DOCTOR OF PHILOSOPHY

The Phytoremediation Abilities of Helianthus annuus and Brassica juncea in Spent Engine Oil and Mine Spoil Co-Contaminated Soi

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Coventry University

By

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The Phytoremediation Abilities of *Helianthus annuus* and *Brassica juncea* in Spent Engine Oil and Mine Spoils Co-Contaminated Soils

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Abstract

Phytoremediation is a biological treatment technology that utilizes plants to extract, stabilize, volatilize, or facilitate the degradation of pollutants in contaminated soils. The aim of this study was to compare the phytoremediation abilities of *Helianthus annuus* and *Brassica juncea* in Spent Engine Oil (SEO)and mine-spoils co-contaminated soils.

This featured four experiments which investigated the effects of Spent Engine Oil and mine-spoils on germination & plant growth parameters, the potential for mixed-cropping to alleviate soil toxicity effects imposed by Spent Engine Oil, the phytoremediation abilities of the chosen species for the treatment of lead (Pb) and petroleum hydrocarbon contamination, and the potential for struvite and NPK fertilizers to deliver exogenous enhancement of the phytoremediation process.

The experiments consisted of greenhouse pollution simulations which featured a range of pollutant concentrations from 0% to 9.2% w/w for SEO single contaminant experiments, and 0.8% SEO + 10% mine-spoils and 1.6% SEO + 10% mine-spoils w/w.

Percentage germination was determined for Spent Engine Oil concentrations ranging from 0%-6% over a 21-day period, and the results showed that the studied species were adversely affected by increasing doses of Spent Engine Oil which manifested through dose-dependent decline in germination for both species as Spent Engine Oil concentrations increased. Similar patterns were also observed for the growth parameters studied in Spent Engine Oil single contaminant treatments, and in Spent Engine Oil and mine-spoils co-contaminated treatments, which manifested in significant reductions in plant height, number of leaves, and laminar leaf area with increases in contaminant doses.

Residual Total Petroleum Hydrocarbons (TPH), Polycyclic Aromatic Hydrocarbons (PAHs) and lead (Pb) concentrations in co-contaminated soils treated with *Helianthus annuus* and *Brassica juncea* were determined by Gas Chromatography with Flame Ionization Detector (GC-FID), Gas Chromatography with Mass Spectrometry (GC-MS), and Inductively Coupled Plasma with Optical Emissions Spectrometry (ICP-OES) respectively. The results showed that two species significantly reduced total PAHs, TPH and Pb, although the extent of removal decreased as contaminant doses increased in soil treatments. The highest removal for all contaminants were observed in *Helianthus annuus* planted soils.

Nutrient supplementation with NPK and Struvite fertilizers proved beneficial for improving the growth, total Pb uptake and dissipation of Pb, TPH and total PAHs in co-contaminated soils. However, struvite fertilizer was most promising in improving contaminant dissipation, Pb uptake and growth under contaminant stress when combined with *Helianthus annuus*.

Overall, a key finding from this study relating to the tolerance and phytoremediation abilities indicate that *Helianthus annuus* could be used for the treatment of low to medium levels of Pb and petroleum hydrocarbon co-contamination in soils. Another key finding from this study was an indication that struvite could be a promising alternative to regular fertilizers for exogenous nutrient supply for phytoremediation enhancement. This could present a tremendous opportunity for contribution to the circular economy with huge benefits for environmental sustainability, with reduced exploitation on natural nutrient reserves, and conversion of waste to resource for the resolution of other environmental challenges. However, further trials are still required with other plant species and various struvite doses and a wider range of soil contaminants to assess its potential for wider applications under a boarder spectrum of conditions, but overall, this study provided a solid launch point and a step in the right direction to further uncover struvite's full potential.

Acknowledgements

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List of Abbreviations

Abbreviation	Definition
ANOVA	Analysis of Variance
BJ	*Brassica juncea*
BOD	Biological Oxygen Demand
CAWR	Centre for Agroecology, Water and Resilience
CCB	Continuing Calibration Blank
CCV	Continuing Calibration Verification
CFIA	Canadian Food Inspection Agency
COD	Chemical Oxygen Demand
DCM	Dichloromethane
DTPA	Diethylenetriaminepentaacetic Acid
EDDS	Ethylenediamine-disuccinc Acid
EDTA	Ethylenediaminetriacetic Acid
EPA	Environment Protection Agency
GC-FID	Gas Chromatography – Flame Ionization Detector
GC-MS	Gas Chromatography – Mass Spectroscopy
H	Height
HA	*Helianthus annuus*
ICP-OES	Inductively Coupled Plasma – Optical Emissions Spectroscopy
LLA	Laminar Leaf Area
MC	Moisture Content
MCERTS	Monitoring Certification Scheme
NL	Number of Leaves
NPK	Nitrogen, Phosphorus and Potassium
NTA	Nitrilotriacetic Acid
OCP	Organochlorine Pesticides
PAH	Polycyclic Aromatic Hydrocarbons
PCB	Polychlorinated Biphenyls
PCDF	Polychlorinated Dibenzofurans
PCF	Plant Concentration Factor
PGPR	Plant Growth Promoting Rhizobacteria
PHE	Phenanthrene
PMVC	Pig Manure Vermicompost
POP	Persistent Organic Pollutants
PRP	Progress Review Panel
RPM	Rotations Per Minute
RWC	Relative Water Content
SDS	Sodium Dodecyl Sulphate
SEO	Spent Engine Oil
STRV	Struvite
TPH	Total Petroleum Hydrocarbons
UKAS	The United Kingdom Accreditation Service
USEPA	United States Environmental Protection Agency

Note: This list of abbreviations is supplemented with chapter specific list of abbreviations where required to refresh the memory of the reader.

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Chapter 1: INTRODUCTION

The fact that the soil is the primary interface between the atmosphere and the earth's crust and the major medium of man's interaction with the environment makes it vulnerable to diverse forms of alteration with pollution being a concomitant culprit (Marques, Rangel, and Castro 2009). Over the years, the soil has been impacted by anthropogenic activities such as energy production, transportation, food production/agriculture and housing with the rapidly growing global population increasing the demand/stress on land (Batty and Dolan 2013). These land-use activities are often associated with varying degrees of soil degradation such as oil spills from crude oil exploration and exploitation for energy production, soil contamination with spent engine oils through improper disposal after the servicing of automobiles, generator sets and other engines, soil pollution with pesticides through farm practices, soil heavy metal pollution from mining sites, soil erosion from construction activities, PCB and heavy metal pollution from transportation activities, etc (Garbuio, Howard, and Dos Santos 2012, Akoto et al. 2023, Žibret et al. 2018, Assennato et al. 2022, Stojic, Pucarevic, and Stojic 2017, Marcotullio, Braimoh, and Onishi 2008, Kollaros et al. 2014, Novák, Balla, and Kamp 2020). For instance, Akoto *et al.* (2023) investigated topsoil heavy metal pollution at Nangodi which is a mining area in the Northern region of Ghana. The results of their study reported elevated concentrations of cadmium, iron, arsenic, lead, and mercury, directly linked to anthropogenic activities when compared to the normal background concentrations for these metals in the control soils. Similarly, Stojic, Pucarevic, and Stojic (2017) also reported anthropogenic related soil contamination by PCBs, copper, and zinc along the railway tracks the western part of the Autonomous Province of Vojvodina, in Serbia. Aside the pollution impacts anthropogenic activities exert on the environment, ecosystem services and habitat loss are also impacted by human activities which is further exacerbated by the ever growing demands to support the needs of the growing global population resulting in an increase in intensity of these activities with concomitant increase in the associated environmental impacts (Marques, Rangel, and Castro 2009). For instance, Assennato *et al.* (2022) in their study of The Impact of Urbanization on Land in Italy reported significant losses in wood production with concomitant effects on carbon storage, habitat quality degradation, alteration of hydrological regime regulation, and decline in pollination, from 2012-2020 as a result of urbanization.

Contamination involving a mix of high levels of petroleum hydrocarbons (including gasoline, spent engine oil, diesel and crude oil) and heavy metals (including As, Cd, Pb, Ni and Zn) have been found in areas affected by spills and leaks from gas stations and storage tanks, former train stations and railroads, mining sites, industrial zones and refinery wastes (Samaksaman *et al.* 2016; Wiłkomirski *et al.* 2011), and amidst the various pollutants that have plagued the soil and groundwater over the years, hydrocarbons and heavy metals are some of the most recurrent contaminants at play (Cavazzoli *et al*. 2022). Various studies (Li *et al.* 2014; Järup 2003; Li *et al.* 2020; Alrumman, Standing, and Paton 2015; Mazzella *et al.* 2007) have reported the deleterious effects these contaminants pose to the ecosystems and human health alike. For example, heavy metals have a significant effect on soil productivity (Singh and Kalamdhad 2011), alter the activities, diversity and population sizes of microbial communities, and could be poisonous to humans and animals via food-chain bioaccumulation and dermal absorption pathways (Li *et al*. 2014; Vazquez-Duhalt 1989). Hydrocarbons are also harmful to the ecosystems and human health with effects like inhibition of enzymatic activities (Alrumman, Standing, and Paton 2015), elimination of certain free marine nematode communities (Mahmoudi et al. 2005), and the risk of cancer to humans if exposed to certain PAHs like benzo[a]pyrene, naphthalene, chrysene, benzo[a]anthracene, benzo[k]fluoranthene and benzo[b]fluoranthene (Abdel-Shafy and Mansour 2016).

Mining is considered to be a prime source of heavy metal contamination in the environment via large volumes of waste minerals and tailings from vigorous extraction of minerals (Karn *et al.* 2021). This has been shown in numerous studies (Li *et al*. 2014; Shahmoradi *et al.* 2020; Wilson and Pyatt 2007; Ge *et al*. 2015; Niu, Gao, and Zhao 2014) which reported high metal levels in soils and plants in areas surrounding mining sites, thus, elevating the risks of bioaccumulation and biomagnification. For instance, a study by Shahmoradi *et al.* (2020) on the effects of iron mining activities on the sediments of the Aqyazi River in Iran reported elevated levels of Cd and Cu concentrations in the river. The results of their geoaccumulation index in tandem with spatial distribution of Cd and Cu concentrations led them to the conclusion that mining activity was the source of the contamination. Similarly, Li *et al.* (2018) also reported concentrations of Cd, Cr, Cu, Pb, Ni and Zn exceeding normal background concentrations in samples from farmland tillage soil surrounding a coal mine in southwestern Shandong province. The results of their micro-domain analysis of toxic metals in a typical area of the coal transportation line revealed acute heavy metal contamination levels on the sides of the coal transportation road, which is indicative of a link between mining activities and heavy metal contamination in the area. When it comes to pollution by petroleum hydrocarbons, petroleum producing, and industrialized countries have a higher prevalence of the occurrence of hydrocarbon pollution. However, pollution caused by its products (particularly spent engine oil spills) occur in every major city across the globe (Agamuthu, Abioye, and Aziz 2010). Spent Engine Oil (SEO) is a hazardous waste generated during the servicing of engines of automobiles and machinery due to a depletion in the effectiveness of the engine oil as a lubricant, imposed by contamination from impurities, and chemical changes due to exposure to high temperatures and combustion by-products (Nte, Chimezie Onyeoziri, and Chukwuma 2020). Annually, copious amounts of spent engine oil are generated across the globe. For instance, the United States of America recycles up to 3200 million litres of spent engine oil annually with significant quantities still being discharged into the environment (Atagana 2011). The situation is even more acute in less developed countries with no systems in place for recycling or proper management/disposal of spent engine oil as seen in Nigeria that generates and discharges up to 80 million litres of spent engine oil into the environment annually. Similar trends were also observed in the Kampala district of Uganda where approximately 1,112,704 litres from garages and 354,900 litres from fuel stations are disposed directly into the environment annually (Ssempebwa and Carpenter 2009). The toxic substances like lead, cadmium, arsenic, zinc, polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), and benzene contained in SEO, coupled with its physical properties like hydrophobicity, density, and viscosity make it particularly hazardous to soil and aquatic environments (Udonne and Onwuma 2014, Pinheiro et al. 2017, Kanokkantapong et al. 2009). For instance, increase in soil bulk density, decrease in soil aeration, reduction in moisture content, nutrient deficiency, soil water logging, and alteration of soil pH are some notable manifestations of the effects of SEO on soil physical and chemical properties, which create sub-optimal soil conditions (Swapna and Vijayammal 2021, Okonokhua, B.O., Ikhajiagbe, B., Anoliefo, G.O. and Emede 2007, Johnbosco, Bibiana.C, and Richard.E 2020, Ifeanyi and Agwu 2014).

In response to the increased risks posed by these pollutants in the environment, several physical and chemical methods (such as soil washing, incineration, thermal desorption, chemical oxidation, and chemical leaching) have been used to remediate polluted soils (Aparicio *et al*. 2022; dos Santos *et al*. 2017; de Percin 1995; *Trellu et al.* 2016). There is literature to show that these technologies have been successful and efficient in the remediation of certain contaminants in the soil. For instance, Liu et al. (2014) reported up to 97.4% removal of PCB after 1hr treatment at 600 °C using thermal desorption. Similar results was reported for soil washing with averages reaching 97.1% and 94.9% removal efficiencies of per- and poly-fluoroalkyl substances (PFASs) from clay soils using perfluoro-carboxylic acids and perfluoro-sulfonic acids respectively (Grimison et al. 2023). However, certain factors associated with these technologies like high costs and environmental pollution (e.g., increased risks of soil pollution via leaching and transport of contaminants to nearby unpolluted areas, ground and surface water pollution via infiltration and surface run-offs, and emission of pollutants into the atmosphere) have left a lot to be desired (Atagana 2011). For instance, cost has been reported to be a significant cause of failure for soil washing projects and some examples were highlighted by Dermont *et al*. (2008).

The quest for an eco-friendly, non-destructive, and cost-effective technology for the in-situ remediation of contaminated sites, has resulted in the emergence of phytoremediation as a viable remediation technology for contaminated environments (Agamuthu, Abioye, and Aziz 2010). Phytoremediation simply refers to the utilization of plants (either natural or genetically altered/enhanced) to extract toxic pollutants such as heavy metals, pesticides, Polyaromatic hydrocarbons and polychlorinated biphenyls from soils and convert them from toxic to safe compound metabolites (Mahar *et al.* 2016). Phytoremediation is a cheap and environment friendly method of detoxification of polluted environments (Reddy and Cameselle 2009, Gomes, Dias-Ferreira, and Ribeiro 2013). This is because it does not incur the high logistic, operational, pre-treatment, capital, and landfill costs incurred by most ex-situ remediation technologies (Trellu *et al.* 2016, Grimison *et al.* 2023, Zhao *et al.* 2019, Song *et al.* 2022, Dermont *et al.* 2008). The cost effectiveness and minimal environmental disturbance of phytoremediation and other in-situ bioremediation technologies make them a preferred choice for the phytoremediation of polluted environments.

Several studies have been conducted using different plants to detoxify soils contaminated with different contaminants (Atagana 2011; Ismail *et al.* 2014; Tariq and Ashraf 2016; Sewalem, Elfeky, and El-Shintinawy 2014). Plants such as *Z. maize, H. annuus, B. Campestris*, *P. sativum*¸ *Helianthus Annuus* and *Chromolaena Odorata (L)* have been used in these studies to degrade hydrocarbons and extract heavy metals from polluted soils. Results from these studies showed varying levels of success. For instance, *Helianthus Annuus* accumulating up to 71% of Pb in its shoots in a study by Sewalem, Elfeky, and El-Shintinawy (2014). Tariq and Ashraf (2016) also achieved impressive results in the phytoremediation of heavy metals, reporting removal efficiencies of 96.23% for Pb, 56.03% for Cd, 68.43% for Pb for *Pisum sativum, Helianthus annuus,* and *Zea mays* respectively.

Helianthus annuus and *Brassica juncea* have been shown in literature to have immense potential for extracting and accumulating heavy metals from polluted soils. This can be seen in numerous studies where both species have been extensively tested with results showing its ability to survive and thrive in heavy metal contaminated soils (Kötschau *et al.* 2013; Mohammadzadeh *et al*. 2017; Adesodun *et al*. 2010; Andreazza *et al.* 2015; Forte and Mutiti 2017; Ashraf, Ahmad, and Ozturk 2010; Pugazholi, Babypriya, and R 2013, Vera Tomé, Blanco Rodríguez; and Lozano 2009; Goswami and Das 2015; Chigbo, Batty, and Bartlett 2013; Bauddh and Singh 2012; Rehman *et al.* 2019; Irfan, Ahmad, and Hayat 2014).

