the M-PEA. This light is emitted by three diodes with a 5 mm diameter focus and 12-bit resolution. Each reading is recorded after 1 second of light emission. After one hour of dark adaptation, eight readings per plant were taken on different leaves of the plants. No particular leaves were measured every week; however leaves were selected at random which were at different stages. Specific physiological parameters, derived from this data by the M-PEA, were analysed to determine the vitality of *H. cannabinus* when cultivated in coal tailings. The M-PEA Plus V1.10 software was used to determine the different parameters. The M-PEA records data regarding prompt fluorescence and modulated 820 nm reflection simultaneously.

Prompt fluorescence

The prompt fluorescence data gives information about the function of PSII in the electron transport chain. An OJIP transient results from the data from which other vital parameters are calculated

(Table 3.2). This study focused on the following OJIP-derived parameters (Bussotti et al., 2020b; Yusuf et al., 2010).

Table 3.2: Prompt fluorescence parameters derived from the OJIP transient

Photochemical phase – Single turnover events		
F_v/F_m	$= (F_M - F_0)/F_M$ where $F_0 = F_{0.03}$ $F_M = F_{300}$	Maximum quantum yield efficiency.
PI_{ABS}	$= \frac{\gamma_{RC}}{1-\gamma_{RC}} \cdot \frac{\phi_{P_0}}{1-\phi_{P_0}} \cdot \frac{\Psi_{E_0}}{1-\Psi_{E_0}}$ $= \frac{RC}{ABS} \cdot \frac{TP_0}{ABS-TP_0} \cdot \frac{ET_0}{TP_0-ET_0}$	Absolute performance index - for energy conservation from photons absorbed by PSII to the reduction of intersystem electron acceptors.
Ψ_{E_0}	$= \frac{ET_0}{TP_0}$ $= (1 - V_J)$	Probability that a photon trapped by the PSII reaction centre enters the electron transport chain – probability that electron moves further than Q_A^- .
Φ_{E_0}	$= \phi_{P_0} \cdot \Psi_{E_0}$ $= \frac{ET_0}{ABS}$	Quantum yield of electron transport.
Thermal phase – Multiple turnover events		
I-P phase	ΔV_{IP}	Amplitude of the contribution of the I-rise to P-rise of the OJIP curve.
δ_{R_0}	$= \frac{RE_0}{ET_0}$ $= \frac{(1-V_I)}{(1-V_J)}$	Probability that an electron is transported from reduced PQ to the electron acceptor side of PSI.
Φ_{R_0}	$= \phi_{P_0} \cdot \Psi_{E_0} \cdot \delta_{R_0}$ $= \frac{RE_0}{ABS}$	Quantum yield for the reduction of end-electron acceptors at the PSI acceptor side.
PI_{TOT}	$= PI_{ABS} \cdot \frac{\delta_{R_0}}{1-\delta_{R_0}}$ $= PI_{ABS} \cdot \frac{RE_0}{ET_0-RE_0}$	Total performance index - for energy conservation from photons absorbed by PSII to the reduction of PSI end-electron acceptors.

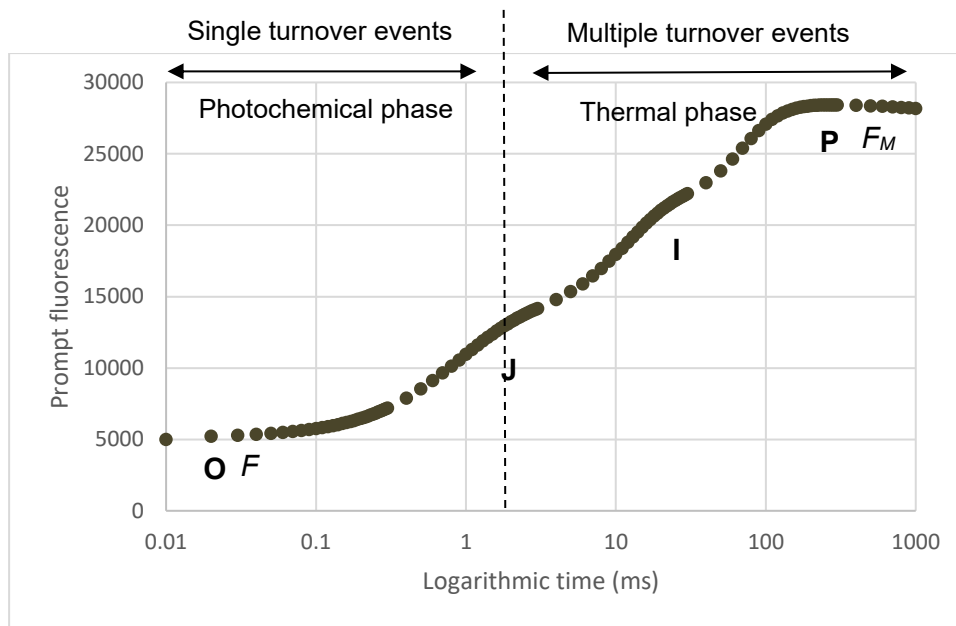


Figure 3.2: Standard OJIP curve depicting the two phases of the electron transport chain and critical inclinations O, J, I, P.

Figure 3.2 illustrates the expected shape of a normal OJIP transient, including the single turnover events, which are part of the photochemical phase of the electron transport chain. Also, the multiple turnover events, which are part of the thermal phase of the electron transport chain.

Modulated 820 nm reflection

Modulated 820 nm reflection readings represent the oxidation and re-reduction of PSI. Thus, the parameters derived from the MR_{820} curve were used to evaluate the state of PSI in the plants. Furthermore, Table 3.3 shows the parameters to analyse the results of the M-PEA measurements (Petrova et al., 2021).

Table 3.3: Modulated Prompt fluorescence parameters derived from the modulated 820 nm reflection curve.

Modulated 820 nm reflection parameters		
MR_o/MR_{min}	$= MR_o/MR_{min}$	The rate at which oxidation and re-reduction of PSI occurs.
Slope of fast phase	$= Slope = \frac{\Delta y}{\Delta x}$ (1-3 ms)	The rate at which oxidation of PSI occurs. $P_{700} \rightarrow P_{700}^+$
Slope of slow phase	$= Slope = \frac{\Delta y}{\Delta x}$ (20-40 ms)	The rate at which re-reduction of PSI occurs.

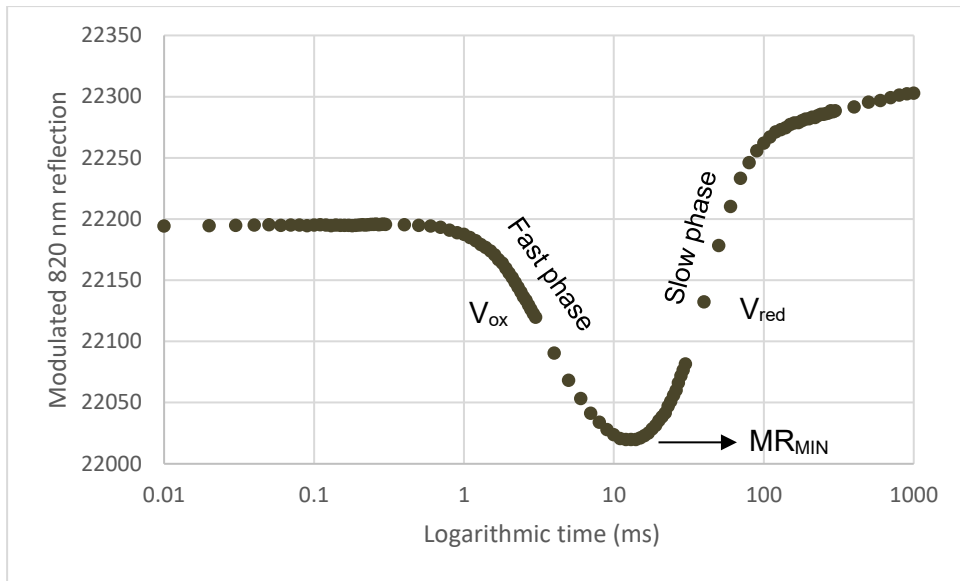


Figure 3.3: Modulated 820 nm reflection curve illustrating the oxidation and re-reduction of PSI.

The modulated 820 nm reflection curve describes the slow- and fast phase of the oxidation and re-reduction of PSI. The abovementioned parameters (Table 3.3) are calculated using the curve in Figure 3.3.

3.3 Chlorophyll content

Chlorophyll content was quantified using a Chlorophyll Content Meter (CCM-300, Opti-Sciences, USA), which accurately measures the chlorophyll fluorescence ratio between 735 nm and 700-710 nm. This ratio is linearly proportional to chlorophyll concentration (mg/m²). The chlorophyll fluorescence ratio (CFR) was calculated using the following formula:

$$CFR = \frac{735 \text{ nm}}{700 \text{ nm}}$$

where:

- CFR = Chlorophyll Fluorescence Ratio
- Intensity at 735 nm = Measured fluorescence intensity at 735 nm
- Intensity at 700 nm = Measured fluorescence intensity at 700 nm

The CCM-300 is particularly advantageous for this study as it can accommodate both small and thick leaf samples without the influence of Kautsky induction effects. In total, five readings were

taken from each pot, encompassing both young and old leaves to ensure a comprehensive assessment of chlorophyll content across different developmental stages.

3.4 Soil and plant analysis

To evaluate the potential of *H. cannabinus* to absorb heavy metals and rare earth elements, soil and plant samples were pooled and sent to Nvirotek Labs for analysis. After each experimental repetition, the plant material was dried and stored in paper bags until all repetitions were completed. Following this, both initial and final soil samples from each treatment were collected and analysed. Additionally, plant material, which included roots, stems, and leaves of *H. cannabinus*, was collected and analysed separately. A standard soil and plant analysis was conducted by Nvirotek for all samples. Analysis for heavy metals and rare earth elements was outsourced to an unidentified specialized laboratory. The concentration of the following elements was determined by the outsourced lab.

Table 3.4: List of heavy metals and rare earth elements that was tested.

Heavy metals		Rare Earth Elements	
Symbol	Element	Symbol	Element
Pb	Lead	La	Lanthanum
Cd	Cadmium	Ce	Cerium
Hg	Mercury	Pr	Praseodymium
Ni	Nickel	Nd	Neodymium
As	Arsenic	Sm	Samarium
Zn	Zinc	Gd	Gadolinium
Bi	Bismuth	U	Uranium
Co	Cobalt	Eu	Europium
Cr	Chromium	Tb	Terbium
Cu	Copper	Dy	Dysprosium
Fe	Iron	Ho	Holmium
Mn	Manganese	Er	Erbium
Sb	Antimony	Tm	Thulium
Se	Selenium	Yb	Ytterbium
Sn	Tin	Lu	Lutetium
V	Vanadium		
Sc	Scandium		
Y	Yttrium		

3.5 Data processing and statistical analysis

All the raw data were first processed in Microsoft Excel, after which it was analysed with statistical software, SigmaPlot (Grafiti LLC, v12.0). Shapiro-Wilk test was used to determine the normality of the data, followed by a One-way Repeated Measures Analysis of Variance (ANOVA) to determine variance. Some of the data were subject to a Two-way Repeated Measures Analysis of Variance (ANOVA). In the case that the variance test showed $p < 0.05$, a Holm-Sidak post-hoc test was applied to determine the specific variance in the data. The data were analysed against a confidence level of 95%, which means statistical significance was considered for p -values < 0.05 . Furthermore, all the graphs depicted in the results chapter were drawn in SigmaPlot.

CHAPTER 4 RESULTS

The results were primarily categorised under two main themes: physiology, and absorption and translocation. In addition, the overall physiological performance of kenaf was assessed in terms of PSI and PSII functionality. More specifically, PSII functionality was further delineated into the photochemical and thermal phases. Each of these categories is defined by the parameters presented in Figure 4.1.