These studies showed that both species were able to germinate and grow in heavy metal contaminated soils although their growth indices were negatively impacted with increased concentration of heavy metals. The studies also showed varying extraction capabilities for different heavy metals which further confirms that this species has significant potential for the phytoremediation of heavy metal polluted sites. For instance, a study by Chauhan and Mathur (2020) reported heavy metal accumulations reaching 158.29, 59.6, 166.5, 101.89, 53.25, and 2.55 mg/kg for Pb, Cd, Zn, Cu, Fe, and As respectively using *Helianthus annuus*. This shows that *Helianthus annuus* is highly efficient in the removal of Pb, Cd, and Zn in heavy metal contaminated soils. Similar results have been reported for *Brassica juncea*. For instance, Singh and Fulekar (2012) reported percentage removals reaching 88.9%, 80%, and 89.8% for Cd, Pb, and Zn respectively in *Brassica juncea* planted soils.

Their abilities to survive and grow in soils with organic contaminants like PAHs, TPH, SEO and other petroleum hydrocarbons have been reported in several studies (Chigbo, Batty, and Bartlett 2013, Marchand *et al.* 2018, Odebode *et al.* 2021, Kluk and Steliga 2019a, Panwar and Mathur 2023, Rahbar, Kiarostami, and Shirdam 2012), which is indicative of their suitability for this study. For instance, *Brassica juncea* and *Helianthus annuus* have been reported to survive 500 mg/kg pyrene and 1800 mg/kg Total Hydrocarbon Content (THC) levels respectively (Rahbar, Kiarostami, and Shirdam 2012, Chigbo, Batty, and Bartlett 2013). A study by Dominguez-Rosado and Pichtel (2004) reported high biomass production by *Helianthus annuus* and *Brassica juncea* in 1.5% w/w SEO contaminated soils, with total decontamination after 150 days. This indicates that not only are they able to survive and grow in soils with organic contaminants, but they have potential to significantly reduce organic contaminant concentrations from soils.

Various technologies for improving the performances of these species have also been explored. These can be seen in studies where *Helianthus* annuus and *Brassica* have been extensively tested with soil amendments, plant growth promoting bacteria and using biosurfactant and bioaugmentation technologies to enhance its phytoremediation efficiency for heavy metal polluted soils, with promising results (Liduino, Vitor S, Servulo, and Oliveira

2018, Mohammadzadeh et al. 2014, Marques et al. 2013, Govarthanan et al. 2018, Turgut, Katie Pepe, and Cutright 2004, Bahadur et al. 2017; Pérez-Esteban *et al.* 2014; Niazi *et al*. 2017; Mahmud *et al.* 2018).

Despite the successful rigorous testing that *Helianthus annuus* and *Brassica juncea* have undergone to determine their phytoremediation abilities in a heavy metal contamination context, there are insufficient studies that have explored their phytoremediation abilities in soils polluted by petroleum hydrocarbons (especially SEO), or soils polluted with mixed contaminants such as soils co-contaminated with petroleum hydrocarbons and heavy metals, particularly SEO and mine-spoils co-contamination. Mixed contamination is an environmental problem that makes up a significant proportion of contaminated sites around the globe. For instance, about 40% of waste sites across the United States exhibit co-contamination, featuring a blend of organic and inorganic compounds (Sandrin and Maier 2003). This proportion forms a substantial part of the reported 37% of contaminated sites in the country known to contain a combination of organic and inorganic pollutants (Springael et al. 1993). The composition of contamination combinations stems from the interplay between historical and present-day land usage patterns, with each site's unique pollution profile intricately linked to its past activities and current utilization. For instance, a study by (Stojic, Pucarevic, and Stojic 2017) established a relationship between railway transportation activities and soil co-contamination with PCBs and heavy metals (Cu, Zn, Cd and Pb). Automobile workshops, timber processing sites, and petrol stations are also known to contain a combination of organic and inorganic contaminants like TPH, PAHs, and heavy metals (Hutchins and Herwijnen 2005, Jolaoso *et al.* 2019, Raskin and Ensley 1999). Although mine spoils and SEO co-contamination has not been widely reported in the literature which could be indicative of a research gap, situations like improper handling of SEO during equipment/machinery servicing and maintenance on metal mining sites could result in leaks and spills with concomitant SEO-heavy metal co-contamination on mining sites. While this might not be a widespread occurrence compared to other forms of contamination as metal mining is not universal across the globe, this combination of organic (TPH & PAHs) and inorganic (Pb) co-contamination has widespread applicability and occurrence across the globe as previously elucidated above.

The fact that majority of the studies (Rathore *et al.* 2017; Jeyasundar *et al.* 2021; Gayatri, Sailesh, and Srinivas 2019; Raj, Kumar, and Maiti 2020; Niazi *et al.* 2017; Liduino, Vitor S, Servulo, and Oliveira 2018; Kötschau *et al*. 2013; Lothe, Hansda, and Kumar 2016) regarding the phytoremediation potentials of these species is directed towards single contaminants (particularly heavy metals) is indicative of a research gap in terms of their tolerance and phytoremediation abilities in hydrocarbon oil and heavy metals co-contamination, and their abilities to clean up soils polluted with hydrocarbon oils.

Phytoremediation enhancements/optimization methods have been conducted using various soil amendments as stated above, but very few studies have investigated the potential of struvite (an industrial waste) to enhance the phytoremediation process. Struvite is Magnesium Ammonium Phosphate ($NH_4MgPO_4 \cdot 6H_2O$) formed in aqueous environments high in phosphorus and ammonium often as orthorhombic crystals (Tansel, Lunn, and Monje 2018). Struvite (often produced as a by-product) is formed in pipes of wastewater treatment plants in areas of frequent rapid pressure alterations (Ifelebuegu *et al*. 2015). Struvite formation in wastewater treatment pipes presents bottlenecks in operation resulting in higher maintenance costs especially from reduced pumping efficiencies caused by blockages in the pipes (Agudosi *et al.* 2018). Struvite being rich in Phosphorus, Nitrogen and Magnesium also causes environmental problems in aquatic environments via eutrophication and increased Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD). However, the composition of struvite (which shows the presence of Nitrogen and phosphorus) presents an opportunity for the agricultural sector as these are fundamental plant nutrients and hence, studies have been ongoing, researching crystallizing struvite in wastewater treatment plants and exploring its application as fertilizers (Degryse *et al.* 2017; Antonini *et al*. 2011; Gong *et al.* 2018; Agudosi *et al.* 2018). Testing this for its phytoremediation potential would be adopting a sustainability approach to problem solving by taking a waste product and utilizing it in solving another environmental problem (optimizing soil conditions for enhanced phytoremediation). The results from this could be a great addition to the body of knowledge and the ongoing research on ways and materials for enhancing phytoremediation efficiency in polluted soils.

Due to limited literature on the phytoremediation of SEO contaminated soils and SEO-mine spoils co-contaminated soils using *Helianthus annuus* and *Brassica juncea*, this PhD research seeks to fill those research gaps and provide valuable information on the phytoremediation abilities of the chosen species for these two contamination scenarios. There are also very limited studies on the potential of struvite (which is discussed in section **2.6**) as an amendment for the enhancement of phytoremediation, and thus, this research seeks to provide some insight on the potential for struvite to enhance the phytoremediation abilities of *Helianthus annuus* and *Brassica juncea* in SEO and mine spoils co-contaminated soils in comparison to NPK fertilizer.

1.1 RESEARCH AIM AND OBJECTIVES

1.1.1 Aim

This research is aimed at investigating the potential of *Helianthus annuus* and *Brassica juncea* as suitable species for the treatment of SEO and mine-spoils co-contaminated soils and to also evaluate the potential of struvite to enhance the phytoremediation efficiency of the chosen species.

1.1.2 Objectives

1. To investigate the germination response of *Helianthus annuus* and *Brassica juncea* to Spent Engine Oil Concentrations as an indication of their suitability for the phytoremediation of SEO polluted soils.
2. To investigate the potential of mixed cropping on reducing the impacts of SEO concentrations (4.6 and 9.2% w/w) on the growth of *Helianthus annuus* and *Brassica juncea*.
3. To determine the effect of mine-spoils and SEO co-contamination on the growth parameters of *Helianthus annuus* and *Brassica juncea*.
4. To investigate the abilities of *Helianthus annuus* and *Brassica juncea* to reduce TPH and PAH concentrations from SEO and mine-spoils co-contaminated soils.
5. To investigate the abilities of *Helianthus annuus* and *Brassica juncea* to uptake and reduce Pb concentrations in soils co-contaminated with SEO mine-spoils.
6. To evaluate and compare the potential for an industrial waste (struvite) in enhancing the growth and phytoremediation abilities of both species in comparison to NPK fertilizer.

Chapter 2: LITERATURE REVIEW

This chapter covers contaminated land, types of contaminants, various technologies that have been used for the remediation of contaminated land and their drawbacks (sections **2.1** and **2.2**). Phytoremediation, its mechanisms, and technologies for assisted phytoremediation are discussed in section **2.3** of this chapter. Struvite as a potential amendment for enhanced phytoremediation and the plant species utilized for phytoremediation in this study are discussed in sections **2.6** and **2.7** of this chapter.

2.1 CONTAMINATED LAND

According to section 57 of the Environmental Act, contaminated land is any land which appears to be in a condition, by reason of substances in, on or under the land to which significant harm is being caused or one in where there exists a significant risk of harm being caused. This also applies to controlled waters that are being polluted or are likely to be polluted by the above. Land being a valuable and finite resource, makes it imperative that its purity is preserved in such a manner that its ability to support quality of life for communities and biodiversity is not compromised.

Over the years, factors like rapid urbanization and industrialization have been associated with increases in quality of life and have contributed immensely to economic growth and development. However, these activities have had contrasting effects which on one hand was beneficial for economic development and on the other hand, detrimental environmental effects with contaminated land being a concomitant feature. Contaminated land is a global environmental problem that is inadvertently linked with socioeconomic advancement. For instance, China has seen an increase in land contamination since its 1978 Economic reforms (Deng et al. 2016). A study by Zhang *et al*. (2019) showed that soil Pb concentrations increased between 1990 and 2001 as a result of industrialization and transportation with major contamination sources attributed to traffic emissions, mining, smelting and e-waste recycling. Ilić *et al*. (2021) had similar findings where PAH contamination in soil and groundwater was recorded at a former cellulose factory in the city of Banja Luka, Republic of Srpska, Bosnia and Herzegovina. Their study showed that significant PAH contamination was recorded at the site in the topsoil and ground water with PAH contamination being significantly higher in ground water to the point that it was no longer fit for almost any purpose. They attributed main contaminant sources to coal combustion, petroleum sources and biomass combustion. Several other studies (Farooqi *et al.* 2021; Kulikova *et al.* 2019; Rachwał, Magiera, and Wawer 2015; Marinho Reis *et al.* 2016; Li *et al.* 2019; Zwolak *et al.* 2019) have shown increase in land contamination from contaminants like heavy metals, petroleum hydrocarbons, PAHs and pesticides with links to industrialization, urbanization, agricultural expansion etc in different parts of the world.

Contaminants associated with contaminated land from sources listed above are largely classed as organic and inorganic contaminants, all with significant risks and adverse effects to health, biodiversity, and the environment, and these are highlighted in sections **2.1.1** and **2.1.2** of this chapter.

2.1.1 Inorganic Contaminants

Pollutants like heavy metals, trace elements, inorganic salts, mineral acids, and metals with organic compounds as complexes, sulphates, and cyanides are inorganic pollutants which form a major class of contaminants released by chemical and allied industries like pharmaceuticals, refineries and fertilizers (Wasewar, Singh, and Kansal 2020). Inorganic pollutants are largely made up of heavy metals and metalloids which possess long tenacity and resistance to degradation, thus, making them a significant hazard to the environment and living systems especially due to their carcinogenic and bio-accumulative properties (Borah, Kumar, and Devi 2020).

Pb has been ranked as one of the top 10 chemicals of public health concern (World Health Organization 2018) making it's contamination of soils a significant global concern especially due to its persistence and toxicity (Etim 2017). Lead exposure can result in acute and chronic illnesses in individuals of all age groups, affecting various organ systems. Chronic lead poisoning is more prevalent than the acute form, with adults having a higher predisposition In to issues such as memory and concentration problems, depression, abdominal and neuromuscular symptoms, fatigue, anaemia, sleep disturbances, hypertension, and cardiovascular diseases, while children with chronic exposure exhibit aggression and apathy (Dobrescu et al. 2022). Although Pb is a fairly stable compound with high resistance to corrosion, its high mobility at low pH creates a significant risk as factors like changes in soil pH and acidic water drainage can mobilize Pb, and Pb migration will result in pollution with elevated risks to the environment and human health (Center for Disease Control and Prevention 1992). Pb when present in high concentrations disrupts plant growth and development, inhibiting root elongation, reducing nutrient uptake, and causing chlorosis (Kumar, Smita, and Cumbal Flores 2017). Additionally, Pb can accumulate in plant tissues, posing health risks for animals and humans if consumed, with elevated risks of biomagnification (Balkhair and Ashraf 2016). As for microorganisms in the soil, Pb acts as a toxic pollutant, impairing microbial activity and diversity, thereby, disrupting crucial soil processes such as nutrient cycling and decomposition, ultimately affecting the overall soil health and ecosystem functioning (Collin et al. 2022). These necessitate a call to action to contain and treat soils contaminated with Pb to avert the imminent risks it poses to the environment and to human health, and this constitutes the primary reason why Pb is the primary metal of interest in this study.

2.1.2 Organic Contaminants

Organic contaminants are toxic molecular compounds found in industrial products such as organic solvents, petroleum hydrocarbons, pesticides, dyes and detergents which could pose a serious threat to humans and wildlife when their permissible limits are exceeded (Geetha and Nagarajan 2021). Organic contaminants have been categorized into Persistent Organic Pollutants (POPs) and non-Persistent Organic Pollutants with the former garnering more attention and concerns due to their high persistence and toxicity in soil which elevate their threat to human health (Meng *et al.* 2021). POPs such as Poly Aromatic Hydrocarbons (PAHs), Polychlorinated Biphenyls (PCBs), and Organochlorine Pesticides (OCPs) are particularly dangerous due to their ability to be absorbed by plants grown on soils contaminated by POPs and the high tendencies for biomagnification (Zeliger 2011).

The environmental hazards coupled with the threat to humans, wildlife and biodiversity imposed by these contaminants has fuelled concerns, policy approaches and calls to action for the remediation of contaminated land in a bid to minimize the concomitant risks and to return them to a state where they can support environmental, social, and economic activities which had been otherwise compromised. Some of the methods and technologies that have been used for the remediation of contaminated land are covered in the subsequent sections of this chapter.

Among the various forms of soil contamination with organic contaminants, SEO contamination has widespread significance due to the use of engine oils in every city across the globe. For instance, Nigeria is reported to generate about 87 million litres of SEO annually

(Tanimu 2019), and the European Union (EU) reported to manage 3 million tonnes of SEO annually, making SEO the most significant liquid hazardous waste in Europe (Pinheiro et al. 2017). This creates the risk of SEO contamination especially in places where stringent hazardous waste management protocols are not enforced or adhered to. SEO is a concerning environmental and health hazard due to its composition of metals and heavy polycyclic aromatic hydrocarbons (Thenmozhi et al. 2013). These elements can contribute to chronic health risks, including mutagenicity and carcinogenicity, with prolonged exposure to high concentrations of waste engine oil possessing associations with the development of liver or kidney diseases, potential damage to the bone marrow, and an elevated risk of cancer (Thenmozhi et al. 2013). SEO contamination in the soil causes significant changes in soil microbiological and physicochemical properties, alters soil drainage regimes and creates unsatisfactory conditions for plant growth which manifests through stunted growth and plant mortality at high SEO concentrations (Silva et al. 2023). Therefore, the remediation of SEO contaminated soils is of utmost importance to safeguard the environment and human health, to restore the health and productivity of contaminated soils, preventing further spread of pollutants and ensuring a sustainable and safe environment for present and future generations. Section 2.2 below reviews various remediation technologies that have been utilized in the remediation of heavy metals and petroleum hydrocarbon contaminated soils with a focus on Pb and SEO contamination.