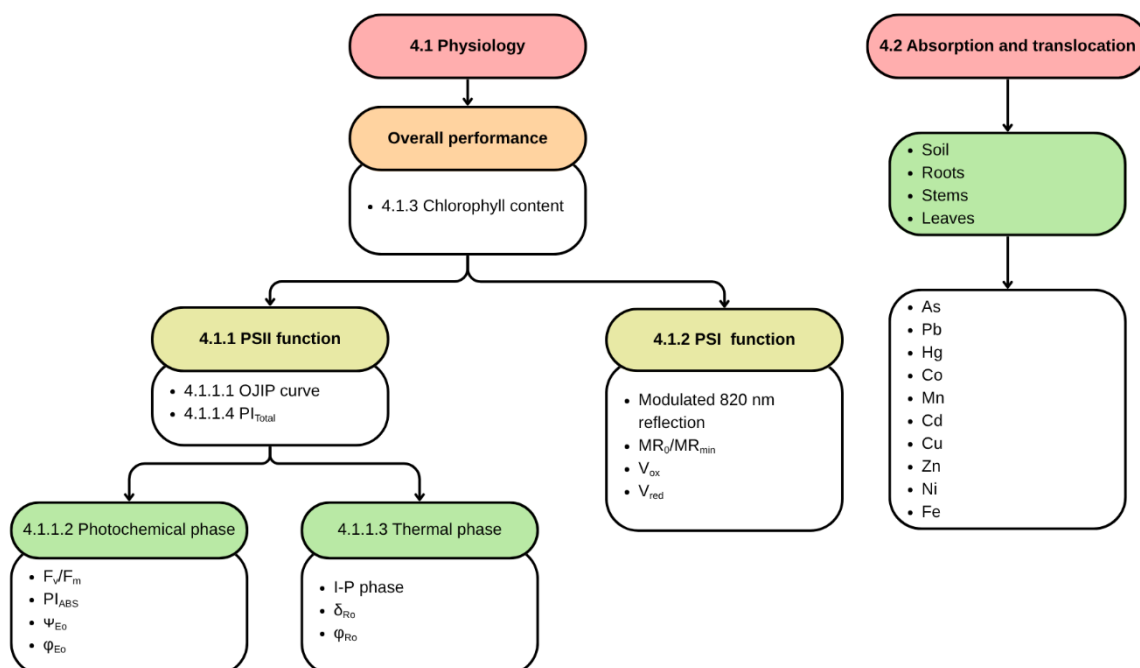


Figure 4.1: Schematic summary of the layout of the parameters and results.

4.1 Physiology

4.1.1 Functionality of Photosystem II

4.1.1.1 OJIP-curve

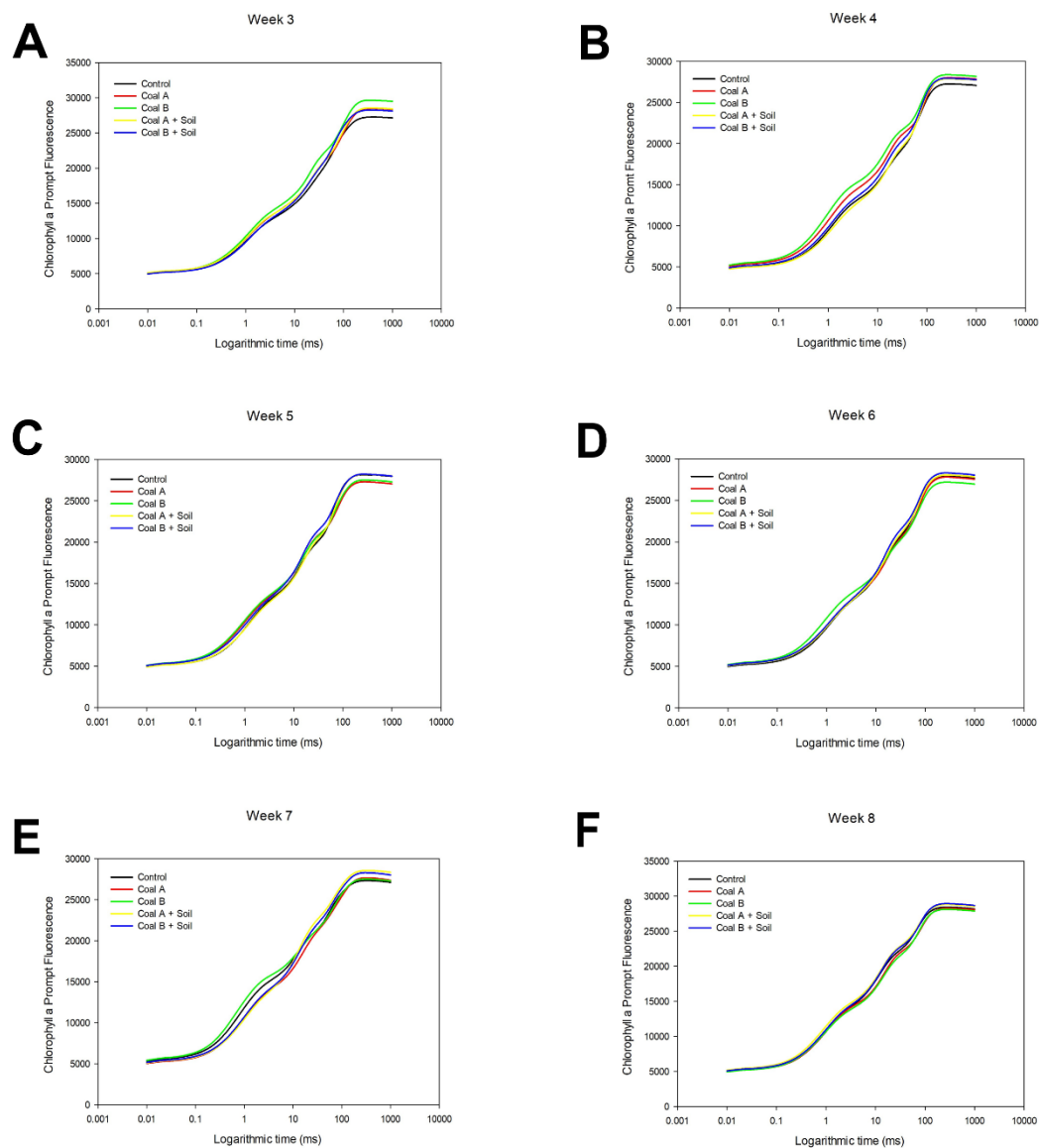


Figure 4.2: The average chlorophyll a fluorescence (OJIP) transient taken over time of the different soil treatments (A) week 3 (B) week 4 (C) week 5 (D) week 6 (E) Week 7 (F) Week 8 after germination of Kenaf.

The OJIP curve, derived from the M-PEA fluorimeter, represents the electron transport chain in photosynthesis. Considering each incline, certain information can be deduced from the critical points of the OJIP transient.

It is evident that the graphs maintained a typical OJIP shape for the two months for all soil treatments (Fig 4.2). During weeks 3 and 4, the shape of the curves of the different treatments seem to vary. To confirm the significance of the critical points, namely O, J, I, and P, the data were subjected to a two-way repeated measures analysis of variance (ANOVA). Following this, the results, according to Table 4.1, showed that there were statistical differences between Coal B and the other treatments in the J-step of the OJIP curve.

Table 4.1: Statistical differences in the critical inclinations of the OJIP curve of Kenaf, indicating p-values for all the treatments, using a confidence level of 95%.

O-step	p<0.05				
J-step	p>0.05				
	Control	Coal A	Coal B	Coal A + Soil	Coal B + Soil
Control					
Coal A	0.939				
Coal B	0.006	0.039			
Coal A + Soil	0.923	0.776	0.001		
Coal B + Soil	0.829	0.906	0.004	0.927	
I-step	p<0.05				
P-step	p<0.05				

Table 4.1 indicates that the only significant differences relative to the OJIP curve, were found in Coal B, which was significantly higher than the other treatments. This indicates that Coal B had the biggest effect on the OJIP curve.

4.1.1.2 Photochemical phase – single turnover events

The electron transport chain can be divided into two phases, namely photochemical- and thermal phase. The photochemical phase can be measured using certain parameters, in this case F_v/F_m , PI_{ABS} , Ψ_{E0} and ϕ_{E0} .

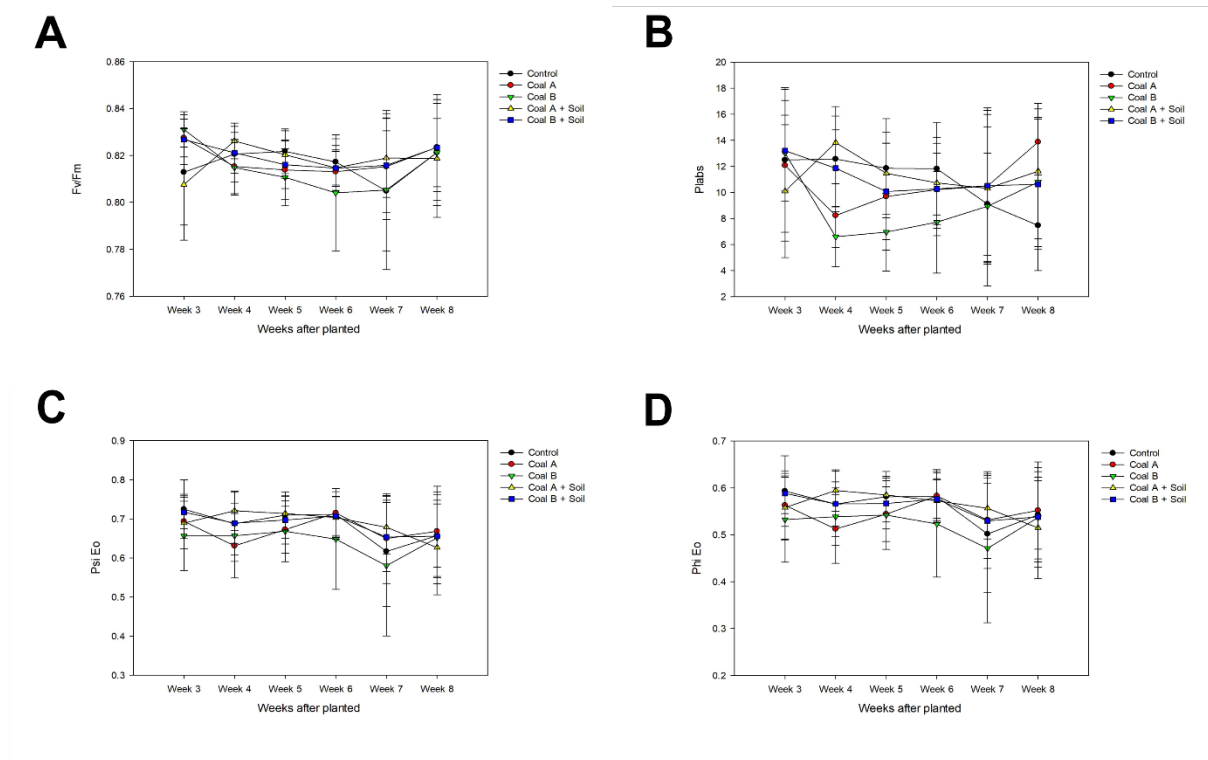


Figure 4.3: Scatter-line graphs illustrating the physiological parameters representing the photochemical phase of the electron transport chain namely (A) Maximum quantum yield efficiency of PSII (F_v/F_m) (B) Absolute performance index (PI_{ABS}) (C) Initiation of electron transport (ψ_{Eo}) (D) Quantum yield of electron transport (ϕ_{Eo}) over eight weeks.

To further explore the parameters related to light absorption, energy trapping, and electron flow, scatter/line plots for each treatment across the experimental period are presented in Figure 4.3. A one-way repeated measures ANOVA was conducted to determine statistical differences among treatments, with significant outcomes reported in Table 4.2.

Table 4.2: Statistical differences in the photochemical parameters derived from the OJIP transient indicating p-values for all the treatments, using a confidence level of 95%.

Photochemical Parameters					
F_v/F_m	p=0.697				
PI_{ABS}	p=0.251				
Ψ_{Eo}	p=0.015				
	Control	Coal A	Coal B	Coal A + Soil	Coal B + Soil
Control					
Coal A	0.817				
Coal B	0.046	0.288			
Coal A + Soil	0.986	0.768	0.029		
Coal B + Soil	0.879	0.801	0.037	0.985	
ϕ_{Eo}	p=0.021				
	Control	Coal A	Coal B	Coal A + Soil	Coal B + Soil
Control					
Coal A	0.815				
Coal B	0.057	0.383			
Coal A + Soil	0.972	0.748	0.04		
Coal B + Soil	0.986	0.752	0.053	0.994	

Statistical analysis revealed that kenaf grown in Coal B exhibited significantly lower p-values for Ψ_{Eo} and ϕ_{Eo} . These parameters represent the probability of electron transport beyond Q_A , indicating that electron flow in kenaf was adversely affected under the Coal B treatment. Table 4.2 shows that p-values of Ψ_{Eo} and ϕ_{Eo} in Coal B were lower than those planted in Coal A + Soil, and Coal B + Soil. The decline in the Coal B treatment parameters appeared after week 6. However, kenaf planted in Coal A did not differ significantly from the other treatments.

4.1.1.3 Thermal phase – multiple turnover events

I-P Phase

As shown in Figure 4.4 (A–F), kenaf in Coal A and Coal B treatments exhibited lower variable fluorescence during week 3, followed by a gradual increase over time, surpassing the other treatments by week 8.

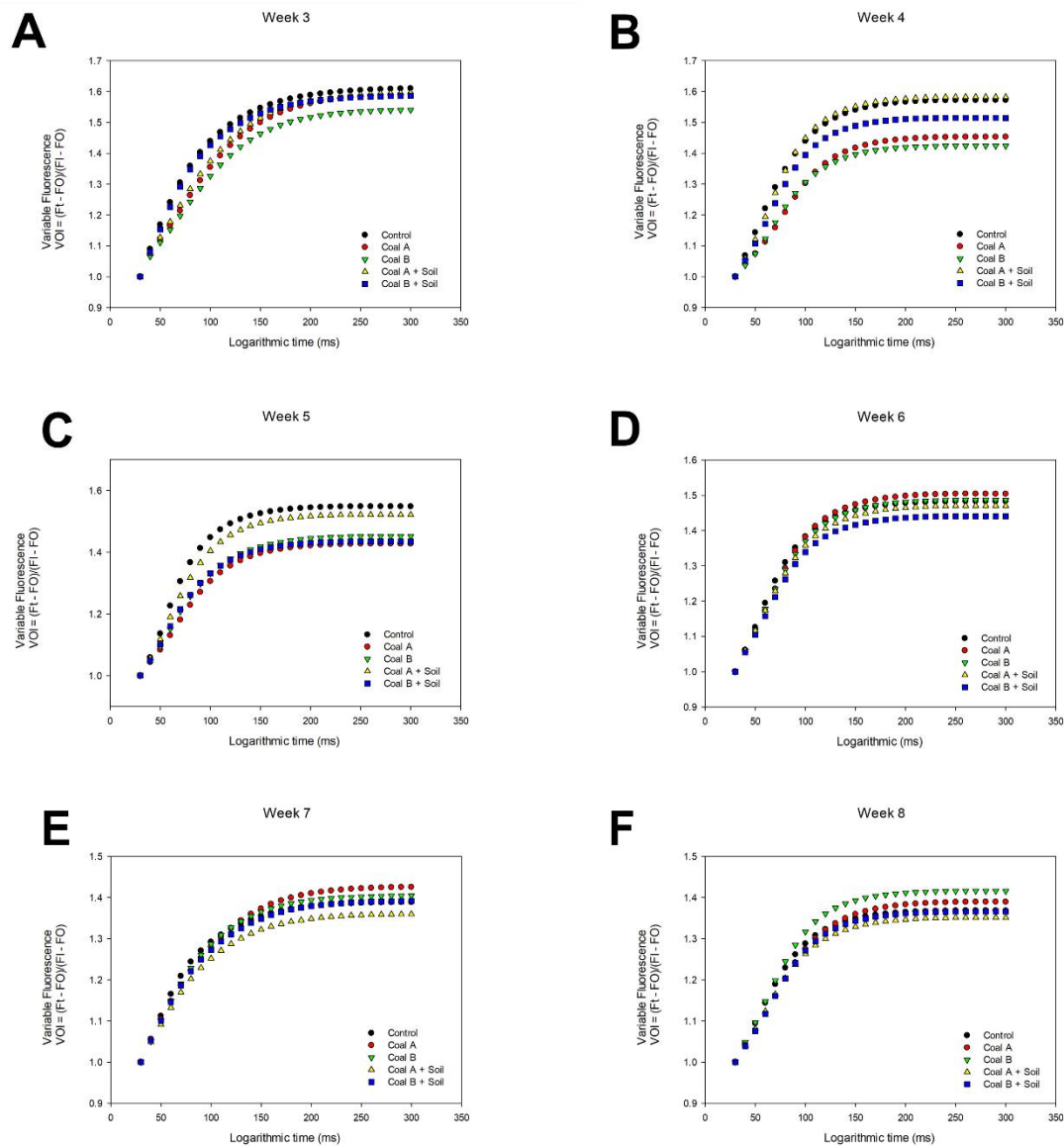


Figure 4.4: Scatter plots of the variable fluorescence between O and I $V_{OI} = (F_t - F_O)/(F_1 - F_O)$, were drawn between 30ms and 300ms of (A) week 3 (B) week 4 (C) week 5 (D) week 6 (E) week 7 (F) week 8 after germination of Kenaf.

Considering Figure 4.4 (A-F), Coal A and Coal B were lower than the other treatments during week 3, but gradually increased until week 8, where it was higher than the rest. However, the p-value was 0.207 after statistical analysis, indicating no significant difference in the I-P phase.

Reduction of end electron acceptors

Other key parameters that describe the thermal phase of photosynthesis are δ_{R_0} and ϕ_{R_0} , which mainly represent the flow of electrons from Q_A^- to end electron acceptors. In both cases, there was a steady decline in these parameters over time (Fig 4.5).

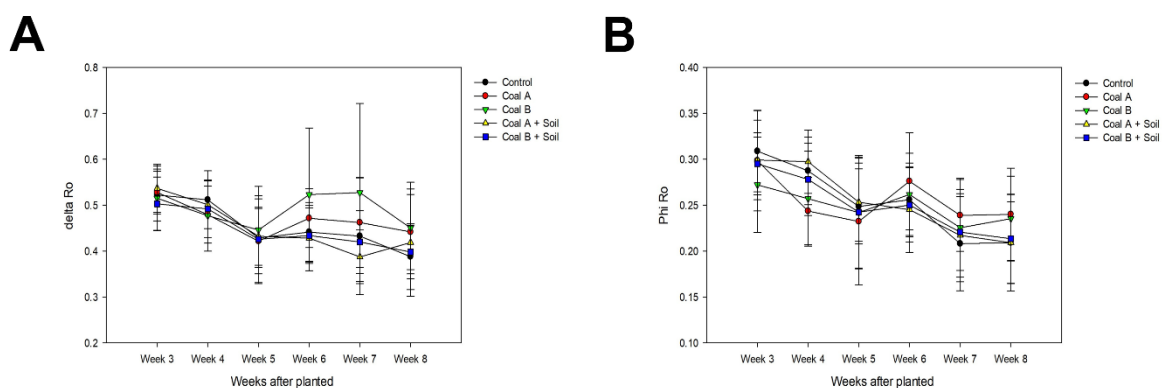


Figure 4.5: Scatter-line graphs of the physiological parameters representing the thermal phase of the electron transport chain namely (A) Flow of electrons past PQ (δ_{Ro}) and (B) Quantum yield for the reduction of end electron acceptors at PSI (ϕ_{Ro}) over eight weeks.

Similarly, δ_{Ro} and ϕ_{Ro} values were initially lower for plants in Coal A and Coal B during the first three weeks, but subsequently increased and exceeded those of other treatments in later weeks. However, δ_{Ro} did not show statistically significant differences. δ_{Ro} was statistically significant only in the early weeks, with no significant variation observed thereafter (Figure 4.5).

Table 4.3: Statistical differences in the thermal parameters derived from the OJIP transient indicate p-values for all the treatments, using a confidence level of 95%.

Thermal Parameters	
I-P Phase	p=0.207
δ_{Ro}	p=0.065
ϕ_{Ro}	p=0.957

4.1.1.4 Plant vitality indexes

The total performance index (PI_{Total}) is the parameter that gives information about the overall electron transport chain, taking both photochemical and thermal phases into consideration.

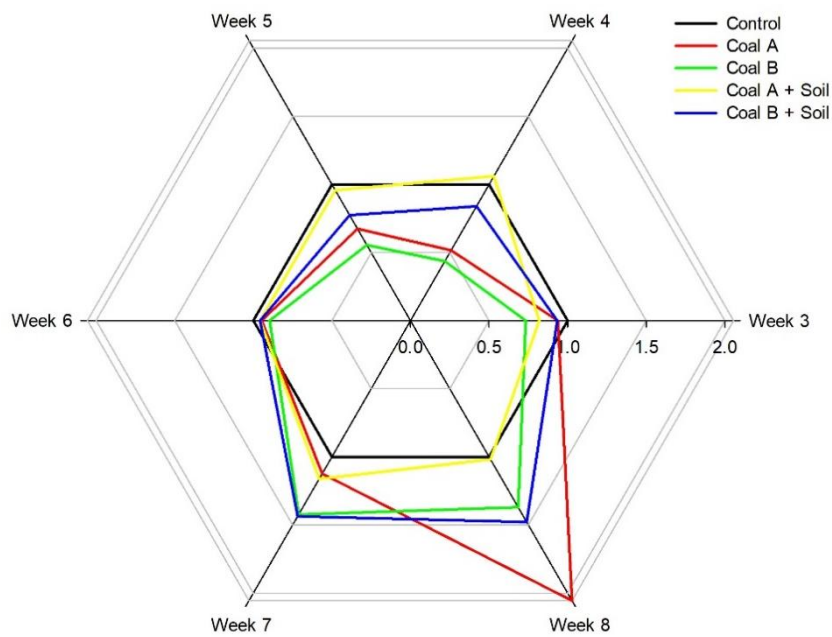


Figure 4.6: Radar plot describing the total performance index (PI_{Total}) of each week. The data were normalized to evaluate the differences in treatments relevant to the Control.

Figure 4.6 indicates that all the soil treatments were lower than the Control group during weeks 3-6, after which they increased until all the treatments were higher than the Control group in weeks 7 and 8. The total performance of the Coal A-Soil mixture remained similar to the Control group. Yet, statistical analysis indicated a p-value of 0.388, resulting in no significant difference between the treatments. Thus, the total performance of PSII was constant across all treatments.

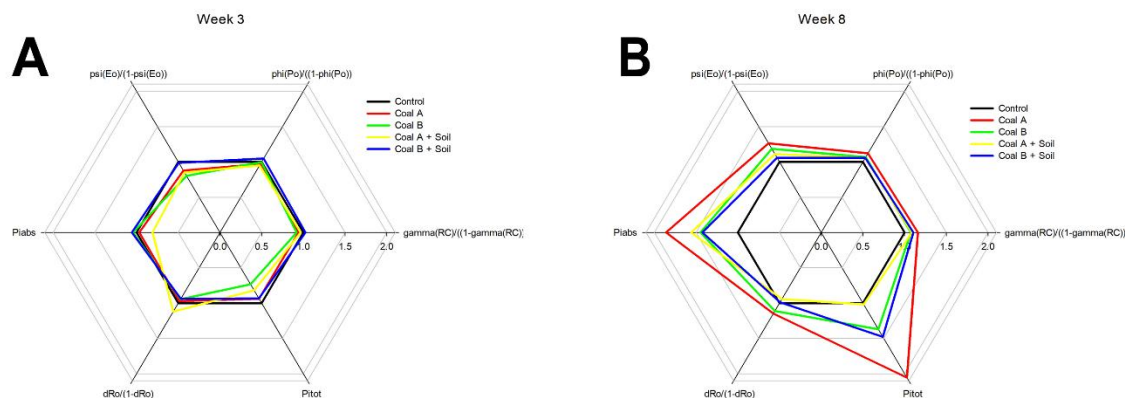


Figure 4.7: Radar plot describing the vitality performance indexes of Kenaf during weeks 3 and week 8. The data were normalized to evaluate the differences in treatments relevant to the Control.

Upon closer examination of the various parameters contributing to the total performance index, the differences between weeks 3 and 8 appear in Figure 4.7. The overall vitality of the plants showed an increase relative to the Control. However, the increase visible on the graph was not statistical in nature and thus not significantly different.

4.1.2 Functionality of Photosystem I

4.1.2.1 Modulated 820 nm reflection curve

Modulated 820 nm reflection shows the plant's PSI electron transport, specifically the plastocyanin redox states and P_{700} in the electron transport chain.