2.2 REMEDIATION OF CONTAMINATED LAND

Soil remediation simply refers to the management of contaminants at a site to prevent, minimize or mitigate impacts to human health or the environment usually preceded by the identification of contaminated soil, determination of remedial objectives and formulation of an appropriate remediation strategy (Fernández, Sánchez-Arguello, and García-Gómez 2022). Over the years, various technologies have been employed to tackle the remediation of contaminated soils and these have been classified into physical, chemical and bioremediation technologies (Song *et al*. 2022; Lv, Bao, and Zhu 2022). These technologies are usually either implemented on-site (in-situ) or excavated and transported to an off-site facility for treatment (ex-situ) with the major downside of ex-situ technologies being high costs from transportation and the main downside of in-situ being the significantly longer time required for complete remediation when compared to ex-situ methods (Khan, Husain, and Hejazi 2004). There are 3 key approaches remediation technologies have and these include containment which aims to isolate the site without necessarily acting on the contaminants, immobilization to minimize contaminant transport within the environment and treatment approaches which aim to lower contaminant concentrations to acceptable limits for the intended land-use (Fernández, Sánchez-Arguello, and García-Gómez 2022). Some of the major remediation technologies employed for the management of contaminated land are covered in sections **2.2.1** to **2.2.3** of this chapter.

2.2.1 Physical Remediation Technologies

Physical remediation simply refers to the employment of physical processes for the remediation of contaminated soils usually requiring comparatively simple equipment, easy operation and relatively cost effective (Lv, Bao, and Zhu 2022; Song *et al*. 2022). Some of the key physical remediation technologies that have been used in the remediation of Pb and petroleum hydrocarbons are discussed briefly in sections **2.2.1.1** and **2.2.1.2** below.

2.2.1.1 Thermal Desorption

This remediation technology involves the separation of volatile and semi-volatile contaminants from soil via direct or indirect heating to appropriate temperatures in a vacuum or into a carrier gars, and the subsequent removal or recycling of the carrier gas in the off-gas treatment system (Zhao et al. 2019). This technology is particularly suited for the remediation of soils contaminated with volatile and semi-volatile organic compounds like PAHs, PCBs, Total Petroleum Hydrocarbons (TPH), chlorinated solvents and volatile inorganic substances like mercury (Hg). The wide range of organic contaminants treatable using this technology, the reduction in the likelihood of secondary pollution because of the effective air pollution control systems in place which also facilitates recyclability of valuable contaminants, equipment mobility and the minimization of the production of toxic secondary pollutants like polychlorinated dibenzofurans (PCDFs) make this an attractive choice for remediation projects (Liu *et al*. 2015; de Percin 1995). However, the higher costs when compared with bioremediation technologies like microbial remediation and bio-ventilation as well as its limited suitability for inorganic contaminants are some key drawbacks of using this technology (de Percin 1995; Zhao *et al.* 2019).

2.2.1.2 Soil Washing

Soil washing is an ex-situ remediation technology involving the excavation of contaminated soil followed by a separation of contaminant using water and/or other extracting agents which facilitate the transport of the contaminants from the soil into the extracting agent which can be recovered and recycled or disposed of (Yi and Sung 2015; Trellu *et al.* 2016; Feng *et al.* 2001). The versatility of this technology in terms of applicability in the remediation of soils contaminated with organic and inorganic pollutants alike contributed to its widespread adoption over the years. The use of additives like surfactants and acids have been effective in speeding up the process as they help in facilitating the leaching of the contaminants via increased solubilization and mobilization of the contaminants from the soil to the soil washing solution (Khalid et al. 2017). For instance, study by Dike *et al.* (2013) reported up to 96% PAH dissipation in soils containing up to 83% SEO concentrations using soil washing with normal household detergent composed of surfactants, sodium carbonate, sodium silicate, sodium sulphate, sodium carboxymethyl, cellulose, enzymes and optical brightener. However, the results they reported for heavy metal removal was underwhelming especially as the heavy metal concentrations were all well below 1 mg/kg. What was worthy of note is that chromium concentration in the SEO was about 0.003 mg/kg which would have been diluted after mixing with the soil. However, the removal rate reported for Cr was 70% at 83.3% SEO concentration. This is indicative that the washing solution utilized is highly effective for PAH dissipation, but sub-optimal for the removal of heavy metals. This could be due to its inability to mobilize the heavy metals in the soil solution. However, promising results have also been achieved with soil washing for heavy metal removal when acidic solutions were utilized. For instance, a study by Masson *et al.* (2022) which reported a removal efficiency of 58.69% in soils contaminated with Pb at 2000 mg/kg concentration using soil washing technology with a 5% w/w saponin solution. Their study showed that the optimum pH for Pb removal was 3.5, and a graded dose response was observed as Pb removal increased with an increase in saponin concentrations in the washing solution. This demonstrates that lower pH solutions are optimal for heavy metal removal using soil washing which is why a better results were obtained by Masson *et al.* (2022) which had Pb concentration that was about 2000 times higher when compared to Dike *et al.* (2013).

However, as successful and effective as this technology is in the remediation of organic and inorganic contaminants, it is associated with the generation of high volumes of contaminated effluents requiring treatment which are accompanied by high costs from energy consumption, transportation and extraction solution recovery, leaving a lot to be desired in terms of economic viability (Trellu et al. 2016).

2.2.1.3 Electrokinetic Remediation

Electrokinetic remediation is a technique used to clean up contaminated soil or groundwater that involves applying an electric field to the affected area, which mobilizes charged contaminants and ions (Adebayo *et al.* 2023). This method utilizes a low-intensity electric field to mobilize the target pollutants, employing transport mechanisms like electromigration, electroosmosis, and electrophoresis (Park *et al.* 2009). It is particularly suitable for treating soils with low-permeability, high salinity, and strong buffering capacity that are contaminated with both organic and inorganic pollutants (Mao, Shao, and Zhang 2019). For instance, 75% remediation efficiency was reported for crude oil contaminated soils using electrokinetic remediation in a laboratory scale study by Korolev, Romanyukha, and Abyzova (2008). They observed that increase in soil porosity was beneficial and the introduction of a leachate solution to simulate soil washing in tandem with electrokinetic remediation increased the remediation efficiency to 95%. Mao, Shao, and Zhang (2019) reported 24% and 55% removal efficiency for Zn and SEO respectively using electrokinetic remediation over a 17-day remediation period. They also reported a graded dose response in remediation efficiency manifested through increase in remediation with increase in voltage gradient, indicating that high energy consumption is required for optimal remediation with this technology. However, it appears that this technology might not be effective for the removal of certain metals like Cr and Pb as some studies have generated subpar results for the said metals using this technology. For instance, Cameselle, Gouveia, and Cabo (2021) reported low solubility for Cr and Pb using this technology even after combining the use of chelating agents with the technology, yielding a maximum removal efficiency of 11.8% and 9.8% for Cr and Pb respectively. However, combining electrokinetic remediation with permeable reactive barriers like aminated electrospun nanofiber membrane for Cr, and reactive materials (such as fly-ash and graphene oxide) for Pb, have yielded removal efficiencies reaching 72.6% and 92.6% for Cr and Pb respectively (Zhou et al. 2021, Wang, J. *et al.* 2021).

High energy demand, scale constraints relating to non-uniformity of the electrical field for larger sites, dependence on adequate soil characteristics (such as permeability and electrical conductivity), time constraints (especially for soils with low permeability), and risk of secondary pollution from elevated contaminant solubility represent some of the key limitations of this technology (Song et al. 2022).

There are several other physical remediation technologies such as soil flushing, soil replacement, incineration, soil isolation, landfilling, and vitrification (Hu *et al*. 2021; Zhu *et al.* 2021; Khan, Husain, and Hejazi 2004; Halmemies *et al*. 2003) all achieving varying degrees of success in the remediation of heavy metals and petroleum hydrocarbons including SEO and Pb. However, as physical remediation technologies are largely devoid of chemicals and chemical reaction processes, it usually requires an additional treatment step for the contaminants that have been concentrated in liquid (water) or gaseous mediums to prevent the occurrence of secondary pollution.

2.2.2 Chemical Remediation Technologies

Chemical remediation technologies rely on the supply of chemical remediation reagents to enhance the availability and transport of contaminants and reduce contaminant toxicity through decomposition, adsorption, reduction, complexation, oxidation, and precipitation chemical reactions (Lv, Bao, and Zhu 2022; Song *et al.* 2022). Some of the chemical remediation technologies are covered briefly in sections **2.2.2.1** and **2.2.2.2** below.

2.2.2.1 Chemical Oxidation/Reduction

Chemical oxidation is primarily an in-situ remediation technology which involves the minimization of contaminant mobility, environmental availability and toxicity to prevent contaminant transport by injecting oxidants deep into the contaminated area and surrounding areas to facilitate reactions between the injected oxidants and the contaminants (Aparicio *et al*. 2022). This technology is particularly useful as it could be employed in the remediation of organic and inorganic contaminated soils (Liang *et al*. 2022; Yang *et al.* 2020; Kurakalva 2022) making it versatile as its applicability spans across a vast spectrum of contaminants. For instance, maximum removal efficiencies of 98%, 95%, and 90% have been reported for chemical oxidation using hydrogen peroxide, ozone, and persulfate respectively as oxidants in diesel contaminated soils (Lim, Lau, and Poh 2016). Because this technology is often carried out at mild temperatures and normal pressure conditions, it has been viewed as an attractive choice for the remediation of contaminated soils and groundwater (Kurakalva 2022). One downside of using this technology is that most in-situ chemical oxidation technologies experience non-selective oxidant consumption (soil oxidant demand) where only a small percentage of the oxidant reacts with the target contaminant (O'Connor *et al*. 2018) which could lead to wastage of oxidant and possibly, secondary pollution. This was observed in a study by (Lee *et al*. 2003) where only 18% of the permanganate oxidant used participated in the oxidation reaction to neutralize 41% of Trichloroethylene. This could mean that significantly higher amounts of oxidants could be required to destroy target contaminants, with the excess oxidants running the risk of secondary aquifer contamination. Other disadvantages of using this technology are high operating costs, secondary pollution and possible negative impacts to microbial communities because of the toxicity of some of the oxidants (Song *et al*. 2022; Chang *et al.* 2022; Sutton *et al*. 2011).

2.2.2.2 Chemical Leaching

Chemical leaching is a technology often used in tandem with soil washing and it involves the injection of extraction agents into contaminated soils to enhance the solubilization, desorption and transport of target contaminants into the extraction agents which is then removed and sent for further treatment (Huang *et al*. 2020). This technology is versatile as it could be used for the remediation of organic and inorganic contaminants alike. For instance, the utilization of 5% acetic acid + 5% potassium chloride washing solution yielded a maximum of 86.9% removal efficiency for Pb within a 6hr washing time in a study by Etim (2017). Similar results have also been reported using EDTA with a reported 77% removal efficiency for Pb in a study by Kabilan and Muttharam (2017). Similarly, Hu *et al.* (2021) found that citric acid significantly increased the removal efficiency of Zn by 34.8% when used as a leaching agent in a soil washing experiment as opposed to distilled water that showed only 6.7% removal efficiency for zinc when used as an eluent. They also found that the removal efficiency by citric acid increased in a dose dependent manner, which could mean that larger quantities of citric acid could be required for efficient leaching of Zn in contaminated soils. Similar findings were made by dos Santos *et al*. (2017) in their study which explored the efficacy of surfactant assisted soil washing on the removal of petroleum pollutants from soil. Their study showed that the addition of 5g/kg soil of Sodium Dodecyl Sulphate (SDS) surfactant to the soil washing fluid led to up to 95% removal of petroleum contaminants in the soil, with the removal efficiency increasing in a dose dependent manner.

Several other chemical remediation technologies such as chemical stabilization, low temperature plasma technology and catalytic oxidation technology (Tendero *et al*. 2006; Rajamanickam and Shanthi 2016) have also been used to immobilize and directly react with contaminants in soil. Although chemical remediation technologies have relatively quick turnaround times, low energy consumption and are more cost-effective when compared to physical remediation technologies, the injection of chemicals into the soil could result in secondary pollution and with deleterious effects on soil microbial communities all present bottlenecks for this class of remediation technology.

2.2.3 Bioremediation Technologies

Bioremediation is a cost-effective and environmental friendly technology that uses microbes and biological processes to detoxify and degrade pollutants in soil and water environments (Mehjabeen et al. 2022a). Plants, microorganisms and plant-microbe associations are the primary agents of bioremediations, with their enzymatic components possessing powerful catalytic properties facilitating the alteration of the structural and toxicological properties of biodegradable environmental pollutants (Gianfreda and Rao 2004). Bioremediation does not alter the natural properties of the soil, requires less man power with the ability to run with minimal human involvement, is cheaper than physical and chemical remediation technologies and promotes rhizospheric microbial biomass production (Mehjabeen *et al*. 2022b), making it a very attractive choice for the remediation of contaminated environments. Bio-stimulation and bioaugmentation are some of the most commonly used bioremediation technologies

(Fernández, Sánchez-Arguello, and García-Gómez 2022), and are briefly discussed in sections **2.2.3.1** and **2.2.3.2** of this chapter.

2.2.3.1 Bio-Stimulation

Bio-stimulation involves the use of nutrient supplementation (particularly nitrogen and phosphorus) to enhance the metabolic activities of indigenous microbial communities which utilize hydrocarbons as carbon sources for their growth, thereby leading to the degradation of those hydrocarbons in polluted soils (Wu et al. 2019). This technology has mainly been used for the remediation of soils with organic contaminants and has shown potential for the successful remediation of SEO contaminated soils. For instance, amending soils contaminated with 5% and 15% SEO concentrations effected 92% and 55% biodegradation of SEO after treatment with brewery spent grain over an 84 day period (Abioye, O P, Agamuthu, and Abdul Aziz 2012). Similarly, a study by Wu *et al.* (2016) showed that bio-stimulation with $(NH_4)_2SO_4$ and KH_2PO_4 at a C:N:P ratio of 100:10:1 showed a 60% reduction in TPH after a 6-week incubation period. Organic substances like glucose, sucrose and volatile fatty acids have also demonstrated potential for bio-stimulation purposes. For instance, a study by Yang *et al*. (2018) reported that bio-stimulation with effluents from hydrogen production containing 9.1mM glucose, 16 mM volatile fatty acids and 3.11 mM ethanol improved the degradation of 2,4-dichlorophenoxyacetic acid.

2.2.3.2 Bioaugmentation

Bioaugmentation is a bioremediation technology that utilizes the supply of exogenous microorganisms and/or their biologically active enzymes to contaminated soils to facilitate the degradation, removal and/or biotransformation of contaminants and toxic substances from contaminated soils (Gao, D. et al. 2022). Bacteria and fungi like *Staphylococcus* *haemoliticus* strain 10SBZ1A, *Rhodococcus* sp. BAP-1, *Pseudomonas stutzeri* and *Betaproteobacteria* have been used in the bioaugmentation of organic pollutants in soils (Zhang *et al.* 2020; Jiang *et al.* 2020; Nzila *et al.* 2021; Crampon, Bodilis, and Portet-Koltalo 2018). For instance, *Staphylococcus haemoliticus* strain 10SBZ1A successfully removed 80% of Benzo[a]pyrene within a 30 day period in a study by Nzila *et al*. (2021). A study by Jiang *et al.* (2020) showed promising results using bioaugmentation, reporting a degradation of fluoranthene by approximately 78% after bioaugmentation with *Rhodococcus sp.* This technology has also been explored for the remediation of Pb contaminated soils and promising results have been reported. For instance, (Hashemi et al. 2018) reported removal rates reaching 57.9 %and 55.2% for Pb and Zn respectively by earthworms after 28 days of Pb exposure. They observed that long term exposure of the earthworms to high Pb and Zn exposure enhanced the removal rates by up to 17% within a 14-day period for Pb and Zn, which is an indication that earthworms respond positively to long term exposure to high doses of Pb and Zn within a bioaccumulation context. However, they also reported a dose dependent increase in earthworm mortality as metal concentrations increased.

The potential for combining bioaugmentation and bio-stimulation technologies for enhanced biodegradation of organic pollutants has been explored. For instance, a study by Behera *et al*. (2022) reported the highest TPH degradation (up to 90% degradation) in treatments with bacterial consortium containing *Dietzia lutea* (IRB191), *Dietzia lutea* (IRB192), *Staphylococcus warneri* (BSM19), and *Stenotrophomonas pavanii* (IRB19) strains combined with poultry litter extract as nutrient amendment for bio-stimulation energy. Sarkar *et al*. (2020) reported similar findings in their study which showed that a combination of bioaugmentation and bio-stimulation with nitrates enhanced TPH degradation (86% TPH degradation) when compared to the control treatments.

As promising as bioremediation technologies are especially when taking their advantages (section **2.2.3**) into consideration, they are not devoid of challenges. Competition between fungal agents and indigenous microorganisms, longer remediation cycles compared to physical and chemical remediation technologies, dependence on soil/environmental conditions and inadequate enzyme durability represent some of the key challenges relating to the utilization of this technology.