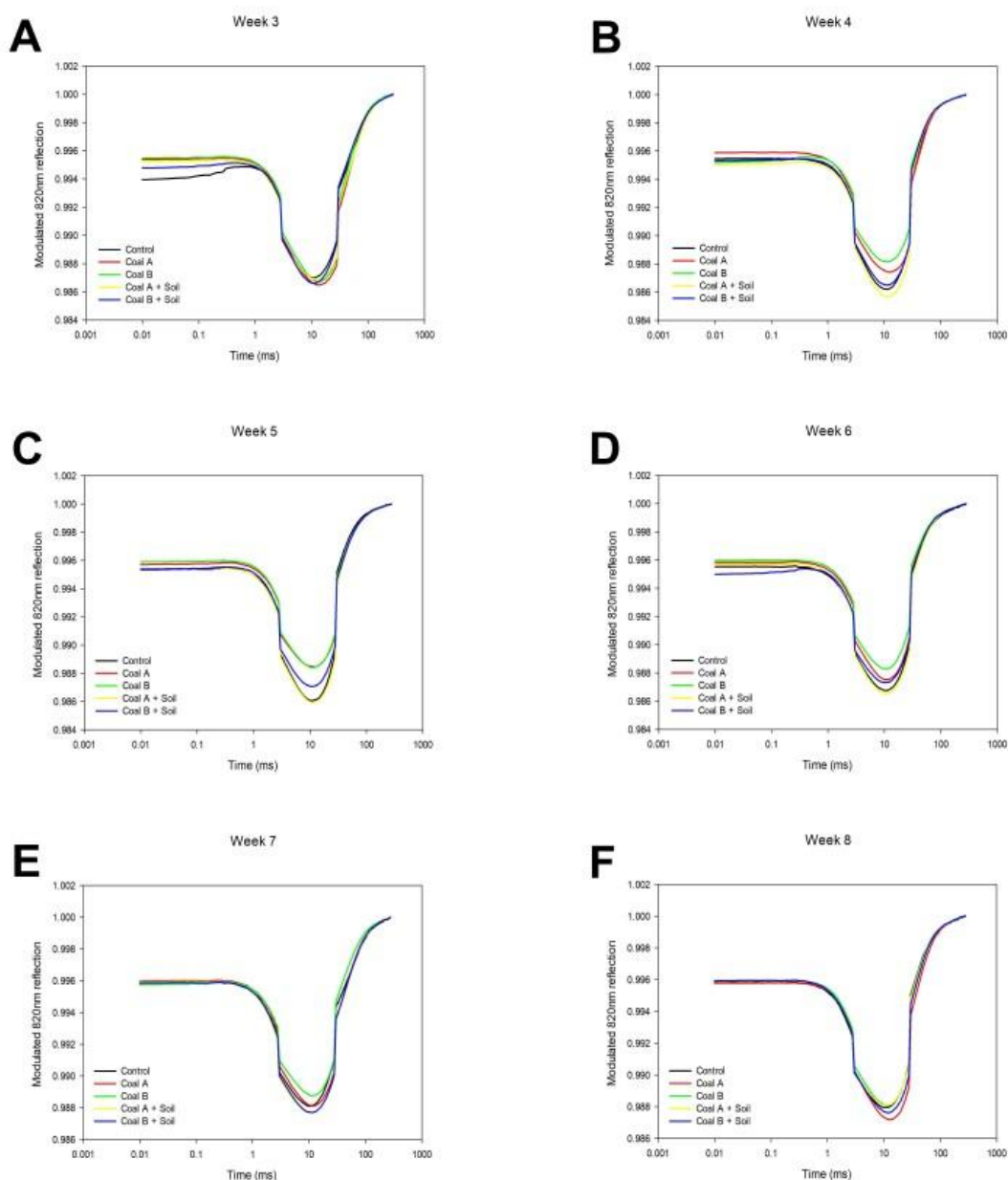


Figure 4.8: Line graphs depicting the average modulated 820 nm reflection transient taken over time of the different soil treatments (A) week 3 (B) week 4 (C) week 5 (D) week 6 (E) week 7 (F) week 8 after germination of Kenaf.

Similar to the OJIP curve, the results showed consistency in the shape of the curve between treatments. Especially considering the MR_{min} value of the curves, the first few weeks showed minor differences where the differences were insignificant during the last two weeks of the experiment. The stability in MR_{min} indicates that the oxidation of P_{700} and re-reduction of PSI remains functional. This indicates no significant impact of the coal tailings on the redox state of kenaf during the eight weeks, which correlates with PSII function (Figure 4.2 (A-F)).

4.1.2.2 Modulated 820 nm reflection parameters

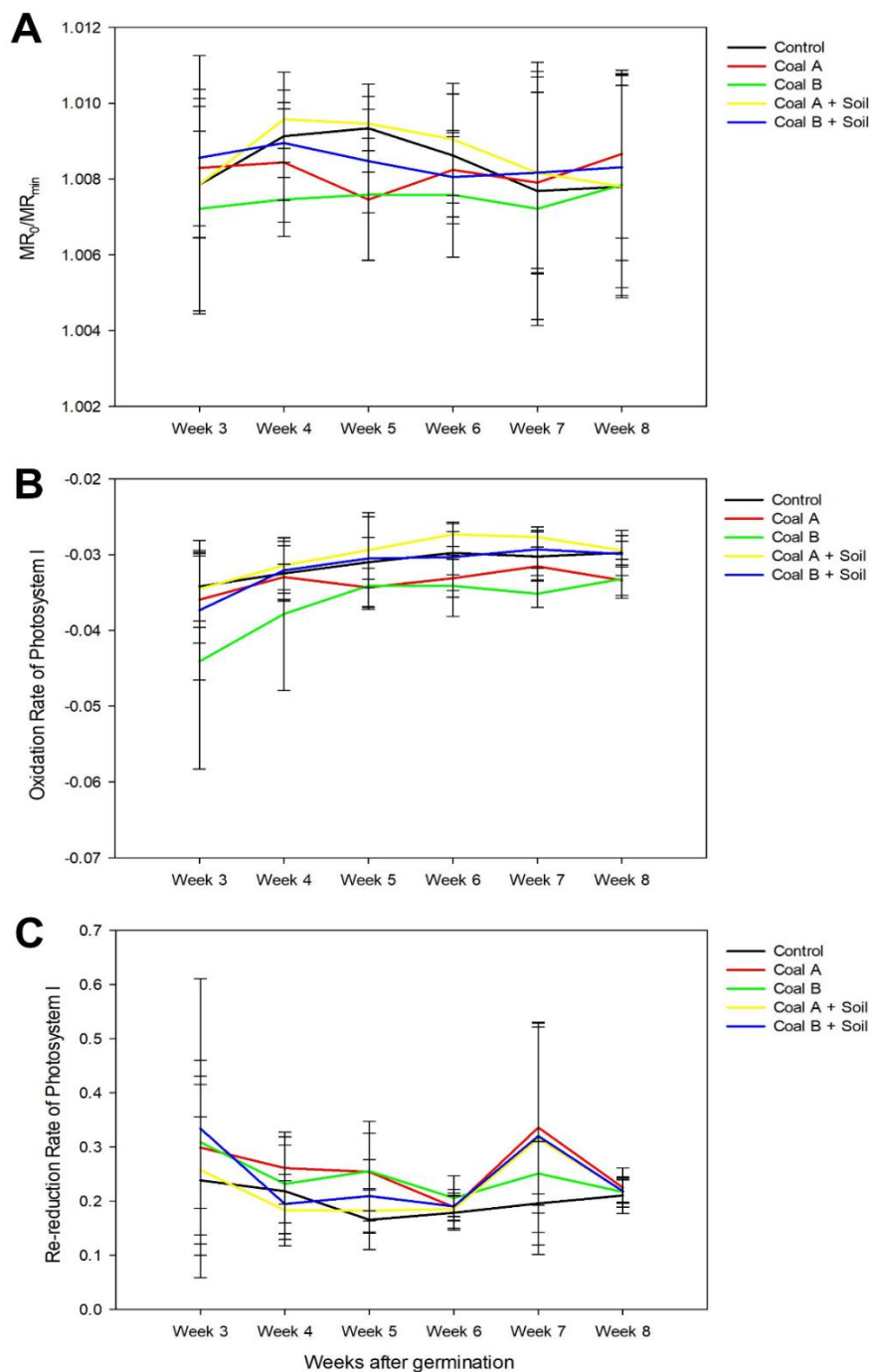


Figure 4.9: Line graphs representing the modulated fluorescence parameters derived from the modulated 820 nm reflection curve namely (A) Oxidation and re-reduction rate of PSI (MR_0/MR_{min}), (B) Oxidation rate of PSI (V_{ox}), (C) Re-reduction rate of PSI (V_{red}) over eight weeks.

Further investigation showed that the oxidation of PSI, and re-reduction of PSI, respectively, were statistically insignificant (Table 4.4). In contrast, MR_0/MR_{min} , describing the rate of oxidation and re-reduction of PSI, displayed significance in the results. Plants treated with Coal B indicated a significantly lower PSI efficiency than Coal A + Soil and Coal B + Soil treatments. Yet, Coal A treatments remained insignificant. This correlates with the results for PSII function in section 4.1.1, where Coal B treatment showed lower values.

Table 4.4: Statistical differences in the MR_0/MR_{min} ratio derived from the Modulated 820 nm reflection curve indicating p-values for all the treatments, using a confidence level of 95%.

MR_0/MR_{min}	p=0.006				
	Control	Coal A	Coal B	Coal A + Soil	Coal B + Soil
Control					
Coal A	0.797				
Coal B	0.033	0.172			
Coal A + Soil	0.869	0.484	0.006		
Coal B + Soil	0.958	0.911	0.033	0.672	
V_{ox}	p=0.370				
V_{red}	p=0.475				

4.1.3 Chlorophyll content

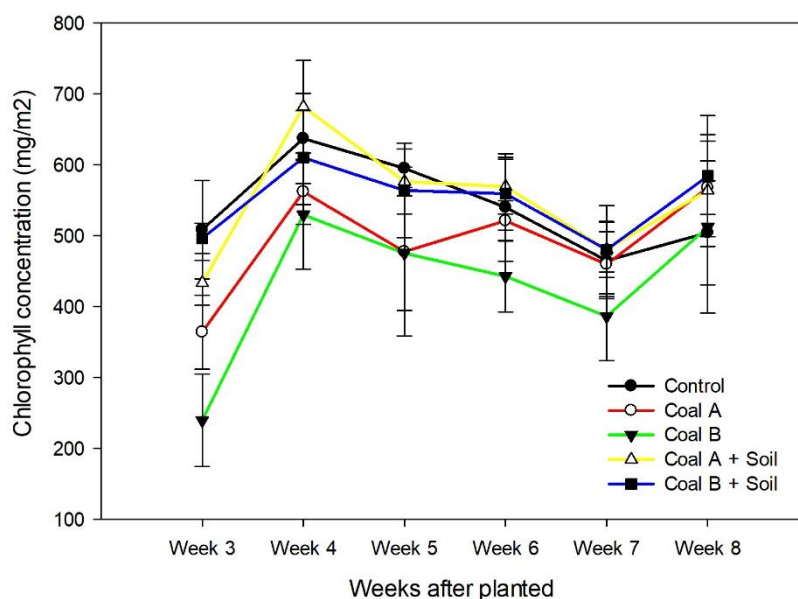


Figure 4.10: Line graph showing the average chlorophyll concentration of Kenaf taken over eight weeks.

Chlorophyll concentrations in kenaf subjected to coal tailing treatments were initially lower than the Control group (Figure 4.10). However, by the eighth week, the Control exhibited the lowest chlorophyll content among all treatments.

Yet, overall, Table 4.5 showed that Coal B was significantly lower than Control, Coal A + Soil and Coal B + Soil, indicating lower chlorophyll concentrations in Coal B. Coal A and Coal B remained comparable throughout the experiment. These results are comparable to the function of PSII and PSI in sections 4.1.1 and 4.1.2, where kenaf planted in Coal B displayed lower values than the treatments of Coal A + Soil and Coal B + Soil.

Table 4.5: Statistical differences of chlorophyll concentration between the soil treatments indicated with p-values for all the treatments, using a confidence level of 95%.

Chlorophyll Content	p<0.001				
	Control	Coal A	Coal B	Coal A + Soil	Coal B + Soil
Control					
Coal A	0.179				
Coal B	<0.001	0.116			
Coal A + Soil	0.973	0.121	<0.001		
Coal B + Soil	0.943	0.121	<0.001	0.934	

Considering the p-values in Table 4.5, statistical differences were seen between Coal B and the rest of the treatments; however, looking at Figure 4.10, the chlorophyll content of Coal A and Coal B was visibly lower than the rest of the treatments. The treated plants, where soil is involved, had increased chlorophyll.

4.2 Heavy metal absorption and translocation of kenaf

Coal tailings are recognized for their elevated concentrations of heavy metals and rare earth elements, including various trace elements that may adversely affect plant growth and physiological processes. Existing literature suggests that kenaf (*Hibiscus cannabinus*) possesses phytoremediation potential, particularly in the uptake of heavy metals from contaminated soils. Since the soil and plant analysis were outsourced, samples were pooled for analysis, resulting in inadequate data points to apply statistical analysis. Thus, Table 4.6 presents the concentrations of selected heavy metals in the soil, roots, stems, and leaves of kenaf cultivated under different treatment conditions, analysed by Nvirotek.

Table 4.6: Table indicating the concentration (mg/kg) of heavy metals in soil, roots, leaves, and stems of kenaf planted in the different treatments namely Control (C), Coal A (A), Coal B (B), Coal A + Soil (AS), and Coal B + Soil (BS).

Analysis	Treatment	As	Pb	Hg	Co	Mn	Cd	Cu	Zn	Ni	Fe
Soil	C	1.468	2.787	0.279	4.923	198.462	0.044	13.582	18.154	92.659	12582.77
	A	4.567	26.119	0.532	7.214	126.965	0.285	23.234	48.905	22.836	30568.81
	B	26.651	30.736	0.66	14.204	82.28	0.183	14.632	15.012	29.311	19447.22
	AS	2.791	10.697	0.421	5.274	197.015	0.132	16.269	34.378	25.075	21622.69
	BS	6.798	14.433	0.498	4.172	216.01	0.284	16.158	27.241	19.557	20214.83
Roots	C	1.312	2.288	0.298	4.493	185.756	0.08	15.024	26.78	111.024	9615.024
	A	2.891	16.161	0.479	23.318	105.213	0.342	64.076	53.46	46.919	15635.45
	B	3.8	9.027	0.363	11.621	84.688	0.158	113.865	55.262	44.738	12917.31
	AS	1.3	2.804	0.313	4.809	179.057	0.084	16.873	26.253	85.856	10919.11
	BS	1.361	2.861	0.267	4.114	189.406	0.055	16.04	27.228	61.386	10559.95
Stems	C	<0.048	0.069	0.237	0.506	36.563	0.091	5.68	28.162	11.456	145.823
	A	<0.046	0.082	0.226	3.128	22.552	0.144	5.708	17.958	10.951	103.991
	B	<0.047	0.08	0.199	0.995	9.718	<0.047	4.883	14.601	5.822	66.009
	AS	<0.046	0.063	0.237	0.379	17.534	0.065	3	14.155	5.251	67.534
	BS	<0.047	0.072	0.218	0.309	17.73	0.084	2.851	15.508	6.809	87.754
Leaves	C	<0.043	0.123	0.244	0.413	63.896	0.073	4.459	19.61	4.892	101.169
	A	0.239	0.163	0.299	6.42	140.148	0.379	9.63	30.864	13.58	149.975
	B	0.835	0.255	0.266	2.869	120.382	0.128	15.704	43.007	9.594	217.47
	AS	<0.050	0.158	0.276	0.668	84.838	0.134	6.284	24.838	6.284	198.404
	BS	<0.046	0.133	0.253	0.677	61.705	0.068	6.498	20.415	18.065	319.585

Based on the data in Table 4.6, heavy metals predominantly accumulated in the roots, with concentrations consistently high across treatments. Nonetheless, notable element-specific patterns were observed:

Arsenic (As):

Treatment with Coal B resulted in a significantly elevated arsenic concentration in the soil, corresponding with increased accumulation in both the roots and leaves compared to other treatments. Coal A similarly exhibited heightened As levels in the roots. In contrast, variation in arsenic concentrations in the stems was negligible across all treatments.

Lead (Pb):

The Coal A and Coal B demonstrated the highest levels of lead accumulation, surpassing all other treatments. The Control treatment consistently exhibited the lowest Pb concentrations throughout all plant parts.

Mercury (Hg):

Mercury concentrations remained relatively uniform across all treatments and plant tissues, with no significant variation detected in the soil, roots, stems, or leaves.

Cobalt (Co):

Although Coal B exhibited the highest cobalt concentration in the soil, Coal A showed elevated Co levels in the roots, stems, and leaves. Coal B also contributed to increased root concentrations. The Control, Coal A + Soil, and Coal B + Soil treatments exhibited similar, comparatively lower Co levels.

Manganese (Mn):

Unlike the other elements, Mn concentrations were lower in the Coal A and Coal B treatments. Coal B, in particular, had the lowest Mn levels in the soil, roots, and stems. However, Mn concentrations in the leaves of Coal B were higher than those of the Control, Coal A + Soil, and Coal B + Soil treatments. Coal A exhibited even greater leaf concentrations than Coal B.

Cadmium (Cd):

Cadmium uptake did not display a discernible trend across treatments. Slightly elevated Cd concentrations were observed in the soil across all treatments, with Coal A and Coal B showing relatively higher levels in the roots. Coal A presented the highest cadmium concentrations overall, though these differences were not statistically significant across plant parts.

Copper (Cu):

Coal A led to marginally increased Cu concentrations in the soil, while Coal B was associated with the highest copper accumulation in the roots and leaves. The Control, Coal A + Soil, and Coal B + Soil treatments had comparable, lower levels of Cu.

Zinc (Zn):

Although Coal B had the lowest Zn concentration in the soil, the roots and leaves showed elevated Zn levels compared to other treatments. Both Coal A and Coal B treatments showed increased Zn accumulation in the roots and stems.

Nickel (Ni):

The Control treatment recorded the highest Ni concentrations in the soil, roots, and stems, relative to other treatments. However, leaf Ni concentrations in the Control were lower than those observed in the other treatments. Coal B exhibited higher Ni levels than Coal A across most plant parts.

Iron (Fe):

Soil samples from the Coal A treatment contained significantly elevated Fe concentrations, while the Control treatment had the lowest. The remaining treatments displayed relatively similar iron levels. Raw coal tailing treatments resulted in the highest Fe accumulation in roots, whereas the Control showed the lowest. Iron concentrations in the stems and leaves varied among treatments without a clear pattern.

The majority of heavy metals mentioned above limit chlorophyll synthesis in plants. In turn, PSI and PSII function are affected by the absorption of these trace elements, as observed in the previous sections. Considering the literature, physiological results, and the soil and plant analysis, kenaf showed characteristics of an accumulator plant.

Furthermore, Figure 4.11 presents graphical representations derived from the data in Table 4.6, with the treatment concentrations normalized relative to the Control.

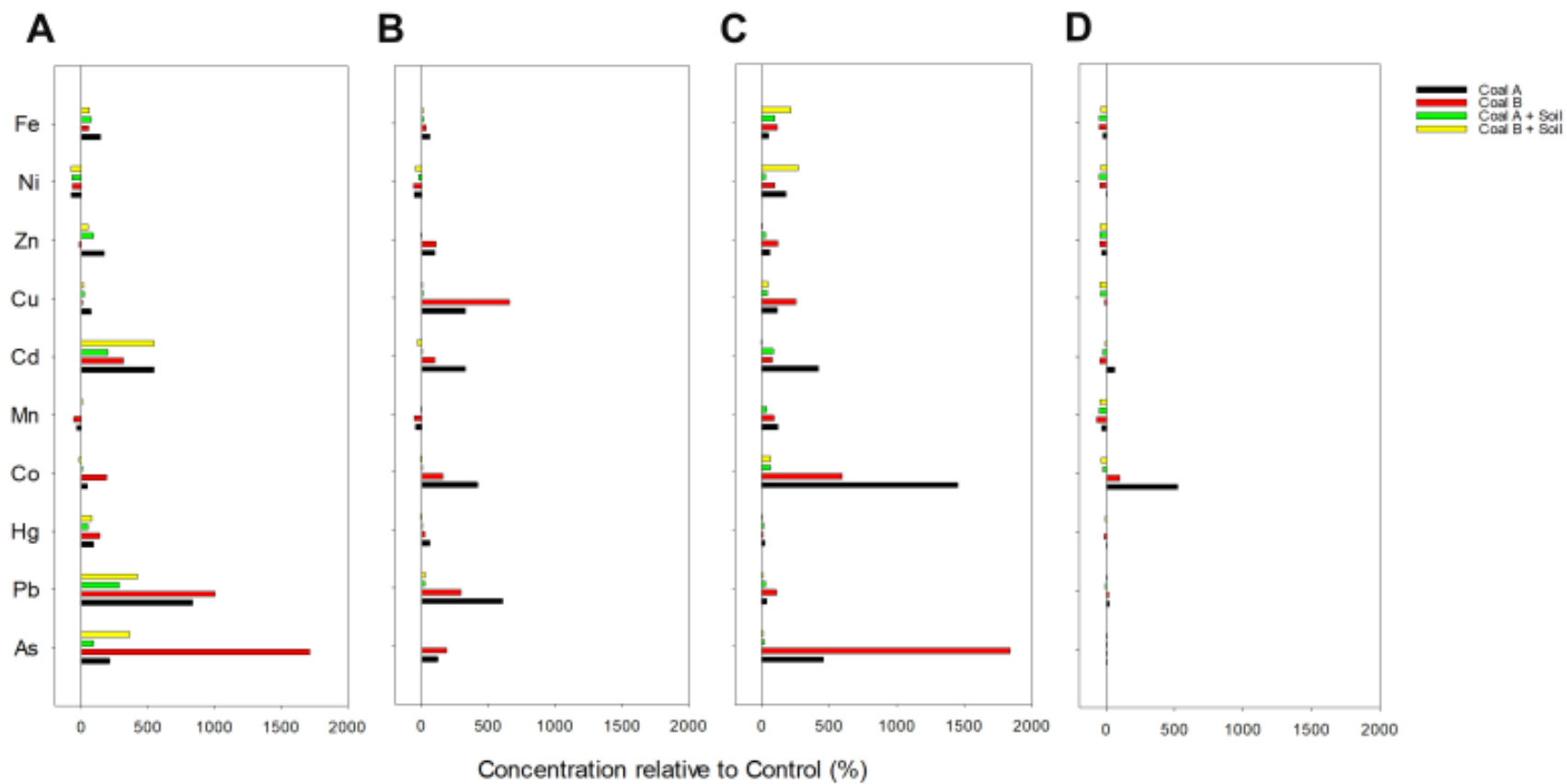


Figure 4.11: The normalized concentration (%) of heavy metals present in (A) soil, (B) roots, (C) leaves, and (D) stems relative to the Control.

Soil

Considering Figure 4.11 (A), the As content of Coal B was notably higher than the other treatments, especially the Control, where it is 1715% higher. Similarly, Coal B showed a high concentration of Pb (1002%), Hg (136%), Co (188%), and Cd (315%). In other cases, Coal A showed high concentrations of Pb (837%), Cd (547%), Zn (169%), and Fe (142%) relative to Control. Coal B + Soil also indicated a relatively high concentration of As (363%), Pb (417%), and Cd (545%). Coal B also has 58% less Mn in the soil than in the control group. Overall, all the treatments had a higher concentration of As, Pb, Hg, Co, Cd, Cu, Zn, and Fe and a lower concentration of Mn and Ni, relative to the Control.

Roots

Figure 4.11 (B) shows the percentage difference in concentration within the roots of kenaf compared to the control. Coal A had higher concentrations in the roots, especially As (120%), Pb (606%), Co (418%), Cd (327%), Cu (326%), and Zn (99%). Coal B showed higher levels of As (189%), Pb (294%), Co (158%), Cd (97%), Cu (657%), and Zn (106%). Also, Mn levels were relatively low in Coal A and Coal B, with 43% and 54%, respectively. Coal A + Soil and Coal B + Soil did not show a large or considerable variation in concentration relative to the Control. The graph indicates that kenaf planted in Coal A stored the most heavy metals in its roots compared to the other treatments.