2.3 PHYTOREMEDIATION

Phytoremediation is a term derived from an ancient Greek word "*Phyto*" which means "plant" and a Latin word "*Remedium*" which means "restoring balance" (Chatterjee *et al*. 2013). Phytoremediation is a low-risk in-situ bioremediation technology that utilizes living plants and their associated microorganisms for the degradation, removal, sequestration of organic and inorganic pollutants from soils, sediments, and water (Mishra and Chandra 2022). This could take place either by the uptake of contaminants by the plants and storing in their roots and shoots (Phytoextraction), enzymatic transformation (phytodegradation) or rhizoremediation which involves the enhancement of microbial activities in the rhizosphere because of the release of exudates from the plant roots (Gomes, Dias-Ferreira, and Ribeiro 2013).

When compared to physical and chemical remediation technologies, phytoremediation is more cost-effective as it requires less machinery, power consumption, capital investment and transport costs. It is also more environmentally sustainable as it generates less secondary waste, less emissions from processes as it relies primarily on biological processes of plant and microbial communities and requires less human involvement (Shen *et al*. 2022; Mehjabeen *et al*. 2022b), and these have resulted in concomitant interest in the technology in the recent decades. Another key advantage of phytoremediation is that it is applicable to a wide range of contaminants including heavy metals (Yang *et al.* 2022), radionuclides (Yan *et al.* 2021), PCBs (Huesemann *et al*. 2009) and organic pollutants like PAHs (Verâne *et al.* 2020), chlorinated solvents (Van Aken and Geiger 2010), TPH (Moreira *et al.* 2013) and pesticides(Hussain *et al*. 2009). This makes it a very versatile bioremediation technology.

2.3.1 Phytoremediation Technologies

There are four main phytoremediation technologies which include uptake of contaminants into plant tissues (phytoextraction), plant induced degradation of contaminants in soil via root exudations which enhance microbial activities in the rhizosphere (phytodegradation), the removal of contaminants in gaseous form by plants (phytovolatilization) and the immobilization of pollutants in soil to prevent the transport and spread of contaminants by plants (phytostabilization). These technologies are discussed in sections **2.3.1.1** to **2.3.1.4** below.

2.3.1.1 Phytoextraction

Phytoextraction is a phytoremediation technology that involves the uptake of contaminants (mostly heavy metals) from the soil by plants which are then harvested and disposed of (Prasad *et al*. 2022). It is a process whereby species with high growth rates, extensive root systems, high biomass production, high tolerance for contaminant concentrations and ability to accumulate contaminants in their roots and shoots (hyperaccumulators) are planted on a contaminated site and after the accumulation process, harvested, treated and disposed of, thereby decontaminating the site (Ranieri *et al*. 2022). Specific plant species known as hyper accumulators are required for effective phytoextractions. Hyper accumulators are a variety of plants from distantly related families, yet share the ability to not only thrive in heavy metal contaminated soils, but also can accumulate astounding quantities of heavy metals in their aerial tissues far beyond what can be seen in the majority of other species without manifesting the effects of phytotoxicity (Rascio and Navari-Izzo 2011).

Successful phytoextraction is dependent on a variety of factors such as soil pH which directly influence the bioavailability of metals for uptake by plants, moisture, temperature, plant biomass production, extensive root systems and rhizospheric microbial activities (Prasad *et al*. 2022). For instance, a study by Wang *et al.* (2006) reported a linear increase in Cadmium (Cd) and Zinc (Zn) accumulation by *Thlaspi caerulescens* as the soil pH decreased, indicating that lowering the pH of the soil was beneficial for phytoextraction. This might be due to an increase in metal solubility as metal bioavailability, solubility and translocation are known to be higher in acidic soils when compared to neutral or alkaline soils (Adamczyk-Szabela and Wolf 2022).

Studies have shown that different species have varying affinities for various heavy metals, whereby some species tend to accumulate more of a certain heavy metal than others. For instance, Tariq and Ashraf (2016) compared the accumulation of Cd, Cu, Co, Ni, Cr and Pb by *Helianthus annuus, Zea mays, Brassica campestris* and *Pisum sativum*. The result of their study showed that *Pisum sativum* had the highest accumulation of Pb (96.23%), *Zea mays* reducing reasonably the concentration levels of all the selected heavy metals but still exhibiting its highest hyperaccumulation ability for Pb (66.36%) and *Helianthus annuus* exhibiting its best phytoextraction potential for Cd among all the other selected metals (56.03%). Hyperaccumulators also tend to accumulate different metals more in various parts of the plant (i.e., roots, shoots, and leaves). For instance, Sewalem *et al*. (2014) studied the phytoremediation of Cd and Pb using sunflower. The results from their study showed that sunflower accumulated a high amount of the total absorbed Cd in the roots (88.84%) while most of the absorbed Pb was accumulated in the shoot (71.39%), and thus, they concluded that they concluded that sunflower would be more efficient in the phytoextraction of Pb and would perform better in the phytostabilization of Cd. This deduction from their study could mean that metal accumulation in above ground parts of plants could be an indication that that species is more suitable for the phytoextraction of that metal, whereas, if most of the accumulation occurs in the roots, that species might be more suitable for the phytostabilization of that specific metal. Therefore, it is worth researching the plant-metal remediation mechanism to help in the selection of appropriate hyperaccumulators for the intended purpose.

Time and growth stage of hyperaccumulators also play a role in the rate of phytoextraction. This means that at a certain stage of growth of a hyperaccumulator specie, the rate of extraction from soil could be more and at other stages of growth, there could be a decline in the rate of metal extraction. This was demonstrated in the study conducted by Adesodun *et al*. (2010) in their study on the phytoremediation potential of sunflowers (*Tithonia diversifolia* and *Helianthus annuus*) for heavy metals in soils contaminated with zinc and lead nitrates**.** They observed substantial accumulation of Zn and Pb in both species within the first 4 weeks after planting. This was followed by a decline in the phytoextraction efficiency of both species. They concluded in their study that the phytoextraction efficiency of *Tithonia diversifolia and Helianthus annuus* for the selected heavy metals is optimum at their initial stages of growth. Knowing this, a good application of this finding when using these species for the phytoextraction of Zn and Pb would be to grow them in soils polluted with these selected heavy metals for about 4-5 weeks when their phytoextraction efficiency is at optimum and harvest after this period after which new seeds can be sown and grown for the same time frame and the cycle continues. This could save time and speed up the process and the time that could have been wasted when their optimum phytoextraction efficiency had been exceeded is put to better use hence ensuring greater efficiency of the project.

Despite the cost-effective and environment friendly allure of this phytoremediation technology, issues like long remediation cycles, metal solubility/bioavailability, limitation to low-medium contaminant levels, potential to introduce toxic contaminants into the food chain and the potential introduction of invasive species represent some of the key bottlenecks of this technology (Prasad et al. 2022). However, strategies like chelation to enhance metal bioavailability and nutrient supplementation have been used to tackle some of these challenges and are discussed in section **2.3.2**.

2.3.1.2 Phytodegradation

Phytodegradation is a process that involves a symbiotic relationship between plants and microorganisms that facilitates the breakdown of organic pollutants within the rhizosphere (Fernández Rodríguez *et al*. 2014). Plant roots release a broad variety of chemical compounds (also known as exudates) into the rhizosphere which attract and select microbial populations in the rhizosphere which in turn impact on the health and performance of the plants by means of various microbial mechanisms (Huang *et al.* 2014). A key advantage of this technology is that it does not have the risk of secondary contamination as it simply involves the breakdown of pollutants to a state where they are no longer toxic.

One of the most important mechanisms of the degradation of pollutants which is the fastest and most effective is the breakdown of pollutants under aerobic conditions (Nevita et al. 2013). **Figure 2.1** shows the main principle of aerobic degradation of hydrocarbons by microorganisms. This begins with an initial intercellular attack on pollutants by an oxidation process, activation, and incorporation of oxygen as the key enzymatic reaction which is catalysed by oxygenases and peroxidases. The procedural conversion of organic pollutants into intermediates of the central intermediary metabolism is carried out by the peripheral degradation pathways (for example, the tricarboxylic acid cycle), cell biomass synthesis takes place in the central precursor metabolytes, and gluconeogenesis are responsible for the synthesis of the sugars required for the various biosynthesis and growth (Das and Chandran 2011). Other mechanisms involved in the microbial breakdown of hydrocarbons include the microbial cell attachment to the substrates and biosurfactant production. **Figure** 2.2 shows the enzymatic reactions involved in the degradation of hydrocarbons.

Figure 2.1 The Main Principle of Aerobic Degradation of Hydrocarbons By Microorganisms (Das and Chandran 2011)

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Figure 2.2 Enzymatic Reactions Involved in the Degradation of Hydrocarbons (Das and Chandran 2011)

The relationship between the activities of plants and microorganisms in the soil is very important as the performances of both in the phytodegradation of pollutants in the soil are affected by the presence of each of the two. For instance, a study by Bordoloi and Basumatary (2015) on the phytoremediation of hydrocarbon-contaminated soil using sedge species showed that the vegetated treatments (i.e. treatments that had *C. rotundus*, *C.brevifolius*, *C. odoratus*, and *C. laevigatus* growing in them) experienced a significant increase in petroleum degrading bacteria at the end of the experiment when compared to the initial population of petroleum degrading bacteria. This could be because of the nutrient exudates released by the roots of the plants leading to an increase in the population of petroleum degrading bacteria (Nevita et al. 2013).

Application of fertilizers be it organic or inorganic, can act as stimulants to the degradation process. Fertilizers enhance/boost plant growth and thus when applied to soils planted with phytoremediation species, it would in turn boost their performance in detoxifying the soil. Studies (Dadrasnia and Pariatamby 2016; Obuotor, Akande, and Bada 2016) have demonstrated that fertilizer application boosts microbial performance in the degradation of petroleum hydrocarbons in polluted soils. For instance, a study by Agarry, Owabor, and Yusuf (2010) evaluated the use of animal manure and chemical fertilizer on the bioremediation of petroleum hydrocarbon contaminated soil showed that after 4 weeks of remediation, poultry manure achieved a 73% remediation, piggery manure 63%, goat manure 50% and NPK fertilizer 39% remediation. Their study demonstrated that although all the fertilizer treatments (both organic and inorganic) were instrumental in the remediation of hydrocarbon polluted soil, all the organic fertilizer treatments were by far more efficient than the chemical fertilizer utilized for the study with poultry manure being the most effective. Chorom, Sharifi, and Motamedi (2010) reported similar findings where the application of NPK fertilizer at 2tons/ha resulted in greater rates of biodegradation of petroleum hydrocarbons after 5 weeks when compared with the control.

The rate of degradation TPH varies between different plant species and is also dependent on time/duration of exposure to the pollutant. This was demonstrated in a study by Idris et al. (2014) where they compared the performances of *Paspalum vaginatum, Paspalum scrobiculatum, Eragrotis atrovirens* and *Cayratia trifolia* in soil polluted with diesel. The results from their study showed that the different species had their peak performances in terms of percentage TPH degradation at different points in time during the study with *E. atrovirens* reaching 68% degradation on Day 7, *P. scrobiculatum* and *C. trifolia* with peak percentage TPH degradations of 74% and 62.9% on Day 72 and *P. vaginatum* had its highest percentage TPH degradation of up to 91.9% on Day 42. Their study also showed that different species are most efficient at different levels of concentration (i.e. some species have their peak percentage

TPH degradation at lower concentrations while others at higher concentrations). This is evidenced in *E. atrovirens P. scrobiculatum* and *C. trifolia* having their peak performance at the lowest concentration level (10g/kg) while *P. vaginatum* had its peak performance at the highest concentration level (30g/kg). Therefore, this study showed that *P. vaginatum, P. scrobiculatum* and *C. trifolia* would be more efficient in reducing TPH levels in soils polluted with diesel at low concentrations while *P. vaginatum* would be more efficient in reducing TPH levels in diesel contaminated soils at higher concentrations.

Proximity to the roots of species greatly influences microbial population and the degradation rate of organic pollutants in the soil. This was evident in the study by Corgié, Joner, and Leyval (2003) which examined the effects of the proximity to the roots of Ryegrass (*Lolium perenne L.*) on microbial population and the degradation of Phenanthrene (PHE) using compartmented pots. They varied distances at 0–3, 3–6, 6–9 mm away from the roots of Ryegrass (*Lolium perenne L.*) as a measure to determine the population of Polyaromatic Hydrocarbons (PAH), total heterotrophic hydrocarbons and the total degradation of PHE. Their results showed that the population of PAH degrading bacteria and heterotrophs was highest at 0-3mm distance from the roots and decreased with increasing distance away from the roots. They reported similar trends in the degradation of PHE with a degradation total of 86% at 0-3mm, 48% at 3-6mm and 36% at 6-9mm. Since a correlation between proximity to roots of phytoremediation species and the population of PAH degrading bacteria has been established, this necessitates the use of species that possess extensive root systems as that could be instrumental in increasing the effectiveness and coverage of phytodegradation projects.

2.3.1.3 Phytovolatilization

This phytoremediation technology involves the plant-mediated uptake, transformation into volatile compounds and final discharge of contaminants into the atmosphere either in their original form or in modified form because of its metabolic and transpiration pull (Wang, M. et al. 2021). Application of this technology extends to organic and inorganic contaminants like Arsenic (Guarino *et al*. 2020), mercury (Ghosh and Singh 2005), 2,4-dibromophenol and 2,4-dibromoanisole (Zhang, Q. *et al.* 2020). For instance, (Zhang, Q. *et al.* 2020) attributed up to 41% of the volatilization of 2,4-dibromophenol from hydroponic solution to phytovolatilization by rice plants. Guarino *et al.* (2020) also reported up to 75% phytovolatilization of arsenic by *Arundo donax L.*

Phytovolatization can either be direct via plant extraction and transport into shoots and leaves prior to volatilization into the atmosphere, or indirect, via plant root activities which increase volatile contaminant flux through mechanisms like increased soil permeability, chemical transport by hydraulic redistribution, lowering the water table and advection with water toward the surface (Limmer and Burken 2016).

Figure 2.3 Direct and Indirect Phytovolatization Processes (Limmer and Burken 2016).

In terms of waste generation, post remediation treatment and disposal of contaminated biomass, phytovolatization seems more advantageous to phytoextraction as harvesting, treatment and disposal are not required by phytovolatilization (Bhat *et al.* 2022). However, the fact that phytovolatilization does not completely remove the contaminants from the environment, but rather, transfers it from one part to another (soil/water to atmosphere) with the likelihood of precipitation with rainfall back to terrestrial ecosystems becomes a key limitation of this technology (Wang, M. et al. 2021).

2.3.1.4 Phytostabilization

Phytostabilization is the use of plants to stabilize contaminants in soils by reducing their bioavailability and mobility, as a containment measure to prevent the spread of pollution to uncontaminated areas (Khalid *et al.* 2017). EPA (2000) defined phytostabilization in two-fold. First as the immobilization and accumulation of soil contaminants by the roots of plants, adsorption of contaminants onto the roots of plants or the precipitation of the contaminants in the plant root zone. Secondly, it defined phytostabilization as the utilization of plants and their roots in the prevention of the spread of contaminants to other environments because of wind erosion, leaching, water erosion and soil dispersion. Reducing or totally preventing the mobility of the contaminant is instrumental to the prevention of air or ground water contamination by pollutants and facilitates the reduction of the bioavailability of the pollutant thereby preventing the spread of the contaminant through the food chain (Branzini and Zubillaga 2010).

The key processes involved are sorption, complexation, precipitation and metal valence reduction with the plants primary function being the reduction of the volume of water percolation through the soil matrix to mitigate soil erosion and the concomitant transport of contaminants to other areas (Yadav et al. 2022). For instance, a study by Bomfim *et al.* (2021) showed that *Leucaena leucocephala* accumulated 100 to 300 mg Fe/dm^3 of soil with 92% of the accumulation being in the roots and 8% in the shoots parts. This demonstrated that *Leucaena leucocephala* is a phytostabilizer for Iron (Fe) as it helped contain the pollution from Fe without transporting significant amounts to the aerial parts of the plant, which helps mitigate the need for further treatment of contaminated biomass or risk poisoning of animals from consumption.

The efficiency of phytostabilization projects can be enhanced by the addition of amendments like compost, mineral fertilizers, and sewage sludge to soils. For example, Ciarkowska *et al.* (2017) studied the effects of mineral fertilizers and sewage sludge on the phytostabilization of Zn-Pb ore flotation tailings with *Dianthus carthusianorum* and *Biscutella laevigata* over a 3-year potted experimental period. Their results showed that the addition of NPK fertilizer and sewage sludge enhanced dehydrogenase and urease activities, reduction in the solubility of Cd, Zn and Pb, and increased nutrient availability, which enhanced phytostabilization.