Leaves

Comparative to the soil, the leaves (Figure 4.11 (C)) showed high concentrations of heavy metals compared to the roots and stems. Coal B had very high As concentrations in the leaves, 1841% higher than Control. Furthermore, concentrations of Pb (107%), Co (594%), Cu (252%), Zn (119%), and Fe (114%) were high in the leaves of kenaf planted in Coal B. Coal A also had elevated concentrations of As (455%), Co (1454%), Mn (119%), Cd (419%), Cu (115%), and Ni (177%) in the leaves. Coal B + Soil had relatively high levels of Ni (269%) and Fe (215%) compared to the control group. On the other hand, Coal A + Soil indicated no drastic difference in heavy metal concentrations relative to Control.

Stems

Lastly, Figure 4.11 (D) showed very little difference in heavy metal concentrations compared to the Control. The only notable difference is Co, which was 518% higher in the kenaf stems planted in Coal A. Although not notable, the overall results on the stems showed decreased concentrations of heavy metals in the stems of kenaf.

CHAPTER 5 DISCUSSION

5.1 Photochemical performance of kenaf

This study aimed to determine whether kenaf can grow on coal tailings while maintaining physiological function. Photosynthetic efficiency was assessed using prompt chlorophyll fluorescence (OJIP transients), modulated 820 nm reflection, and chlorophyll content across different treatments. Data were collected after an eight-week growth period and analysed statistically.

Photosynthesis is central to plant vitality, and its efficiency reflects overall plant health. This complex process involves a series of biochemical reactions that can be evaluated through specific physiological parameters corresponding to distinct phases and complexes within the photosynthetic electron transport chain (ETC). Heavy metals are well-documented for their adverse effects on plant growth and photosynthetic function, often limiting biomass accumulation and physiological resilience (Yan et al., 2020; Zhao et al., 2022). Nevertheless, literature suggests that kenaf possesses advantageous traits, including rapid growth, high biomass production, robust root systems, and high fibre content, that may confer tolerance to environmental stressors (Cleophas et al., 2023). Accordingly, a key question addressed in this study is: How do coal tailings influence the physiological performance of kenaf?

5.1.1 Photosystem II function

Prompt fluorescence was used to construct OJIP transients, which reflect the fast kinetics of chlorophyll *a* fluorescence and provide insight into the photochemical performance of Photosystem II (PSII). The OJIP curve, plotted on a logarithmic time scale, includes four key steps: O (0.03 ms), J (2 ms), I (30 ms), and P (300 ms), each representing different stages of electron transport within PSII (Salvatori et al., 2014). Results indicated that soil composition did not alter the general shape of the OJIP curve (Figure 4.2), which remained consistent with the standard profile illustrated in Figure 3.2. This suggests that electron flow through PSII to the final electron acceptors was largely unaffected across treatments.

Statistically significant differences were, however, observed at the J-step, particularly in the Coal B treatment, which displayed elevated values compared to other treatments (Table 4.1). This effect was most prominent in week 4 (Figure 4.2 (B)) and indicates a transient bottleneck in electron flow beyond the primary quinone acceptor (Q_A^-).

Supporting this, the photochemical phase of photosynthesis, encompassing the O–J interval, includes energy absorption and trapping by the light-harvesting complex (LHC) and initiation of electron flow beyond Q_A . Key parameters derived from this phase include the maximum quantum efficiency of PSII (F_v/F_m), the absolute performance index (PI_{ABS}), the probability of electron transport initiation (Ψ_{E_0}), and the quantum yield of electron transport (ϕ_{E_0}). A significant decline in Ψ_{E_0} and ϕ_{E_0} was observed in the Coal B treatment (Table 4.2), particularly from week 6 onwards (Figure 4.3 (C–D)), indicating a reduction in electron transport efficiency beyond Q_A .

In contrast, the thermal phase of electron transport (I–P step), associated with electron flow beyond plastoquinone (PQ), and the reduction of end-electron acceptors, showed no significant differences among treatments (Table 4.3). Parameters such as δ_{R_0} (efficiency of electron transfer past PQ) and ϕ_{R_0} (quantum yield for end-acceptor reduction), I- and P-steps remained stable, further indicating that coal tailings did not impair downstream ETC processes. These findings suggest that while electron initiation was affected in Coal B, kenaf demonstrated recovery and adaptability in later stages of electron transport. This supports the hypothesis that kenaf can adapt and maintain an acceptable health for growth and survival.

This conclusion is further supported by the total performance index (PI_{Total}), which did not show a statistically significant difference across treatments ($p = 0.388$). Interestingly, between weeks 3 and 8, PI_{Total} appeared higher in the coal tailing treatments compared to the Control, although this difference was not statistically significant (Figure 4.7). These findings imply that while Coal B reduced initial PSII efficiency, kenaf maintained overall photosynthetic functionality.

Although the OJIP transient primarily reflects PSII activity, the thermal phase also provides indirect insights into PSI performance, particularly regarding reducing final electron acceptors. However, the function of PSI is best evaluated through modulated 820 nm reflection.

5.1.2 Photosystem I function

Modulated reflection at 820 nm provides information on the redox dynamics of plastocyanin (PC) and P_{700} , the reaction centre of PSI. The shape of the reflection curve remained consistent across treatments and throughout the experiment (Figure 4.8), indicating stable PSI functionality. The MR_0/MR_{min} ratio, representing the rate of oxidation and re-reduction of PSI, was significantly lower in the Coal B treatment ($p = 0.006$), compared to Coal A + Soil and Coal B + Soil (Table 4.4). This suggests that Coal B may reduce PSI efficiency by limiting electron transfer from PC to P_{700} and slowing the re-reduction of P_{700} .

Despite this, no significant differences were found in V_{ox} (oxidation rate of PSI) or V_{red} (re-reduction rate), implying that kenaf maintained effective PSI redox dynamics. These results are consistent with the PSII data, where Coal B had an observable effect on electron initiation and PSI activity but did not compromise overall photochemical stability.

5.1.3 Chlorophyll content

5.1.3 Chlorophyll content

Photosynthetic function is closely linked to chlorophyll content, as chlorophyll pigments within the LHC are essential for absorbing and transferring light energy. These pigments are arranged in an antenna-like structure that optimizes light harvesting. Heavy metal contamination has been shown to inhibit chlorophyll synthesis, ultimately reducing photosynthetic capacity (Gajić et al., 2018). In this study, chlorophyll content was lower in plants grown in Coal A and Coal B compared to treatments containing soil (Figure 4.10). Notably, the Coal B treatment exhibited a statistically significant reduction in chlorophyll content relative to the Control, Coal A + Soil, and Coal B + Soil (Table 4.5). This reduction is likely attributable to elevated concentrations of toxic heavy metals such as arsenic and lead in the Coal B substrate.

5.2 The absorption, translocation, and physiological effects of heavy metals on kenaf

Fibre crops have been identified as potential candidates for the remediation of coal tailings, given their demonstrated tolerance to metal-contaminated soils (Cleophas et al., 2023). In various studies, kenaf has been proved to have phytoremediation potential in multi-metal polluted soils (Cleophas et al., 2023; Rumjeet, 2019). Previous studies also suggest that the tolerance mechanisms of such crops are attributed to the restricted upward translocation of heavy metals from roots to aerial parts (Fernando et al., 2015). This section aims to address three primary questions: Does kenaf absorb heavy metals from coal tailings? To what extent are these metals translocated to the stem and leaves? And how do they affect photosynthetic performance?

Standard outsourced soil and plant analyses (Tables 6.1-6.3 in the Annexure) revealed no drastic differences in the chemical and physical properties among treatments. Additionally, the analysis of heavy metals and rare earth element uptake (Figures 6.1-6.4 in the Annexure) demonstrated that kenaf can absorb and translocate these elements to various plant tissues. Overall, plants grown in Coal A and Coal B substrates exhibited higher concentrations of heavy metals compared to the Control group, particularly in root tissues.

This study, however, specifically focused on trace elements abundant in coal tailings, namely arsenic (As), lead (Pb), mercury (Hg), cobalt (Co), copper (Cu), zinc (Zn), nickel (Ni), and iron

(Fe). Physiological data indicated that kenaf grown in the Coal B treatment experienced the most tremendous negative impact, presumably due to the higher concentrations of toxic heavy metals. Elevated As, Pb, Hg, Co, and Cd levels were detected in Coal B (Figure 4.11 (A)). Except for Co, these metals are known to inhibit chlorophyll biosynthesis and disrupt photosynthetic function (Cleophas et al., 2023; Gajić et al., 2018; Saleem et al., 2020; Yan et al., 2020; Zhao et al., 2022). While Co can be toxic to plants, this typically occurs at concentrations exceeding 50 mg/kg (Table 2.5), which was not observed in this study. Thus, Co is unlikely to have contributed significantly to the reduced photochemical performance in Coal B.

As, Pb, Hg, and Cd are highly toxic to plants and exhibited varying degrees of absorption and translocation. According to Figure 4.11, none of these elements were significantly translocated to the stems, and the movement of Pb, Hg, and Cd from roots to shoots was notably restricted (Figures 4.11 (C–D)). The literature offers conflicting reports regarding Pb translocation in kenaf. For instance, Cleophas et al. (2023) reported higher Pb concentrations in stems and leaves compared to roots following soil application. Conversely, Yan et al. (2020) found that Pb uptake was inhibited entirely due to its low bioavailability. These contrasting results suggest that soil pH and other physicochemical properties strongly influence heavy metal uptake, which can vary substantially between soil types and treatments. In contrast, the results for Cd are consistent with those reported by Cleophas et al. (2023), where Cd concentrations were highest in the roots, with minimal translocation to aerial tissues (Figure 4.11 (B)). Moreover, overall absorption of Cd and Hg was limited, indicating the presence of exclusion mechanisms in kenaf roots that restrict their uptake and systemic movement.

Arsenic absorption and translocation displayed a distinct pattern. In the Control, Coal A + Soil, and Coal B + Soil treatments, As concentrations in the soil were low, and accordingly, uptake was limited. However, this may also reflect active exclusion mechanisms in kenaf. Notably, prior studies have identified kenaf as a species with phytoremediation potential for arsenic-contaminated soils, capable of sequestering As in its biomass (Nizam et al., 2016). In the present study, As concentrations were highest in the leaves (Figure 4.11), suggesting that kenaf can translocate arsenic efficiently from roots to leaves. This characteristic supports the feasibility of periodically harvesting kenaf leaves for safe removal and disposal of accumulated As.

Considering both the physiological and heavy metal absorption data, arsenic appears to have exerted the most pronounced adverse effect on kenaf performance in the Coal B treatment. The high concentration of As in the leaves, combined with its known inhibitory effects on chlorophyll synthesis, suggests that it played a major role in reducing photochemical efficiency. Despite these

challenges, kenaf demonstrated the capacity to adapt and stabilize under coal tailing conditions, maintaining sufficient physiological function to support continued growth.

5.3 Limitations of the study

While the findings of this study strongly support the hypothesis that kenaf can be cultivated on coal tailings in South Africa, several limitations must be acknowledged. First, the experiment was conducted over an eight-week period, which does not encompass the whole growth cycle of kenaf. Extending the study duration may yield different or more comprehensive insights into the long-term physiological responses of the plant.

Second, the research was conducted as a pot trial under controlled environmental conditions. While this approach enabled precise monitoring, it limits the applicability of the findings to field conditions, where ecological variables such as temperature, humidity, carbon dioxide concentration, and water availability fluctuate naturally and may significantly influence plant performance.

Lastly, individual replicates were not analysed separately due to time and budgetary constraints. Instead, plant material was pooled before analysis, which may have masked variability among replicates and limited the resolution of statistical comparisons.