The absence of secondary waste generation which negate the requirement for post-treatment and the facilitation of ecosystem restoration via soil fertility improvement, represent some of the key benefits of the phytostabilization technology (Bolan *et al.* 2011). However, phytostabilization is more of a containment technology than a remediation technology as it does not seek to remove or treat the contamination, but rather, focuses on preventing the spread of the contamination. This necessitates adequate monitoring of phytostabilization sites to ensure that optimal stabilization conditions are maintained, and periodic reapplication of additives and amendments might be imperative if they were deployed in the phytostabilization process (Keller *et al*. 2005).

2.3.2 Assisted Phytoremediation

Various factors such as low bioavailability of nutrients, low contaminant solubility and stunted growth are some of the unsatisfactory conditions imposed by contaminant toxicity in soils (Li et al. 2021). This section discusses some of the key methods that have been studied to overcome the challenges to efficient phytoremediation as imposed by contaminant toxicity.

2.3.2.1 Chelate-Assisted Phytoremediation

Chelate-assisted phytoremediation is a technique that uses chelating agents to mobilize heavy metals in soil, thereby making them readily available for plants uptake from soils (Sidhu *et al.* 2017). This technique involves the amendment of soils with chelating agents like citric acid and malic acid, ethylenediaminetriacetic acid (EDTA), nitrilotriacetic acid (NTA), diethylenetriaminepentaacetic acid (DTPA), ethylenediamine-disuccinic acid (EDDS), to accentuate metal bioavailability in soil, enhance metal desorption to soil solution from the soil matrix and to facilitate metal transport to the xylem and translocation of metals from roots to shoot (Fine *et al.* 2014; Attinti *et al.* 2017; De Araújo and Do Nascimento 2010; Liu *et al*. 2008; Mahmud *et al*. 2018; Duarte, Freitas, and Caçador 2011).

The technique of chelate-assisted phytoremediation was based on the limitations imposed by limited bioavailability and solubility of heavy metals in soils which in turn, affects heavy metal uptake by plants in heavy metal contaminated soils. Essentially, this technique was developed to optimize soil conditions for enhanced metal uptake rates by hyperaccumulator plants in heavy metal extraction (Suthar, Memon, and Mahmood-Ul-Hassan 2014).

EDTA is one of the most popular chelators that has been deployed to enhance the uptake of heavy metals because of its efficiency in enhancing the uptake of Pb, Cd, Zn and Cu (Gabos, de Abreu, and Coscione 2009, Suthar, Memon, and Mahmood-Ul-Hassan 2014, Rathika et al. 2021, Li, F. li et al. 2020). For instance, a 4-week potted experiment by Li *et al.* (2020) showed that the uptake of Pb by *Brassica juncea* was 13.5 mg/kg higher in treatments containing 100 mM of EDTA when compared with the control. This was in line with the findings of Liu *et al*. (2008) who also reported a significant increase (137.3 mg/kg) in the shoot concentration of *Sedum alfredii hance* in treatments containing 5 mM of EDTA when compared to the control. Concerns relating to the high risk of heavy metal pollutants being leached from soil into groundwater due to the low biodegradability of EDTA stimulated research into highly biodegradable chelating agents like citric acid, oxalic acid and EDDS (Chen, Yang, and Wang 2020). These have shown promising results in the enhanced uptake of heavy metals in various studies (Duarte, Freitas, and Caçador 2011; Evangelou, Ebel, and Schaeffer 2006; Chigbo and Batty 2013; Turgut, Katie Pepe, and Cutright 2004). For instance, the results from a study by Duarte, Freitas, and Caçador (2011) showed that citric acid application significantly increased the concentration of Zn in the root tissues of *Spartina maritima* by up to 85%, and Cu by 31%.

Nevertheless, chelating agents can also exert negative effects like inhibition of plant growth, biomass production, and can harm soil microorganisms when applied at certain doses (Vigliotta et al. 2016; Bareen, Saeed, and Afrasiab 2017; Chigbo and Batty 2013; Chen, Yang, and Wang 2020). For instance, a study by Vigliotta *et al.* (2016) reported a 37% and 49% reduction in leaf and stem biomass respectively for maize plants in soils amended with EDTA at 5.0 mmol/kg soil. Similar findings were reported in a study by (Guo et al. 2019) where EDTA application at 5.0 mmol/kg and 10 mmol/kg reduced the biomass of potherb mustard by 58% and 76% respectively. Other studies (Saifullah *et al*. 2009; Zhang *et al.* 2016) have reported symptoms such as chlorosis and necrosis, abscission, shoot desiccation and reduced transpiration were usually observed after EDTA application/amendment which could be a result of increased metal toxicity via leaching and/or EDTA toxicity. EDDS has also shown similar effects. For instance, a study by Liu *et al.* (2008) showed reductions in shoot dry weight by up to 22.6% and 33.5% after being amended with EDTA and EDDS, respectively. A similar observation was made by Attinti *et al.* (2017) in who observed that the addition of EDDS resulted in negative effects on fescue plants which showed symptoms of phytotoxicity. Interestingly, in the same study, the amendment with EDDS did not have negative effects on the growth of vetiver plants. This could be suggestive that there are certain plants that could have high tolerance for synthetic chelators and pairing synthetic chelators with high tolerant plants could eliminate the risk of stunted growth and other negative effects most synthetic chelators have on the growth and biomass production of plants.

2.3.2.2 Nutrient Assisted Phytoremediation

Nutrient supplementation involves the addition of nutrients (in the form of organic or inorganic fertilizers) to enhance plant growth in unsatisfactory soil conditions imposed by pollutants, and also to provide the carbon substrate and other nutrients needed to enhance microbial activities within the soil (Srinuykong and Sampanpanish 2018). Studies have shown that organic amendments like pig manure vermicompost (PMVC) (Wang *et al.* 2012) and bio-waste like tea leaves, potato skin, soy cake, banana skins, brewery spent grain and spent mushroom compost (Dadrasnia and Pariatamby 2016; Abioye, Agamuthu, and Abdul Aziz 2012), have been effective improving plant growth under contaminant stress, improving plant metal uptake and enhancing the microbial degradation of pollutants. For instance, Wang *et al.* (2012) recorded a 2.27 and 3.93-fold increase in root and shoot biomass of *Sedum alfredi* respectively in PMVC treatments when compared with unamended treatments. They also reported an increased Cd accumulation of up to 1.97-fold and an enhanced PAH degradation of up to 0.49%, 5.84% and 7.15% for Phenanthrene, Pyrene and Anthracene, respectively in PMVC amended treatments. Similar observations were made by Dadrasnia and Pariatamby (2016) where bio-wastes (tea leaves, potato skin and soy cake) in conjunction with *Dracaena reflexa* enhanced the microbial degradation of petroleum hydrocarbons by up to 43% when compared with unamended treatments after 180 days. This could be as a result of the combined stimulation of petroleum degrading microbial activities via enhanced root exudation and nutrient supply to the soil (Obuotor, Akande, and Bada 2016, Nevita et al. 2013).

Inorganic fertilizers like NPK, nitrogen and phosphate fertilizers have been efficient in minimizing plant growth inhibition under contaminant stress (Li et al. 2012; Dheeba, Sampathkumar, and Kannan 2014; Merkl, Schultze-Kraft, and Arias 2005; Atma *et al.* 2016). For instance, (Li et al. 2012) reported up to 3.8-fold increase in the biomass of *Amaranthus hypochondriacus* after NPK fertilizer supplementation. They also reported significant increase in Cd uptake which could be related to the enhanced biomass production since efficient phytoextraction relies and high biomass yield (Ranieri et al. 2022).

The impact of nutrient amendment could be dependent on nutrient levels already present in the soil. This implies that a significant improvement in plant growth and phytoextraction abilities would be more pronounced in nutrient deficient soils and less significant in nutrient rich soils. Choi and Chang (2009) illustrated this in an investigation of the effects of nitrogen fertilization on the degradation of aged diesel in composted drilling wastes over a four-year period. Their results showed that significant TPH degradation occurred only in the 1st and 4th year of nitrogen fertilization (ammonium sulphate) in media (compost) with low nitrogen supply. This could be because the initial ammonium sulphate in the composts for the first and fourth year were low (8.3 N/kg and 17.7 N/kg for the first and fourth year respectively) and the initial ammonium sulphate in the second and third year were 68.7 mg N/kg and 325.3 mg N/kg. This could imply that the addition of nitrogen supplementation in the first and fourth year showed significant increase in TPH degradation because there already existed a nitrogen deficiency in the compost, whereas in the second and third year, an abundance of nitrogen in the compost limited the improvement in TPH degradation compared with the unamended control.

2.3.2.3 Surfactant Assisted Phytoremediation

Surfactants are amphiphilic with both hydrophobic and hydrophilic groups, which lower surface/interfacial tension between two liquids or between a liquid and a gas/solid (Alvarez and Schechter 2017). Their unique properties have facilitated their vast deployment across a wide range of industries including petroleum industries, detergent and personal care industries, soil and water remediation, food industries and excavation industries (Gong, Chen, and Pu 2019). Their ability to solubilize contaminants like petroleum hydrocarbons (dos Santos *et al.* 2017), chlorinated hydrocarbons (Tian *et al.* 2018), PAHs, TPH and heavy metals (Liduino, Servulo, and Oliveira 2018; Mekwichai *et al.* 2020) has favoured experiments in their potential for enhanced phytoremediation. Surfactant-enhanced phytoremediation improves the desorption of hydrophobic contaminants from soil particles via the amphiphilic structures of surfactants, and improves phytoremediation efficiency through increased contaminant bioavailability (Liu *et al.* 2013).

The possibility of synthetic surfactants like Sodium Deodecyl Sulfate (SDS), polyoxyethylene(23)dodecanol (Brij35), and tween80 to enhance the phytoremediation of contaminants have been explored in various studies (Liao *et al.* 2016; Pierattini *et al.* 2018; Lu *et al.* 2019). For instance, Cheng, Lai, and Wong (2008) reported an 18% increase in pyrene removal in *Agropyron elongatum* planted soils amended with tween80 when compared with unamended treatments. Similar observations were made by GAO *et al.* (2007) who reported that tween80 application at less than 13.2 mg/L concentrations significantly enhanced pyrene and phenanthrene by ryegrass with Plant Concentration Factors (PCFs) reaching 216% when compared to unamended treatments. SDS on the other hand, although has been vastly used in the desorption of organic contaminants from soils (dos Santos et al. 2017), has not shown significant potential for enhancing phytoremediation when compared with tween80. For instance, Somtrakoon and Chouychai (2018) reported that the addition of SDS did not stimulate the removal of phenanthrene and pyrene from the soil. This is consistent with the findings of Gao *et al*. (2007) who reported that the presence of SDS in soil did not stimulate the removal of pyrene from soil.

As useful as synthetic surfactants can be for enhanced phytoremediation, their poor biodegradability, negative effects on soil microorganisms, plant toxicity and reduction in oxygen demand in aquatic environments represent some major disadvantages related to their usage, thereby making the environment friendly and highly biodegradable biosurfactants an attractive choice (Johnson *et al.* 2021).

Biosurfactants are ampiphillic compounds excreted extracellularly that contain hydrophobic and hydrophilic moeities, allowing them to accumulate between the fluid phases on an organism and thus, reduce the surface and interfacial tension (Fadhile Almansoory *et al.* 2015). They are biodegradable, low-toxicity, eco-sustainable and very stable biomolecules produced by microorganisms, with the ability to maintain activity in a wide range of harsh environmental conditions (Sonowal *et al*. 2022).

Various biosurfactants like rhamnolipid and soybean lichitin have shown promising results in the enhancement of the phytoremediation of soils containing organic and inorganic pollutants (Liao *et al.* 2016; Liduino, Servulo, and Oliveira 2018). For instance, Liao *et al.* (2016) evaluated the usability and possible risks associated with surfactant-enhanced phytoremediation of soils contaminated with hydrocarbon oils using rhamnolipid and soybean lichitin. The results of their study showed that removal efficiencies were 10% and 6% higher in rhamnolipid and soybean lichitin treatments respectively, when compared with the unamended treatments. They also reported no phytotoxicity effects in plants with biosurfactant application. They reported degradation as the predominant removal mechanism which was observed in the saturated hydrocarbon fractions (reduction from 60% to 36%) whereas the aromatic and asphaltene fractions were resistant to the treatment. They predicted that this phenomenon could be that aromatic and asphaltene TPH fractions were more toxic to the soil microbes than the saturated hydrocarbon fractions.

Rhamnolipids have also shown promising results in the removal of heavy metals in soils, with a reported the 41%, 30%, 29% and 20% reduction in the concentrations of Ni, Cr, Pb and Zn respectively in treatments amended with rhamnolipid and *Helianthus* annuus L (Liduino, Vitor S., Servulo, and Oliveira 2018). Similar findings were reported by Mekwichai *et al.* (2020), with up to 39 Mg/kg Cd reduction in *Zea mays* planted soils amended with rhamnolipids.

Rhizobacteria-derived biosurfactants from microorganisms like *Pseudomonas sp., Bacillus sp. Pseudomonas fluorescens, Bacillus subtilis, Serratia marcescens* and *Rahnella sp. JN6* have also shown promising results in enhancing phytoremediation (Mulligan 2017; He *et al*. 2013; Govarthanan *et al*. 2017; Fadhile Almansoory et al. 2015; Lal *et al*. 2018). For instance, Fadhile Almansoory *et al*. (2015) evaluated the potential of *Serratia marcescens*-derived biosurfactant to enhance the phytoremediation of gasoline contaminated soil. Their results showed that adding the *Serratia marcescens*-derived biosurfactant at 10% concentration increased TPH solubility and removed up to 93.5% TPH in *Ludwiga octovalvis* planted soils. They noted that the biosurfactant treatment yielded higher TPH removal when compared with the synthetic surfactant SDS which facilitated 86.3% TPH removal. A study by

Govarthanan *et al*. (2017) showed that biosurfactant extracted from the heavy metal resistant *Rahnella sp. JN6* has potential to remove heavy metals with removal rates of 74.3%, 72.5% and 70.1% recorded for Cu, Cr and Pb respectively.

From the above, surfactant-assisted phytoremediation is a promising technique for enhanced phytoremediation of organic and inorganic contaminants, and despite the challenges (e.g., phytotoxicity and poor biodegradability) posed by synthetic surfactants, the emergence of biosurfactants have relieved those concerns, making it an attractive technique for enhancing the phytoremediation of contaminated soils.

2.3.2.4 Bioaugmentation Assisted Phytoremediation.

Bioaugmentation is the addition/inoculation of microorganisms to enhance a specific biological activity (Vogel 1996). It involves the introduction of indigenous or genetically modified microorganisms to contaminated sites to enhance the removal/degradation of the undesired (toxic) compounds (Mrozik and Piotrowska-Seget 2010; Wani and Khan 2010; Aung *et al*. 2015).

Certain microbes like *Pseudomonas sp.* Lk9, *Pseudomonas koreensis* AGB-1, *Bacillus sp*. J119, *Herbaspirillum sp*. GW103, and *Bacillus subtilis* strain SJ-101 have shown promising results in enhancing plant growth and biomass production, increase heavy metal solubilization, increase soil microbial biomass and significantly enhance phytoextraction of heavy metals (Romeh and Hendawi 2017; Ma *et al*. 2016; Chen *et al.* 2014; Babu *et al.* 2015; Sheng *et al.* 2008; Praburaman *et al.* 2017; Zaidi *et al.* 2006). For instance, a 14% increase in the biomass of *Solanum* nigrum L., accompanied by a 46%, 16.4% and 16% increase in the accumulation of Cd, Zn and Cu in the shoot of *Solanum* nigrum L. in treatments containing *Pseudomonas sp.* Lk9 was reported by Chen *et al*. (2014). They attributed this to the biosurfactant production by *Pseudomonas sp.* Lk9 which facilitated metal solubilization, enhanced metal bioavailability and significantly improved soil Fe and Phosphorus (P) mineral nutrient supplies which could have aided enhanced plant growth. Similar results were reported by Babu *et al.* (2015) where inoculation with *Pseudomonas koreensis* AGB-1 enhanced the biomass of *Miscanthus* sinensis by up to 54% in heavy metal contaminated mine site and enhanced heavy metal uptake in inoculated treatments when compared with the uninoculated control.