CHAPTER 6 CONCLUSION AND RECOMMENDATIONS

This study evaluated the growth and phytoremediation potential of *Hibiscus cannabinus* (kenaf) on various soil treatments, including sandy loam, two raw coal tailings from distinct mining sites, and mixtures thereof. The primary objective was to assess kenaf's ability to grow on coal tailings, monitored through physiological parameters using an M-PEA fluorimeter and CCM-300 chlorophyll content meter. These tools provided insight into PSII and PSI function, as well as chlorophyll biosynthesis, key indicators of plant health and growth. Results demonstrated that kenaf can grow on coal tailings, maintaining stable PSII and PSI activity across treatments. Despite a reduction in photochemical performance on the Coal B substrate, attributable to elevated arsenic concentrations, plants adapted and sustained growth throughout the experimental period. Arsenic exposure inhibited chlorophyll synthesis and reduced PSII and PSI efficiency, yet Fv/Fm values remained above 0.8, indicating functional PSII photochemistry. A secondary objective was to examine kenaf's capacity to absorb heavy metals from the substrate. Kenaf effectively accumulated heavy metals and rare earth elements, corroborating literature that supports its use in phytoremediation.

In summary, kenaf grown in Coal B demonstrated the lowest performance across PSII and PSI functions and chlorophyll content. However, the species exhibited notable adaptability, particularly in its ability to maintain downstream electron transport and overall photochemical function despite initial limitations. Treatments incorporating soil—Coal A + Soil and Coal B + Soil—consistently outperformed coal tailing-only treatments, highlighting the mitigating effect of soil on heavy metal toxicity. These findings support the growth potential of kenaf as a phytoremediation species, albeit with reduced efficiency in unamended coal tailings due to heavy metal stress.

These findings suggest that kenaf holds promise as an industrial crop for rehabilitating metal-contaminated soils. Its potential to extract pollutants while supporting economic applications presents a sustainable solution for coal tailings management. Implementing kenaf cultivation on such substrates could promote environmental restoration and local employment opportunities.

From a practical standpoint, using kenaf for mine site rehabilitation offers a feasible and environmentally sound strategy for managing coal tailings. Traditional remediation methods such as soil excavation, chemical treatments, or capping are often expensive and resource-intensive. In contrast, cultivating kenaf represents a low-cost, nature-based approach that reduces the ecological risks associated with trace metal contamination and contributes to landscape stabilization and erosion control. As demonstrated in this study, the integration of soil

amendments can further enhance plant performance, offering a scalable method for transforming post-mining landscapes into productive and safer ecosystems. Thus, incorporating kenaf into mine closure plans aligns with the principles of ecological restoration and circular land use.

Beyond environmental recovery, the widespread adoption of kenaf cultivation on rehabilitated mining land could have significant socio-economic implications. By converting post-mining areas into zones for industrial crop production, local communities, often economically impacted by mine closures, may benefit from new employment opportunities in agriculture, fibre processing, and bio-based industries. This transition supports economic diversification and resilience in regions historically dependent on extractive industries. Moreover, local engagement in phytoremediation initiatives may foster community ownership of land stewardship efforts and promote sustainable agriculture and environmental management skill development. Therefore, the practical application of this study not only addresses the ecological challenges of mine remediation but opens pathways for inclusive, community-driven economic regeneration.

Recommendations

Given that this study was conducted as a preliminary, pot-based experiment under controlled environmental conditions, future research efforts must aim to validate these findings under more realistic, field-based scenarios. Specifically, field trials should be undertaken to evaluate the long-term physiological and ecological performance of *Hibiscus cannabinus* (kenaf) when cultivated on coal tailings over its complete growth cycle, extending beyond the eight-week period examined in this study. Such trials would enable the assessment of the plant's adaptive responses to fluctuating environmental variables and biotic interactions typical of natural ecosystems.

Moreover, future investigations should incorporate key ecological parameters, most notably total biomass yield and fibre quality, to provide a holistic understanding of kenaf's agronomic potential and commercial viability on coal tailings. Emphasis should be placed on evaluating fibre characteristics, such as tensile strength, cellulose content, and industrial applicability, to determine whether cultivation on remediated substrates affects the economic value of the harvestable product.

A multidisciplinary approach integrating physiological metrics (e.g., chlorophyll content, photosynthetic efficiency, and trace element uptake) with ecological and biochemical assessments will yield a more comprehensive framework for evaluating kenaf's suitability for phytoremediation and sustainable land reclamation. In this regard, further exploration of kenaf's capacity to remediate different types of contaminated substrates, beyond coal tailings, will be essential to ascertain its broader applicability.

Future research should also be stratified across distinct disciplinary perspectives to fully elucidate the complex interactions between trace element contamination and plant performance. Collaboration between plant physiology and chemical engineering disciplines will be particularly valuable. Such interdisciplinary efforts can illuminate kenaf's biological responses to metal stress and the geochemical transformations of contaminants in the soil-plant system, thus advancing the scientific foundation for its deployment in phytoremediation initiatives.

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ANNEXURE

Table 6.1: Results from soil analysis of the different treatments namely Control, Coal A, Coal B, Coal A + Soil, and Coal B + Soil.

		Control	Coal A	Coal B	Coal A + Soil	Coal B + Soil
P Bray1	mg/kg	63	17	30	30	34
K AmAc	mg/kg	102	50	55	65	68
Na AmAc	mg/kg	51	51	44	152	105
Ca AmAc	mg/kg	757	6874	1475	1855	840
Mg AmAc	mg/kg	278	466	221	266	226
Ca% AmAc	%	57.82	89.18	77.48	75.5	62.86
Mg% AmAc	%	34.8	9.92	19.01	17.77	27.7
K% AmAc	%	3.97	0.33	1.48	1.34	2.59
Na% AmAc	%	3.4	0.58	2.03	5.39	6.84
Ca:Mg AmAc		1.66	8.99	4.08	4.25	2.27
(Ca+Mg)/K AmAc		23.32	300.42	65.22	69.4	34.96
Mg:K AmAc		8.76	30.07	12.85	13.22	10.7
S-VALUE AmAc	cmol(+)/kg	6.55	38.54	9.52	12.29	6.68
Na:K AmAc	cmol(+)/kg	0.86	1.74	1.37	4.01	2.64
T-VALUE AmAc	cmol(+)/kg	6.55	38.54	9.52	12.29	6.68
S AmAc	mg/kg	46.38	5841.05	528.52	1134.3	172.74
Ca(1:2)	mg/l	26.01	600.1	154.46	507.46	76.07
Mg(1:2)	mg/l	15.38	150.66	40.17	96.11	30.79
K(1:2)	mg/l	5.84	5.67	15.85	5.79	3.24
Na(1:2)	mg/l	20.59	11.51	11.19	76.9	48.87
Cu(1:2)	mg/l	0.05	0.03	0.03	0.02	0.02
Zn(1:2)	mg/l	0.02	0.32	0.01	0.01	0.01
Mn(1:2)	mg/l	0.17	6.7	0.55	1.12	0.13
Fe(1:2)	mg/l	0.35	32.54	0.17	0.07	0.21
SO4(1:2)	mg/l	88.02	2082.27	534.42	1600.29	311.58
B(1:2)	mg/l	0.07	0.12	0.16	0.07	0.07
P(1:2)	mg/l	3.21	0.1	0.2	0.39	0.77
S(1:2)	mg/l	29.34	694.09	178.14	533.43	103.86
PO4(1:2)	mg/l	9.63	0.3	0.6	1.17	2.31
SAR(1:2)	-	0.79	0.11	0.21	0.82	1.19

Table 6.2: Results from plant analysis of kenaf planted in the different treatments namely Control, Coal A, Coal B, Coal A + Soil, and Coal B + Soil

Treatment		N	Ca	Mg	K	Na	S	P	Fe	Mn	Cu	Zn	Mo	B
		%	%	%	%	mg/kg	%	%	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
Roots	Control	1.1	0.37	0.38	0.92	2921	0.22	0.16	7274	158	18	30	1.81	13
	Coal A	1.18	0.76	0.51	0.75	2604	0.73	0.3	9804	87	48	60	2.75	14
	Coal B	1.28	0.74	0.5	0.61	3373	0.46	0.2	8211	82	107	41	1.99	16
	Coal A + Soil	1.05	0.4	0.37	0.92	2492	0.29	0.16	7227	151	17	29	1.91	12
	Coal B + Soil	0.96	0.45	0.37	0.94	1827	0.32	0.14	6009	125	14	27	1.73	11
Leaves	Control	2.87	0.74	0.37	1.32	210	0.23	0.3	143	97	6	22	0.98	61
	Coal A	3.03	1.02	0.6	1.16	271	0.45	0.31	204	146	8	29	1.13	71
	Coal B	3.4	1.09	0.6	1.15	269	0.38	0.38	149	101	11	34	1.2	82
	Coal A + Soil	3.06	0.73	0.42	1.44	217	0.28	0.32	274	139	6	25	1.14	59
	Coal B + Soil	3.05	0.85	0.43	1.46	291	0.31	0.31	197	123	7	26	1.1	63
Stems	Control	1.14	0.36	0.32	1.45	674	0.14	0.19	95	19	3	14	0.72	14
	Coal A	1.22	0.44	0.7	0.96	1190	0.52	0.23	60	20	6	15	1.12	19
	Coal B	1.2	0.42	0.52	1.04	1101	0.33	0.27	58	11	6	13	1.05	18
	Coal A + Soil	1.3	0.42	0.39	1.42	615	0.26	0.22	58	23	4	16	0.69	15
	Coal B + Soil	0.94	0.38	0.3	1.4	644	0.2	0.17	87	14	3	15	1.01	14

Table 6.3: Chemical and physical properties of the different soil treatments namely Control, Coal A, Coal B, Coal A + Soil, and Coal B + Soil.

	pH (KCl)	Density g/mL	Clay %	Silt %	Sand %
Control	7.19	1.34	9	2	89
Coal A	6.11	1.18	3	6	91
Coal B	6.81	0.91	6	3	91
Coal A + Soil	6.52	1.31	7	5	88
Coal B + Soil	5.98	1.31	9	2	89

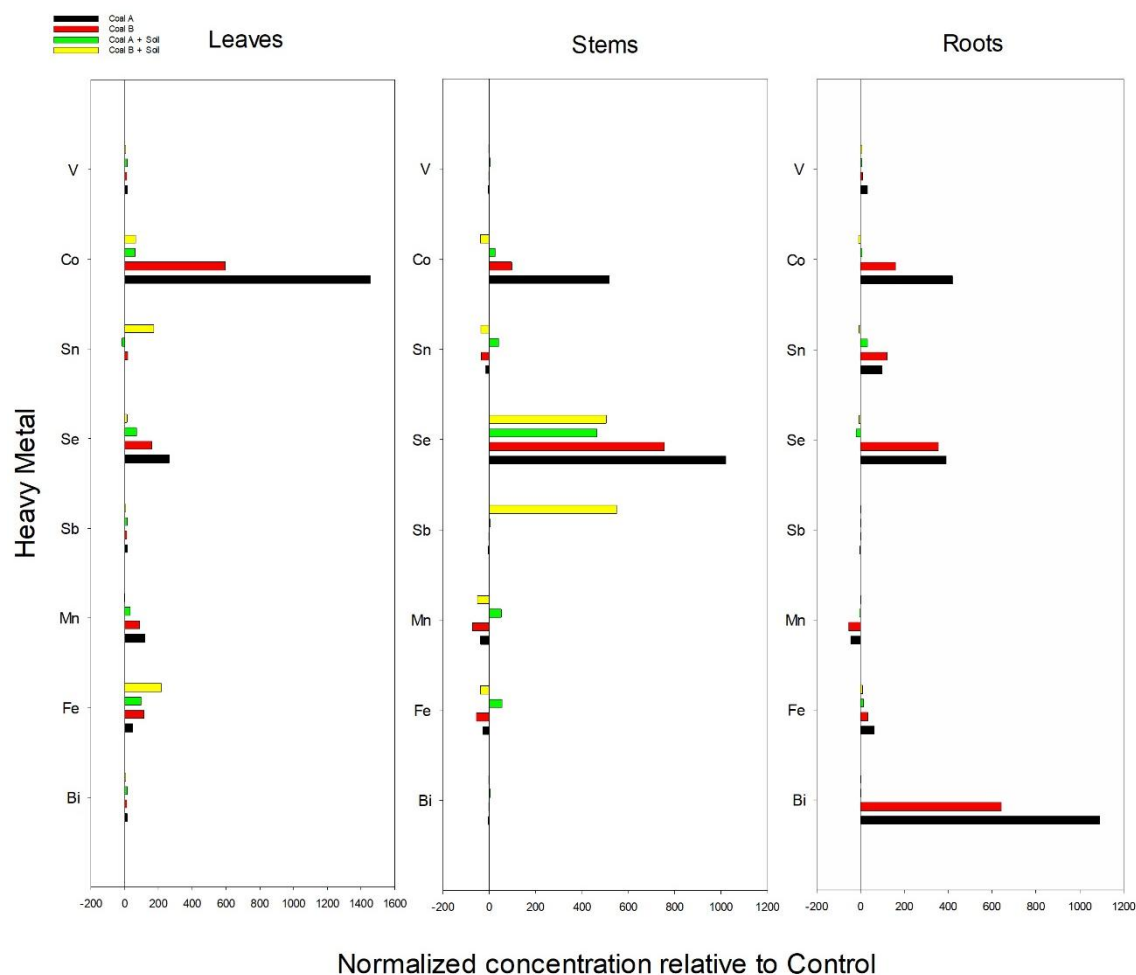


Figure 6.1: The concentration of non-toxic heavy metals present in different parts of Kenaf after two months of growth. Data is normalized so that Control is 0 and the rest of the treatments are relative to Control.