Bacterial inoculants can be instrumental in alleviating the phytotoxicity effects of heavy metals by creating favourable growth conditions and more effective phytoremediation via secretion of chelating agents, enzymes, acidification, and growth-promoting substance thus, they are widely known as plant growth promoting rhizobacteria (PGPR) (Kurniawan et al. 2022). For example, a study by Sheng *et al.* (2012) showed that Plant Growth Promoting Rhizobacteria (PGPR) (*Burkholderia sp*. GL12, *Bacillus megaterium JL35* and *Sphingomonas sp*. YM22) significantly reduced Cd stress in *Zea mays* evidenced by up to 83% and 57% increases in root and shoot dry weight respectively when compared with control treatments. They also recorded increase in Cd concentration by up to 107% and 86% in roots and shoot respectively in inoculated treatments when compared to uninoculated treatments. These agree with findings of Babu *et al*. (2015) and Chen *et al.* (2014) who also reported similar results as pertains to enhancement of biomass production and phytoextraction efficiency after bacterial inoculation. Several other studies (Mello *et al.* 2020; Yahaghi *et al.* 2018; Benson *et al.* 2017; Khan *et al.* 2018; He *et al.* 2020; Jin *et al*. 2019) have reported comparable results, further demonstrating the efficacy of this technique in enhancing heavy metal accumulation in plants and alleviating heavy metal stress via enhanced biomass production.

However, the effectiveness of bacterial inoculation for enhanced phytoremediation has not been limited to heavy metal contamination alone. For example, a study by Kotoky and Pandey (2020) reported Benzo(a)pyrene degradation of up to 87.42% and 86.08% in treatments inoculated with *Bacillus flexus* S1I26 and *Paenibacillus* sp. S1I8 respectively. Similar findings can be seen in a study by Teng *et al*. (2011), who reported 14.2% reduction in PAH concentrations, a boost in microbial activity, a rise in count of culturable PAH degrading bacteria and the carbon utilization ability of the soil microbial community in treatments containing *Alfalfa* and *Rhizobium meliloti* when compared with the controls. They attributed these to the interactions between plant and soil microbes which had concomitant effects on the degradation of hydrocarbons.

Pairing the right microorganisms with the right plant species is efficacious in optimizing the degradation of petroleum hydrocarbons in soils and can achieve significantly higher degradation than when plants or microorganisms are used in isolation. This was demonstrated by Fatima *et al.* (2018) in their study on the efficacy of developing plants (*Leptochloa fusca* and *Brachiaria mutica*) - endophytes (*Acinetobacter sp*. strain BRSI56, *Pseudomonas aeruginosa* strain BRRI54 and *Klebsiella sp*. strain LCRI87) synergism for the efficient remediation of crude oil contaminated soils under field conditions. Their results showed that the highest TPH degradation was observed in treatments that combined vegetation and endophyte augmentation, with TPH degradation reaching 78% - 85% degradation in *Brachiaria mutica* and *Leptochloa fusca* treatments respectively when compared with stand-alone treatments. Overall, their study demonstrated that with the right combination of plant species and endophytic bacteria, significant improvements in the degradation of petroleum hydrocarbons can be achieved.

2.4 PHYTOREMEDIATION OF LEAD CONTAMINATED SOILS

Lead, a frequently encountered heavy metal, has been utilized in various applications, leading to higher concentrations of lead in soil. Prolonged exposure to elevated levels of lead has caused several physiological consequences, with the most well-known effect being its impact on the central nervous system of children, potentially resulting in hindered brain development (Cho-Ruk *et al.* 2006).

Various approaches have been commonly employed for the remediation of Pb-contaminated soils, aiming to mitigate the environmental impact of this toxic heavy metal. Some of the common methods that have been used include the deployment of suitable amendments are added to the soil to form stable complexes with Pb to immobilize and reducing its toxicity, utilization of solvents or surfactants to solubilize and remove Pb from the soil and the excavation and transportation of contaminated soil for off-site treatment. These diverse approaches offer promising solutions to address the pervasive issue of Pb contamination, however, limitations like high costs of remediation, potential for secondary pollution from excessive leaching, and the disruptive nature of some of the traditional remediation technologies have necessitated the exploration of cost effective, less disruptive, and environment friendly technologies for the remediation of Pb contaminated soils (Butcher 2009, Wang *et al.* 2007, Thompson *et al.* 2021).

Hyperaccumulator plants are a unique group of plants that have the remarkable ability to absorb and store high concentrations of certain metals and minerals from the soil without being adversely affected (Rascio and Navari-Izzo 2011). These plants have been used to explore their potential for the removal of Pb from soils. For instance, (Cho-Ruk et al. 2006) explored the potential to deploy *Alternanthera philoxeroides*, *Sanvitalia procumbens*, and *Portulaca grandiflora* to extract Pb from soil contaminated with Pb at 75 mg/kg. After a 45-

day remediation period, they reported the highest Pb extraction (29.9%) by *Alternanthera philoxeroides*. Although a 29.9% uptake as the maximum extraction for their study is relatively benign, *Alternanthera philoxeroides* demonstrated 1.3 – 1.8-fold higher performance than *Portulaca grandiflora* and *Sanvitalia procumbens* respectively. *Glycine max* L demonstrated better performance in the extraction of Pb with a 41.9% uptake of Pb reported in a study by (Aransiola, Ijah, and Abioye 2013). However, when the initial Pb concentration (25 mg/kg) is considered, the percentage of Pb extracted becomes less impressive. Similar results were obtained with *Trachelospermum asiaticum* which accumulated only about 6% Pb in soil contaminated with 500 mg/kg soil (Thompson et al. 2021). However, impressive results were reported in the same study with *Pteris vittate* accumulating up to 90% Pb in its leaves. This demonstrated that Pteris vittate is a viable choice for the phytoextraction of Pb. However, factors like low solubility of Pb in tandem with the toxicity effects which antagonize plant growth have led to subpar extraction of Pb (Testa et al. 2023), thus necessitating the need for exploring alternative approaches to circumvent some of these limitations.

Chelator assisted phytoextraction has been explored for the remediation of Pb contaminated soils. This method involves the amendment of the soils to lower the soil pH, thereby increasing the solubility of the target metal in the soil for uptake by the plant. For instance, Wang *et al.* (2007) reported an 81% increase in extractable Pb after amending the soil with 3 mmol/kg of EDTA, which resulted in a 64% increase (1, 225 mg/kg increase) in Pb uptake by *Bidens maximowicziana* in soils at 2000 mg/kg Pb. This result was impressive as EDTA improved the maximum Pb extraction from 34% to 95%, all without *Bidens maximowicziana* manifesting any toxicity effects of EDTA. Worthy of note is that this plant-chelator combination was perfect as *Bidens maximowicziana* was able to extract majority of the solubilized Pb from the soil. Similar results have been achieved with 10 mmol/kg citric acid and 10 mmol/kg ammonium nitrate with a reported increase in Pb phytoavailability of 85.2% and 75% respectively at 1, 500 mg/kg Pb soil concentration (Gul *et al.* 2020). However, compost, and Titania nanoparticles reduced the solubility of Pb in the soil. The level of solubilization of Pb reported in these studies could easily turn problematic if the phytoremediation specie deployed is unable to extract the abundant soluble Pb, thereby, creating the risk of secondary pollution via Pb migration to other areas previously uncontaminated, or leaching into groundwater.

Stunted growth is one of the manifestations of Pb toxicity in plants, with concomitant reduction in the uptake of Pb by plants. To circumvent this limitation, studies have explored the potential for nutrient supplementation to enhance plant growth under Pb induced stress and enhance phytoextraction. For instance, Meeinkuirt *et al.* (2012) where cow manure and Omscote fertilizers yielded the extraction of about 15, 000 mg/kg Pb by *Pterocarpus macrocarpus*. Their study also demonstrated a relationship between biomass production and phytoextraction, indicating that nutrient supplementation with cow manure and Omscote fertilizer could be a viable option for attenuating the inhibitory effects of Pb while optimizing Pb uptake.

Several plants have been tested for their efficacy in the phytoremediation of Pb contaminated soils (Huang *et al.* 1997, Butcher 2009, Testa *et al.* 2023, Gul *et al.* 2020, Aransiola, Ijah, and Abioye 2013, Herlina, Widianarko, and Sunoko 2020) with varying levels of efficacy as shown in the examples above, however, *Helianthus annuus* and *Brassica juncea* have also produced interesting results in the remediation of Pb. For instance, *Brassica juncea* accumulated up to 677 mg/kg Pb in its shoots in Pb polluted soils amended with 5 mmol/kg EDTA (Lim, Salido, and Butcher 2004). Similar results were reported in a study by Gayatri, Sailesh, and Srinivas (2019b) which showed a 71% reduction in Pb contaminated soils using *Brassica juncea* with a

Pb uptake of 151 mg/kg following an 81-day treatment period. These along with the promising results in several other studies (Di Gregorio *et al.* 2006, Singh and Fulekar 2012, Rathika *et al.* 2021, Salido *et al.* 2003) are indicative of the impressive abilities of *Brassica juncea* for the remediation of Pb contaminated soils. Similarly, impressive results have also been reported for *Helianthus annuus* in the phytoremediation of Pb contaminated soils. For instance, Aybar *et al.* (2023) reported a 66% reduction in soil Pb concentrations with 146 mg/kg Pb concentration in its tissues. Similar trends were reported by Al-Jobori and Kadhim (2019) who reported Pb concentrations reaching 215 mg/kg in the tissues of *Helianthus annuus* in Pb contaminated soil. These alongside the findings of several other studies (Niu, Li, and Mahamood 2023, Alaboudi, Ahmed, and Brodie 2018, Kalyvas *et al.* 2022, Forte and Mutiti 2017) reveal the suitability of *Helianthus annuus* for the phytoremediation of Pb contaminated soils.

The suitability of *Helianthus annus* and *Brassica juncea* for the remediation of Pb contaminated soils aligns with the objectives of my study which relate to investigation of their ability to grow, reduce Pb concentrations and uptake Pb in soils co-contaminated with SEO and mine spoils containing copious amounts of Pb. As much as they have demonstrated immense potential for the phytoremediation of Pb contaminated soils, the literature is limited in terms of their ability to decontaminate Pb and SEO co-contaminated soils.

2.5 PHYTOREMEDIATION OF SEO CONTAMINATED SOILS

In response to the widespread soil contamination with SEO, and the need for an eco-friendly approach for the remediation of SEO contaminated soils, various plants have been deployed to evaluate their efficacy and potential as viable phytoremediation species for SEO contaminated soils. For instance, a study by Escobar-Alvarado *et al.* (2018) compared the efficiency of *Opuntia ficus* to *Lolium perenne* and *Aloe barbadensis* in the phytoremediation of SEO and Pb co-contaminated soil obtained from an auto repair shop. They reported the maximum TPH reduction of 47% after 40 days in the *Lolium perenne* planted soils. However, this was underwhelming when compared to the unplanted controls which showed a 33% degradation of TPH. What was most interesting about their study was that for a starting TPH concentration of 31 823 mg/kg, a 33% (10, 501.59 mg/kg TPH) attributed to natural attenuation within a 140-day window compared to 47% maximum reduction with plants almost negates the requirement for phytoremediation in this instance. As a matter of fact, it can be argued that following the pace of natural attenuation, complete remediation might be possible without effort in this case. In terms of Pb accumulation, the highest accumulation recorded in their study was 900 mg/kg, about 10.8% of the total Pb content in their study.

Similar results were reported for *Zea mays, Vicia faba* and *Triticum aestivuml* with TPH reductions of 16.8%, 30% and 13.7% respectively, while 8.2% - 10.5% TPH reductions were observed in unplanted controls (Diab 2008). The subpar results reported so could be related to the deleterious effects of SEO concentrations on plant growth which has been reported in numerous studies (Nonyelum Helena and Felicia Uchechukwu 2018, Agamuthu, Abioye, and Aziz 2010, Olajuyigbe, Fayinminnu, and Ayoade 2020).

More promising results have been reported for *Jatropha curcas* with SEO degradation of 56.6% and 67.3% at 2% and 1% soil SEO concentrations in a study by (Agamuthu, Abioye, and Aziz 2010). Furthermore, their study demonstrated that biostimulation with brewery spent grain was increased the removal efficiency of *Jatropha curcas* to 89.6% and 96.65% at 2% and 1% soil SEO concentrations respectively. This is indicative that a combination of biostimulation and phytoremediation could be efficacious in enhancing the phytoremediation of SEO contaminated soils. However, it is important to note that the concentration of SEOs in this study was significantly lower than that of Escobar-Alvarado et al. (2018) whose study also had copious amounts of Pb which could have contributed to the poor phytoremediation efficiency manifested in their study.

A study by Dominguez-Rosado and Pichtel (2004) explored the phytoremediation of 1.5% w/w SEO contaminated soils with mixed cropping. The species mixtures used include (*Glycine max* + *Phaseolus vulgaris*), (*Helianthus annus* + *Brassica juncea*), (*Festuca rubra* + *Festuca arundinacea;* + *Lolium perenne* + *Zea mays*), and (*Trifolium pratense* + *Trifolium repens*) and the results showed that the best oil removal was affected by *Trifolium pratense* + *Trifolium repens* combination after 150 days of treatment with the complete oil removal. In comparison, *Helianthus annus* + *Brassica juncea* had the next best performance with a 67% oil removal after 150 days. However, when the treatments were supplemented, *Helianthus annus* + *Brassica juncea* had the best removal efficiency when compared to the other specie combinations with a 100% oil removal. High biomass production was also reported for *Helianthus annus* + *Brassica juncea* in SEO contaminated soil, demonstrating their ability to grow and survive in SEO contaminated soils which is a key requirement of phytoremediation species. This not only demonstrates the potential for *Helianthus annus* + *Brassica juncea* to decontaminate SEO contaminated soils, but it also indicates that mixed cropping could be beneficial in a phytoremediation setting.

These findings are in line with the objectives of my study which relate to evaluating the possibility of mixed cropping *Helianthus annus* + *Brassica juncea* in attenuating the toxicity effects of SEO at higher concentrations. It also aligns with the objective of my study which investigates their potential to reduce TPH and PAH levels in soils co-contaminated with SEO and mine-spoils containing copious amounts of Pb. As much as the above is indicative of potential for the phytoremediation of SEO contaminated soils, the literature is limited in terms of their ability to decontaminate Pb and SEO co-contaminated soils.

2.6 STRUVITE

Nutrient rich aqueous wastes (containing copious amounts of nitrogen and phosphorus) are common in sewage sludge, urban and industrial wastewater, farms and agricultural establishments that use inorganic fertilizers and animal manure, and uncontrolled effluent discharge can have catastrophic environmental consequences like harmful algal blooms in water bodies and eutrophication which pose significant risk to human health and environmental ecosystems (Achilleos, Roberts, and Williams 2022). As a mitigation measure, various biological and physicochemical approaches like biological denitrification, anaerobic ammonium oxidation, ammonium stripping, reverse osmosis, adsorption and ion exchange have been employed for the abatement of nutrient concentrations in effluent streams (Siciliano et al. 2020). Struvite also known as magnesium ammonium phosphate (MgNH4PO4) is a nutrient rich mineral which is primarily made up of phosphate, magnesium, and ammonium (Vasa and Pothanamkandathil Chacko 2021). It is a tenacious mineral formed from the combination of nitrogen, phosphorus and magnesium ions contained in sludges during the biological nutrient removal process (Doyle and Parsons 2002). Struvite formation/precipitation in waste water treatment plants has caused problems like blockage of pipes via struvite deposits which significantly reduce the flow of sludge through the pipes, often leading to high pumping costs and reduction in plant capacity and efficiency (Borgerding 1972). This has been a persistent problem at Slough wastewater treatment works UK, where flushing pipes with 10% sulfuric acid was employed to combat the situation (Williams 2010). However, because struvite is very rich in phosphorus, some circular economic opportunities have been explored with applications like green fertilizer production which could reduce the depletion of phosphate rocks by converting waste to resource (Achilleos, Roberts, and Williams 2022).

Phosphorus is one of the primary limiting nutrients for plants because of its vital function in energy digestion, photosynthetic processes and genetic components (Vasa and Pothanamkandathil Chacko 2021; Smil 2003), and thus, makes struvite a potential source of phosphate supplementation. Struvite utilization as fertilizer has been explored. For instance, (Rech et al. 2020) created struvite-NH4 and struvite-K from poultry manure via nutrient extraction in water, incineration of the solid phase, magnesium supplementation and pH adjustment and acidification. They stated that the final product was a nutrient-rich, pathogen free inorganic fertilizer suitable for largescale agricultural use. (Zhang, T. et al. 2020) reported that after testing crystalized struvite fertilizer with *Zea mays*, phosphorus utilization was 19% and argued that appropriate dosage applications could enhance root growth. Struvite being a slow release fertilizer has a significantly lower risk of nutrient leaching when compared with mainstream phosphate fertilizers, thereby making it a more environment friendly alternative as the risk of pollution is less due to its lower solubility (Hertzberger, Cusick, and Margenot 2020).

However, as much as studies are ongoing regarding struvite deployment as an environment friendly substitute for phosphate fertilizers, there is very limited research on their application as amendments for nutrient enhanced phytoremediation. Therefore, this study seeks to explore the possibility of using this phosphorus rich mineral to enhance the phytoremediation of SEO contaminated soils as well as in a mixed contamination scenario.

2.7 PLANTS USED

Various plants have varying capabilities relevant to the phytoremediation of contaminated soils. Studies have shown that different plants have different capabilities for phytoextraction of heavy metals (Tariq and Ashraf 2016), different phytostabilization abilities (Sewalem, Elfeky, and El-Shintinawy 2014) and in inducing phytodegradation of pollutants. However, for plants to be used for phytoremediation of polluted environments, they need to demonstrate ability to germinate and grow in polluted soils and decontaminate polluted soils either through phytoextraction, phytostabilization or phytodegradation. These formed the major basis for the selection of plants used. Sections **2.7.1** and **2.7.2** below covers some properties of the selected plants and how they contributed to their selection for this research.

2.7.1 Brassica juncea

Brassica Juncea which is popularly known as brown mustard, Chinese mustard, Indian mustard, oriental mustard, and vegetable mustard is a plant species in the mustard family (Shekhawat and Singh 2020). Although its origin is uncertain, it is speculated to be a hybrid of *Brassica* nigra and *Brassica* rapan and hence, could have originated where there was an overlap in the distribution of both species such as the Middle East and its environs (CFIA 2005). Its distribution cuts across various parts of the world from Africa (Kenya, Tanzania, Angola, Zimbabwe), temperate and tropical Asia (China, Japan, Philippines), Australia, Europe (Estonia, Lithuania, Austria, Germany, Hungary, Romania), Northern and Southern America (Brazil, United States, Argentina, Paraguay) (Shekhawat and Singh 2020). It is mainly used economically for food and environmentally for pollution control because of its potential for the hyperaccumulation of heavy metals (Dominguez-Rosado and Pichtel 2004) but this research focuses solely on its phytoremediation abilities.

Brassica juncea has demonstrated heavy metal tolerance and hyper-accumulation potentials through several studies where it survived relatively high doses of heavy metal pollution and accumulated heavy metals in its tissues. This can be seen in a study by Goswami and Das (2015) where *Brassica juncea* survived Cadmium concentrations up to 400mg Cd/kg soil although tolerance indexes (root and shoot length, tissue biomass and leaf chlorophyll) reduced with increasing doses of Cadmium. This study also showed the ability of *Brassica* *juncea* to accumulate Cadmium in its roots and shoot. Worthy of note is the fact that the rate of accumulation was not directly proportional to increases or decreases in doses of the contaminant in their study. This was evidenced by the highest Cadmium shoot and root accumulation occurring in treatments at 200mg Cd/kg soil and in leaves at 100mg/kg concentration after 21 days of treatment. Similar observations were made in a study by Bauddh and Singh (2012) where *Brassica juncea* survived concentrations of Cadmium up to 150mg/kg for up to 60 days. However, like Goswami and Das (2015), they observed a negative response in root and shoot biomass production to Cadmium doses in soil with a decline in root and shoot biomass production being more acute as Cadmium doses increased. Their study also showed Cadmium accumulation in roots and shoot at various concentrations with the highest accumulation of Cadmium (49-51 µg Cd/plant) achieved in roots at 100mg CdCl/kg soil. The study also demonstrated that at some point, increase in contaminant doses can reduce metal extraction and this can be seen in the reduction in Cadmium extraction to 41.24 µg Cd/plant in 150mg CdCl/Kg soil. Several other studies (Rathore *et al.* 2017; Jeyasundar *et al.* 2021; Lim, Salido, and Butcher 2004; Gayatri, Sailesh, and Srinivas 2019; Raj, Kumar, and Maiti 2020; Niazi *et al.* 2017) have also demonstrated the abilities for *Brassica* juncea to survive heavy metal contamination as well as accumulate heavy metals in its shoots and roots. This tolerance for heavy metals and hyper-accumulation ability is indicative of its potential and suitability for the phytoremediation of heavy metal polluted soils.

Since it has been established in studies (Bauddh and Singh 2012, Gayatri, Sailesh, and Srinivas 2019a) that heavy metal pollution makes the soil unsatisfactory for *Brassica juncea*'s growth and also negatively impacts on its phytoextraction capabilities, several attempts have been made to explore ways of boosting its performance in polluted soils. A good example is a study by Mahmud *et al*. (2018) where they evaluated the effect of two dozes (0.5nM and 1mM) of citric acid in improving the growth and phytoremediation abilities of *Brassica juncea* in cadmium contaminated soils. Their study showed that under cadmium stress, the addition of citric acid improved the growth of *Brassica juncea* seedlings. This was evidenced in the citric acid induced enhancement of leaf Relative Water Content (RWC), reduction in oxidative damage, and increasing ascorbate (AsA) and glutathione (GSH) reserves. According to the study, the addition of citric acid at 1.0 mM significantly increased the accumulation of cadmium in the roots and shoot of *brassica juncea* as well as enhanced the translocation of cadmium from roots to shoot when compared to treatments without the addition of citric acid. The study showed no significant improvement in cadmium accumulation when 0.5mM of citric acid was administered when compared to treatments that were not administered with citric acid. This could be indicative that citric acid induced growth and phytoremediation enhancement of *Brassica juncea* in contaminated environments could be dose dependent.

Similarly, Niazi *et al.* (2017) evaluated the effect of phosphate supplementation on the growth and phytoremediation efficiency of *Brassica juncea* in arsenic contaminated soils. Their study featured potted experiments with soils dosed with Arsenic concentrations at 25 mg/kg, 50 mg/kg and 75mg/kg and potassium phosphate supplementation was added to all treatments at 50 mg/kg and 100mg/kg. Phosphate supplementation at 100mg/kg at all concentrations of Arsenic showed very similar results to Mahmud *et al.* (2018) in terms of plant response although the amendment and contaminants used differed. They reported the highest impacts of phosphate when it was dosed at 100mg/kg. The study by Niazi *et al*. (2017) showed that 100mg/kg phosphate supplementation in soils at 25-75mg/kg arsenic concentration significantly enhanced growth parameters (shoot and root dry weight), increased the shoot concentration of arsenic by 19% and 17% in the 50mg/kg and 75mg/kg, increased shoot uptake by 52% and 455%, and increased root Arsenic uptake by 0.04mg-0.13mg/pot for

50mg/kg and 75mg/kg arsenic treated soils respectively. Worthy of note is the fact that they recorded a reduction in the concentration of arsenic in *Brassica juncea* shoot as the soil Arsenic concentration increased despite the addition of phosphate. This agrees with the findings of Mahmud *et al.* (2018) in which severe cadmium stress led to a 13% and 4% decline in shoot and root cadmium accumulation respectively regardless of the citric acid supplementation. In both studies, however, increases in supplementation doses showed better results when compared to treatments that received less supplementation doses. This could be an indication that increasing supplementation doses as concentration of pollutants increase could help alleviate the reduction in the phytoremediation performance of *Brassica juncea* for heavy metals.

A striking observation is the fact that *Brassica juncea* responds differently to different soil amendments in terms of its phytoremediation mechanisms. This was demonstrated in a study by Novo, Covelo, and González (2013) where they evaluated the effects of compost and technosol supplementation on the phytoremediation of copper mine tailings using *Brassica juncea*. The results of their study showed that compost had more significant effect on growth parameters (shoot and root fresh and dry weight biomass) than technosol. This resulted in compost treatments having higher extraction of metals by *Brassica juncea* due to high biomass production alongside enhancement of other growth parameters. They stated however, that a technosol would be best suited for phytostabilization since it enhances ecophysiological conditions, facilitates plant propagation and exhibits favourable metal accumulation patterns. However, it could have been note-worthy to point out that compost is excellent for both phytostabilization and phytoextraction mechanisms in *brassica juncea* as demonstrated in a study by Pérez-Esteban *et al*. (2014). Their study evaluated the use of organic compost (horse and sheep manure) not only improved the soil fertility leading to higher biomass production, but it also reduced the bioavailability of copper thereby reducing copper concentration in *Brassica juncea* shoot. This is indicative that organic compost provides a more rounded benefit when it comes to phytoremediation as it enhances both phytoextraction and phytostabilization when supplemented with *Brassica juncea*.

Organic composts could also be a more cost-effective choice for phytoremediation projects where *Brassica juncea* is used as the phytoremediation species. This can be seen in the study by Novo, Covelo, and González (2013) in which for the root length, the same results were obtained for treatments containing 30% (v/v) and 50 % (v/v) compost and technosol respectively. This could be an indication that more doses of technosol would be required to have the same effects as lesser concentrations of compost when it comes to enhancing the growth of *Brassica juncea* in heavy metal polluted soils. Implications for phytoremediation projects could mean that using technosol as a sole soil amendment would require more quantities/volumes of supplementation which might not be cost effective for large scale projects, especially as it doesn’t yield multiple benefits results like organic compost when used with *Brassica juncea*.

Overall, *Brassica juncea* has shown enormous potential for the phytoremediation of heavy metal contaminated soils which could even be enhanced when supplemented with soil amendments as evidenced in the rigorous testing that has been done with regards to heavy metals. However, there are insufficient studies on its phytoremediation abilities in SEO contaminated soils and multi-contaminated soils and the present study aims to explore its abilities in the phytoremediation of SEO contaminated soils and in SEO and mining soil multi-contamination.

2.7.2 Helianthus annuus

Helianthus annuus also known as sunflower is another plant species that has multiple uses. It has been used for aesthetic purposes, for food to produce oils (sunflower oil) and for environmental pollution control (Prapagdee, Chanprasert, and Mongkolsuk 2013). Of all its uses, its use in the combating of pollution problems (Phytoremediation) is prioritized for this study.

Helianthus annuus like *Brassica juncea* has also demonstrated through numerous studies, its ability to survive several ranges of heavy metal concentrations in soils and accumulate these contaminants in its harvestable parts. An example of its potential to survive metal doses can be seen in a study by Ahmad, Ashraf, and Hussain (2011) where it survived nickel doses up to 40 mg/l. Although root and fresh biomass as well as micro and macro nutrients declined with increased nickel concentrations, no plant mortality was recorded in their study. A study by Kötschau *et al*. (2013) carried out in a former uranium mining site also showed comparable results as pertains to the high tolerance abilities of the species to metal concentrations. In their study, the species survived heavy metal concentrations of up to 0.7 2µg/g Cd, 26.2 µg/g Co, 45.8 µg/g Cr, 29.8 µg/g Cu, 54.8 µg/g Ni, 74.8 µg/g Zn, 10.1 µg/g Th, and 4.68 µg/g U. What was even more striking about this study was that throughout the 24 weeks of vegetation, they didn't observe any toxicity symptoms on the species, and this is indicative of the high tolerance level of the species for heavy metals. A similar trend was also observed in a study by Liduino, Vitor S, Servulo, and Oliveira (2018) in which the species were able to germinate and thrive in soil with heavy metal and petroleum hydrocarbons co-contamination. The ability of this species to germinate and grow in heavy metal contaminated soils has resulted in its being extensively tested for its phytoremediation abilities particularly with heavy metal contaminated soils. Their study also demonstrated the ability of *Helianthus* *annuus* to accumulate heavy metals in its tissues. they observed this in their study which evaluated biosurfactant-assisted phytoremediation of multi-contaminated industrial soil using *Helianthus Annuus L.* over a 90-day period. The results of their 90 days study showed that *Helianthus* annuus was able to accumulate up to 30 mg/kg Nickel, 32 mg/kg Lead, 20 mg/kg Chromium, 300 mg/kg Zinc and 15 mg/kg Vanadium without biosurfactant supplementation. Overall, their study did not show any significant difference in accumulation of heavy metals (with exception of Zinc and Vanadium) between treatments with biosurfactant supplementation and treatments without biosurfactant supplementation. This showed that *Helianthus annuus* has immense potential to remove considerable amounts of heavy metals from polluted soils even without biosurfactant supplementation. A similar study by Lothe, Hansda, and Kumar (2016) showed a similar trend for the phytoremediation capabilities of *Helianthus annuus* in heavy metal contaminated soils where it demonstrated a 29% removal efficiency for copper in a copper contaminated soil. The slow pace of the phytoremediation process has led research being carried out to investigate various methods of improving the removal efficiencies of various species including *Helianthus* annuus. For instance, a study by Seth et al. (2011) which investigated the influence of EDTA on the Lead removal efficiency of *Helianthus annuus*. The results of their study showed that addition of 500µM of EDTA improved the Lead accumulation in the roots from 575µg/g to 645µg/g and in the shoot from 135µg/g to 225µg/g after 28 days of exposure. Although this experiment was not carried out on soil substrate, the results could be indicative of the potential for EDTA doses to increase the removal efficiencies of *Helianthus* annuus in Lead contaminated media including soils. A similar trend was observed in a study by Turgut, Katie Pepe, and Cutright (2004) where they investigated the influence of two chelators (EDTA and citric acid) on the phytoextraction abilities of *Helianthus annuus* in Cadmium, Nickel, and Chromium polluted soils. Interestingly, for both chelators used, lower concentrations yielded better results. This was evidenced in 0.3g/kg EDTA effecting less metal uptake (0.4mg) when compared to the metal uptake (0.73mg) at 0.1g/kg EDTA treatment and 0.3g/kg citric acid being toxic to the species thereby resulting in stunted growth of the plant and reducing metal uptake. The use of citric acid as a chelator didn't prove to be productive as even when administered at 0.1g/kg did not lead to a statistically significant improvement in plant metal uptake when compared against the control.

Bioaugmentation has also been tested to boost the phytoremediation process with *Helianthus annuus*. For example, Prapagdee, Chanprasert, and Mongkolsuk (2013) investigated the potential of inoculating with plant growth promoting bacteria in enhancing the pace and efficiency of the phytoremediation process. The results from their study showed that although the inoculation with plant growth promoting bacteria enhanced plant growth which was evidenced in the observed enhanced root elongation and plant biomass production. They also observed that inoculation with *Micrococcus sp*. enhanced accumulation in the roots and leaves of *Helianthus annuus* when compared to the untreated soils. A striking discovery from Marques *et al*. (2013) study was that unlike the results of EDTA addition as seen in the study by Chandra *et al*. (2011) where it enhanced the removal efficiency of *Helianthus annuus* in a hydroponic culture, inoculation with plant growth promoting bacteria in this study rather enhanced the phytostabilization abilities of the species and helped maintain rhizospheric bacterial populations throughout the experiment. This demostrated that different amendments have different effects on the species with respect to the substrate being used. Using plant growth promoting bacteria to enhance phytostabilization of metals is key in preventing the spread of contamination especially as it prevents the accumulation of heavy metals in the above ground parts of the species.

From the studies shown above, *Brassica juncea* and *Helianthus annuus* demonstrated their abilities to germinate and grow in soils with predominantly heavy metal pollution. They also showed abilities to extract and accumulate heavy metals in their tissues as well as improve their performances when used with a range of soil amendments. Possessing these qualities, this study seeks to investigate their potential for phytoremediation of SEO and mine-spoils co-contaminated soils since this area has not been sufficiently studied, and this formed the basis for their selection as the phytoremediation species for this research project.