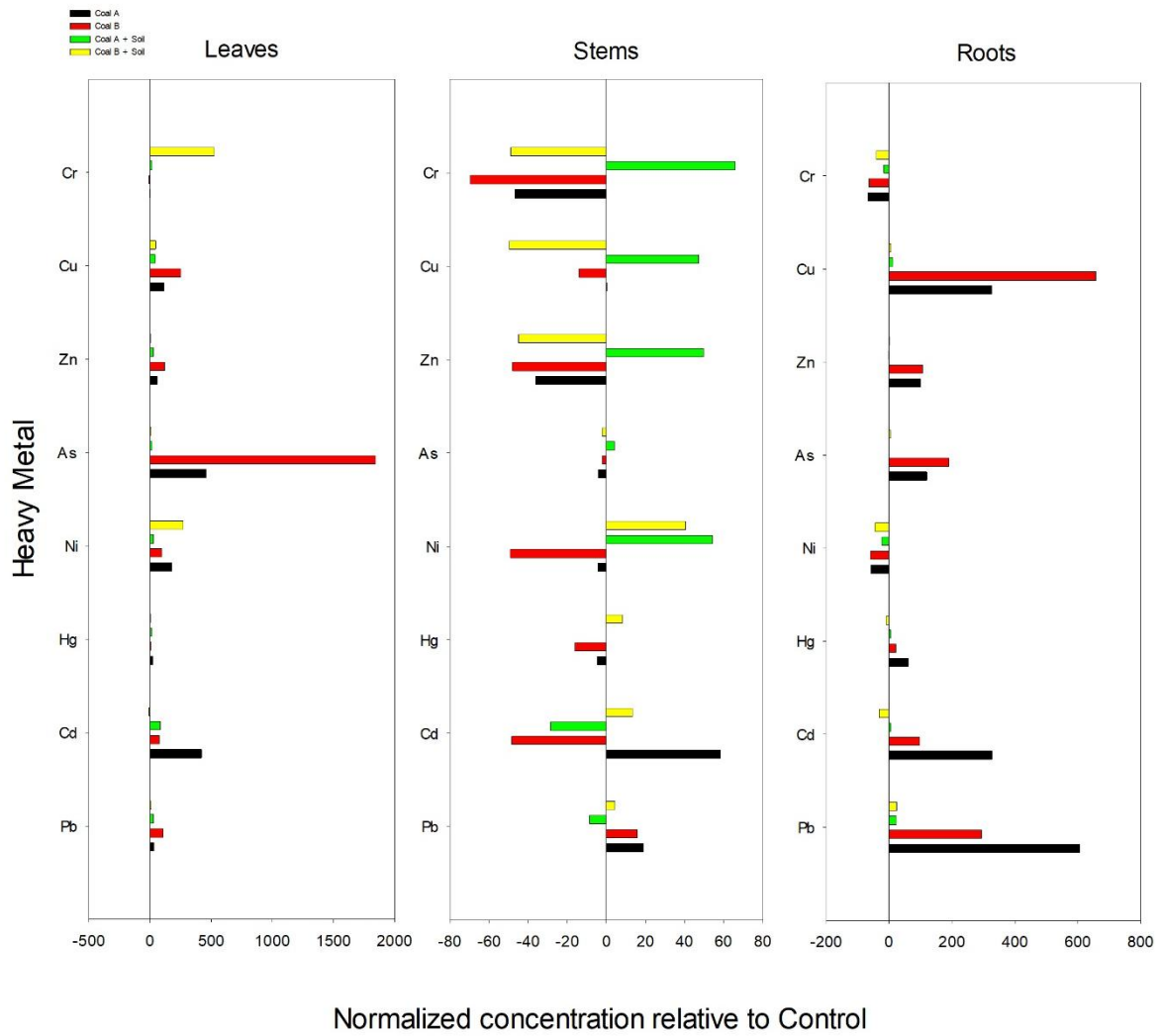


Figure 6.2: The concentration of toxic heavy metals present in different parts of Kenaf after two months of growth. Data is normalized so that Control is 0 and the rest of the treatments are relative to Control.

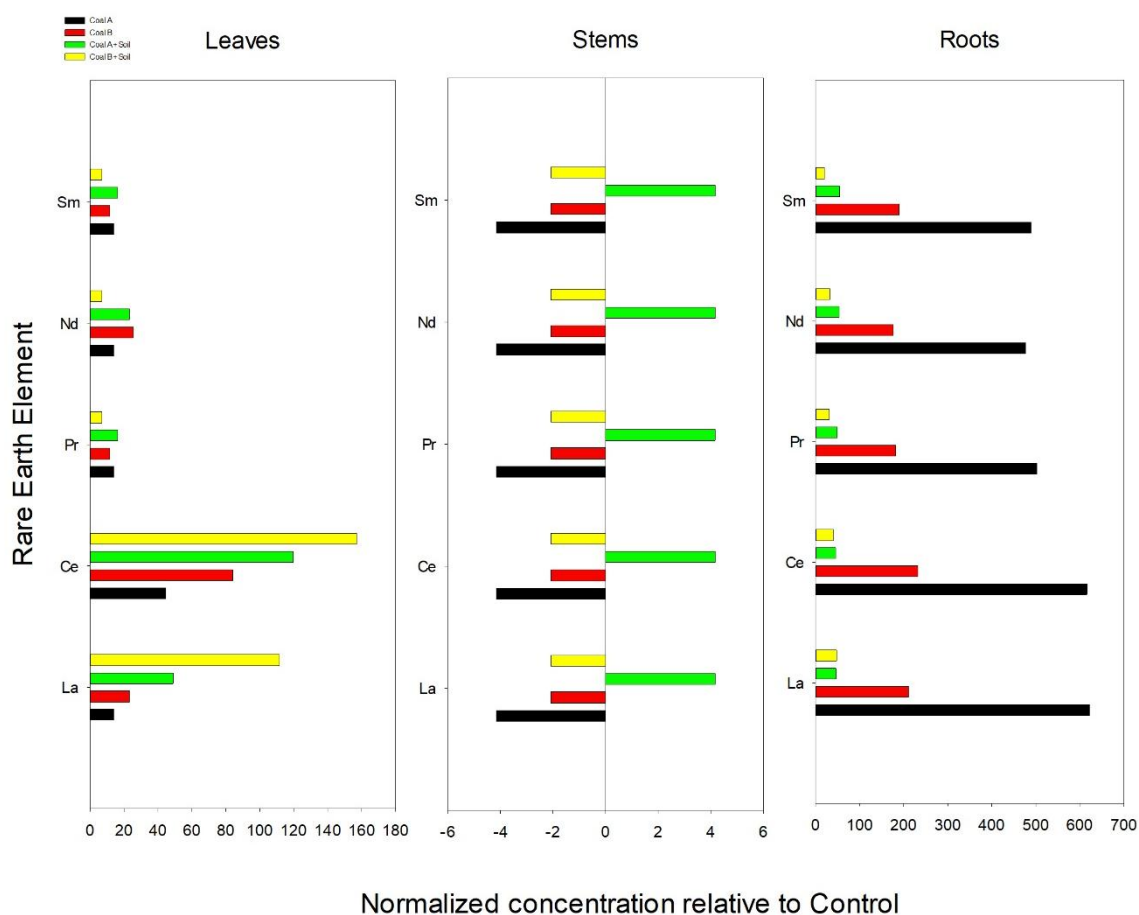


Figure 6.3: The concentration of LREEs present in different parts of Kenaf after two months of growth. Data is normalized so that Control is 0 and the rest of the treatments are relative to Control.

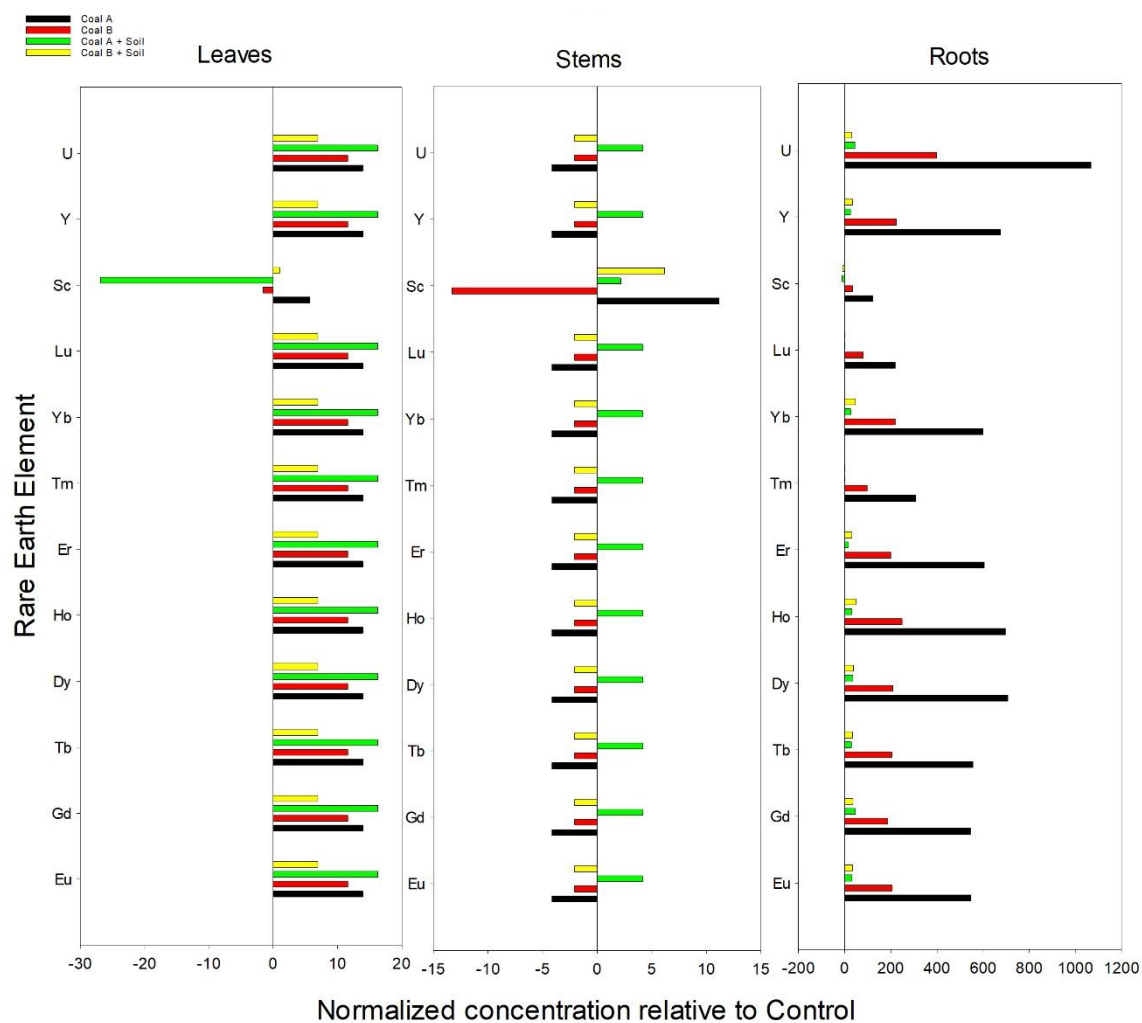


Figure 6.4: The concentration of HREEs heavy metals present in different parts of Kenaf after two months of growth. Data is normalized so that Control is 0 and the rest of the treatments are relative to Control.