Table 2.1 Advantages and Disadvantages of Remediation Technologies

Remediation Technology	**Contaminant Selection**	**Advantages**	**Limitations/Disadvantages**	**References**
Soil Washing	Pesticides, heavy metals, hydrocarbon oils	High removal efficiency, versatile across contaminant and soil types, soil reusability, minimal environmental impacts, short treatment time compared to soil replacement, and site restoration.	Selectivity in effectiveness, high start-up costs, generation of waste streams requiring further treatment, high water consumption, limited applicability for deep contamination, and potential off-site environmental impacts via waste management	(Song *et al.* 2022, Abumaizar and Smith 1999)
Thermal Desorption	PAHs, TPH, PCBs, Hg, DDT	Suitable for a wide range of organic contaminants and contaminant media, minimal site disturbance when compared to technologies like excavation, high removal efficiency, short treatment time, and long-term effectiveness.	High cost, high energy consumption, increased emissions and air pollution, limited application (not widely applicable for inorganic contaminants), noise & disturbance, alteration and damage of soils, site-specific challenges like low permeability and high-water content.	(Zhao *et al.* 2019, Bykova *et al*. 2021)
Electrokinetic Remediation	Heavy metals, hydrocarbon oils,	Minimal waste generation, targeted treatment, in-situ benefits, versatile across	High capital costs, slow treatment rate, high energy consumption, generation of problematic	(Liu *et al.* 2022, Song *et al.* 2022, Aparicio *et al.* 2022)

		contaminant types, and high removal efficiency.	by-products, alteration of properties of the remediated environments (e.g., pH) with concomitant ecological imbalance.	
Soil Replacement	Heavy metals, pesticides, PCBs, Petroleum hydrocarbons and other organic contaminants.	High removal efficiency, quick results, proven and well-established technology with widescale usability, versatile and applicable to all contaminant types, and cost effective.	Generation of high volumes of hazardous waste, soil compaction, ecosystem disruption/ecological imbalance, depth limitations, and short-term efficiency for mobile contaminants.	(Khan, Husain, and Hejazi 2004)
Chemical Oxidation	Petroleum hydrocarbon oils, chlorinated solvents, BTEX compounds, pesticides, herbicides, VOCs, and PCBs	versatile and applicable to a wide range of contaminant types, in-situ benefits, targeted treatments, confidence as it is a proven technology with real-world utilization, effective in contaminant oxidation.	Oxidant selectivity, generation of secondary by-products requiring further treatment, high running costs, greenhouse gas emissions, risk of groundwater contamination, health & safety risks, oxidant selectivity.	(Sui *et al.* 2021, Lim, Lau, and Poh 2016)
Chemical Leaching	Heavy metals	Suitable for severely contaminated sites, adaptability for combination with other remediation methods, speed, and quick turnaround times, effective in solubilizing persistent contaminants.	Secondary contamination, risk	(Qiu *et al.* 2021, Hamby 1996)
Chemical Stabilization	Heavy metals	Minimal site disruption, cost-effective, attenuation of contaminant migration.	Limited primarily to heavy metal contamination, requires specialty knowledge & expertise, alteration of soil properties with concomitant ecosystem imbalance, generation of waste streams, contaminants are not removed which creates the likelihood of contaminant migration with any changes in soil conditions.	(Alpaslan and Ali Yukselen 2002, Song *et al.* 2022)

Biostimulation	Petroleum hydrocarbons, chlorinated solvents, PAHs, Pesticides, heavy metals	Eco-friendly, cost-effective, limited site disturbance, minimal waste generation, versatility.	Time consuming/slow process, potential for secondary pollution via production of harmful intermediate metabolites, variability/uncertainty in effectiveness.	(Rigoletto *et al.* 2020, Y *et al.* 2019, Aparicio *et al.* 2022)
Bioaugmentation	Petroleum hydrocarbons, chlorinated solvents, PAHs, PCBs, Pesticides.	Targeted remediation, eco-friendly, minimal waste generation, minimal site disruption, compatible with other technologies	Dependent on microbial survival in new environments, competition with native species and risk of introduction of invasive species, dependence on environmental conditions, limited applicability, unpredictable results due to high level dependence on other factors.	(Gao, D. et al. 2022)
Phytoremediation	Petroleum hydrocarbons, chlorinated solvents, PAHs, PCBs, Pesticides, heavy metals.	Cost-effective, eco-friendly, long-term sustainability, aesthetic value, minimization of secondary pollution, minimal site disruption, community acceptance, easy to implement without requiring special expertise, versatility, compatible with other technologies.	Slow/time consuming process, risk of invasive species, weather dependency, concerns relating to the fate of phytoremediation plants, site specific limitations	(Mishra and Chandra 2022, Khan, Husain, and Hejazi 2004)

Table *2.1* above captures the advantages and disadvantages of the key remediation technologies. As is already apparent, a perfect remediation technology does not exist, and each technology has its strengths and weaknesses. It is however pertinent that the choice of remediation technology be informed by the key priorities of the decision maker in the selection process, and a combination of technologies might be useful in balancing out the weaknesses in other remediation technologies. Priorities relating to low environmental impact, cost-effectiveness, versatility, sustainability, and potential for circular economic contribution were they key considerations for the selection of phytoremediation in tandem with biostimulation (nutrient supplementation) that informed the choices made for this study.

Chapter 3: EXPERIMENTAL DESIGN AND METHODOLOGY

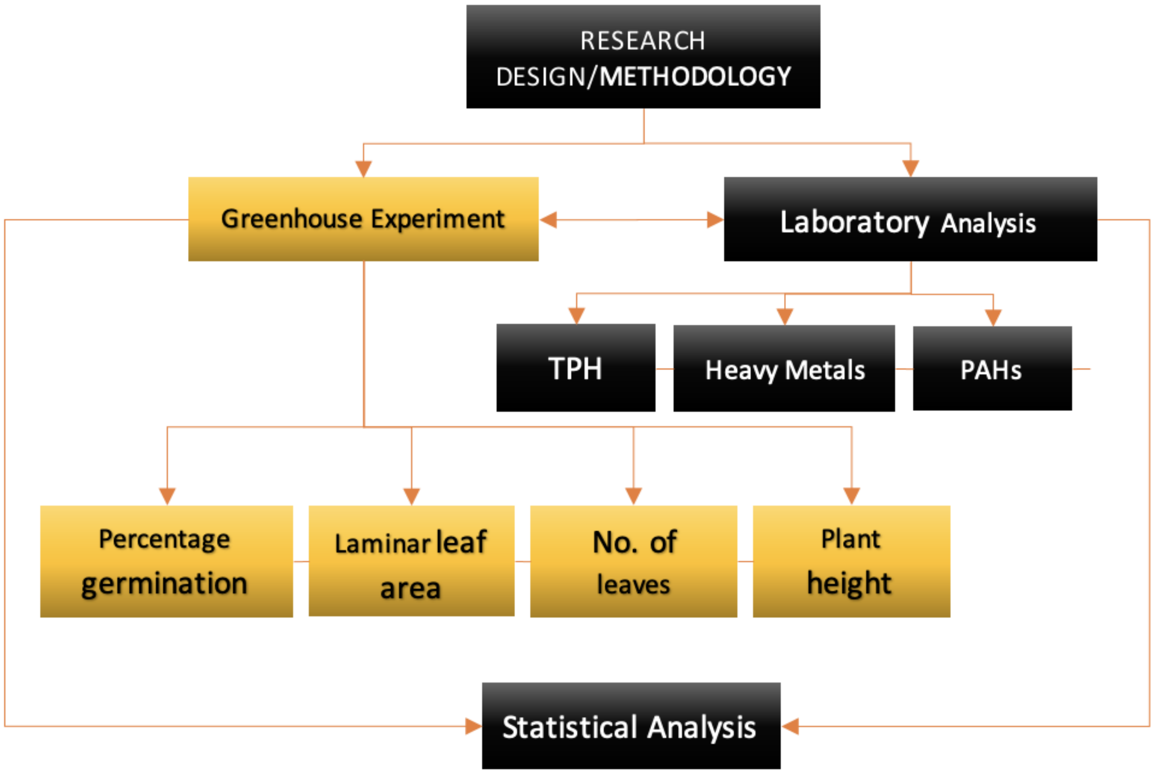

Figure 3.1 Experiment Design Process Flow

This chapter covers the methods for the various experiments that were conducted to fulfil all the objectives of this research, and the overall structure of the experimental design is summarized in **Figure *3.1*** above. The following sections of this chapter cover these methodologies which include collection and characterization of soil used for planting and spoils from a mining site, plant and soil analysis for metal content, measurement of plant growth parameters, and the analysis of PAHs and TPH content in soils. The experimental designs for the various greenhouse experiments are also covered in this chapter.

3.1 SOIL & SPOIL COLLECTION

The soil used for the greenhouse plant trial experiments was clean agricultural topsoil collected using a cleaned shovel from Ryton Gardens. Large quantities of this soil were collected, air-dried, and stored in plastic drums prior the experiment. To facilitate the creation of SEO and mine-spoil co-contaminated soils, mine spoils were procured from Frongoch Mine (shown in **Figure *3.2***).

Figure 3.2 Frongoch Mine

Frongoch Mine, situated near Pont-rhyd-y-groes, Ceredigion, covered approximately 11 hectares and operated from the late 1700s to the early 1900s, producing lead and zinc ore. After falling into disuse, it was reworked from 1924 to 1930 to reclaim previously uneconomical minerals (Natural Resources Wales 2016). According to The Hudson Institute of Mineralogy, the ore in the Frongoch mine seems to have formed where veinlets intersected, and there were two separate ore shoots that were exploited—one on the south side and the other on the north side of the fault system. The primary ore minerals, galena, and sphalerite were distributed across the mined area, but as depth increased, sphalerite became more prevalent while galena decreased. The mine caused significant pollution, impacting downstream watercourses and fish populations, failing environmental quality standards set by the European Water Framework Directive (Natural Resources Wales 2016). The mine-spoil collection was carried out by random sampling covering the entire perimeter of the mine with the aim of obtaining samples representative of the site's pollution profile while eliminating the likelihood of bias in the sample collection process. The spoils were collected in airtight plastic containers using a shovel and transported to an on-site storage at the Centre for Agroecology, Water and Resilience (CAWR) to await further analysis and processing. Worthy of note the fact that several remediation attempts have been made on the mine between 2013 to 2015, however, these have largely been containment measures to attenuate secondary pollution via the transport of copious quantities of metal load from the mine to local receptors (Natural Resources Wales 2016). The nature of remedial activities that took place in the mine, which encompassed reshaping and capping with clay and soils to prevent water ingress and promote re-vegetation, may lead to discrepancies in the recorded metal levels during the characterization of the mine spoils in this study. Consequently, these readings might not entirely reflect the true metal concentrations contained in the mine.

3.1.1 Mixing Procedure for Soil from Frongoch Mine

Large batches of mine-spoils from Frongoch mine were collected as described in **3.1** and homogenization was necessary prior to being integrated into the greenhouse study. The homogenization process involved mixing all the collected soil from the mine in a cement mixer (illustrated in **Figure *3.3***).

Figure 3.3. Homogenization of Frongoch Mine-Spoils Using a Cement Mixer

The mixing process in the cement mixer was carried out for a duration of 1 hour at 30 RPM and then stored in air-tight plastic boxes prior to use in greenhouse studies.

3.2 SAMPLE COLLECTION AND STORAGE

Because of the differences in storage requirements for organic and elemental analysis, samples were collected and stored separately for the various analysis. These are described in the subsequent subsections below.

3.2.1 Sample Collection and Storage for Heavy Metals Analysis

Soils were collected in appropriately labelled sealable polyethene bags and stored in a fridge prior to heavy metal analysis. Plants were carefully removed from the pots to ensure roots were not damaged, washed thoroughly in distilled water to remove all soil and other impurities and oven-dried at 65 °C for 48 hours. These were allowed to cool at room temperature and then ground to a homogenous mixture and then stored in appropriately labelled sealable polyethene bags prior to nitric acid assisted microwave digestion. Because of the low plant material content in the contaminated soils, the plants were not separated into stems, roots, and leaves. For this reason, the plants were ground and analysed whole, rather than in specific parts.

3.2.2 Sample Collection and Storage for TPH and PAH Analysis

Soil samples were collected in appropriately labelled amber glass jars and stored in the freezer at below 4 °C prior to collection in coolers by a commercial laboratory, ELab, who were contracted for analysis.

3.3 SOIL CHARACTERIZATION

After collection and processing of clean soil and spoils from Frongoch mine as described in sections **3.1** and **3.2** above, both were characterized in terms of soil pH, moisture content, total carbon and total nitrogen. The methodologies used are described in the following sections.

3.3.1 Soil pH

Approximately 20g ± 0.1 of prepared soil sample was weighed into a 50 mL beaker, 20 mL of distilled water was added, and the suspension was stirred for about 5 minutes and allowed to stand for 1 hour to enable the suspended particles to settle (USEPA method 9045D). Soil pH in supernatant solution was recorded using the Hanna Bench Top pH Meter with pH Electrode Temperature Probe and mV Meter. It was calibrated using buffer solutions of pH 4.0 and 7.0 and pH 7.0 and 10 at 25 °C.

3.3.2 Soil Moisture Content

Soil moisture content was determined in line with the AS 1289.2.1.1-2005 method (Standards Australia 2005). Soil moisture content was determined by weighing 50g ± 0.01 moist soil (W1) into weighing tins. This was then placed in an oven at 110 °C and dried to a constant weight and the final weight recorded (W2). The moisture content (MC) was then determined as follows.

$$\boldsymbol{MC}\% = \frac{(\boldsymbol{W1} - \boldsymbol{W2})}{\boldsymbol{W1}} \times \mathbf{100}$$

3.3.3 Total Organic Carbon and Total Nitrogen

Sample preparation and analysis for total organic carbon and total nitrogen were by an elemental analyser (Primacs $^{\text{SNC-100}}$, Skalar Analytical, Breda, The Netherlands). Approximately 110 mg ± 10 of prepared soil samples were weighed into crucibles. Calibration standards (0.2% Total carbon and 0.1166% Total Nitrogen) and (1% Total Carbon and 0.583% Total Nitrogen) were prepared and appropriate volumes were pipetted into designated crucibles fitted with glass wool. Appropriate masses (mg) of dry glycine calibration standards were weighed into designated crucibles and all the crucibles were loaded into the auto sampler and analysed using the Primacs $^{SNC-100}$ Carbon Nitrogen analyser.

3.4 MEASUREMENT OF PLANT GROWTH PARAMENTS

The ability of plants to demonstrate high tolerance to contaminant toxicity in soils is indicative of their suitability as phytoremediation species, and to test the tolerance of the chosen species for SEO and mine-spoils co-contamination, key plant growth metrics were determined for both species. The plant growth parameters measured in this study were plant height, number of leaves, laminar leaf area and plant dry biomass. The attributes of plant height, leaf count, leaf area, and biomass production play integral roles in phytoremediation. These growth metrics are essential for effective pollutant uptake, fostering microbial interactions in the rhizosphere, facilitating pollutant sequestration within plant biomass, ensuring the stability and longevity of the remediation process, and contributing to ecological and aesthetic aspects of remediation projects. The decision to measure the selected growth metrics offers a rigorous assessment of their suitability for phytoremediation of the soils co-contaminated with SEO and mine-spoils within a tolerance context. This would yield valuable insights to inform future planning of phytoremediation projects as the results from these assessments will be pivotal in highlighting the importance of growth response on overall phytoremediation outcomes.

Plant height was measured simply using a ruler from the base at the soil to the tip of the plant and the number of leaves were simply counted by visual observation. Laminar Leaf Area (LLA) was determined using the formula ***LLA = 0.5 (Length X Breadth of leaf)*** as seen in (Lale, Ezekwe, and Lale 2014). Plant dry biomass was measured by oven drying the plant samples at 65 °C for 48 hours and weighing the dried plant material.

3.5 SOIL AND PLANT ANALYSIS

This section covers the methods used for analysis of soil samples for heavy metals, TPH and PAHs and covers the methods used for the analysis of plant heavy metal content. These include extraction procedures and instrumental analytical procedures.

3.5.1 Heavy Metals

Soil heavy metal analyses were carried out twice with one at the beginning and the other at the end of the greenhouse Experiments. Heavy metals were extracted from soil samples using Nitric Acid Microwave Digestion as described in the USEPA Method 3050A and the selected heavy metals were determined for soil samples using Inductively Coupled Plasma – Optical Emission Spectrometry (ICP-OES) as described in USEPA Method 6010C.

Heavy metal extraction from plant samples were carried out at the end of the greenhouse experiment using Nitric Acid Microwave Digestion as prescribed by the USEPA Method 3051A and the selected heavy metals analysed using ICP-OES as done for the soil samples.

The reagents, glassware, laboratory machines/instruments and other materials that were used in the laboratory for the extraction and determination of selected heavy metals for soil and plant samples are detailed as follows.

Reagents

All the reagents that were used for the laboratory analysis were laboratory grade and were used as provided. Reagents used include.

- Concentrated Nitric acid.
- Reagent Water
- Standard stock solutions prepared from ultra-high purity grade chemicals or metals (99.99% pure or greater)

- Mixed Calibration Standard Solutions
- Blanks (method blanks and calibration blanks)

Glassware

- Beakers 250ml
- Filter funnel
- Volumetric Flasks (100ml and 1000ml)
- Positive displacement pipets (graduated in ml and µl)

Machines/Instruments

- Microwave oven digester
- Filter Paper – Whatman No. 41 or equivalent
- Analytical Balance – Capable of weighing to the nearest 0.01g
- Fume Cupboard
- Inductively Coupled Argon Plasma fitted with an Optical Emission Spectrometer (ICP-OES)

Personal Protective Equipment

- Eye protective goggles
- Lab coats
- Protective gloves
- Nose masks

3.5.1.1 Soil Extraction for Heavy Metal Analysis

The extraction method used for soil samples was the microwave assisted nitric acid digestion as prescribed by the USEPA Method 3051A. the procedure is detailed below.

1. 0.5g (0.25g for oil contaminated soil) of dry and adequately homogenized soil samples were weighed to the nearest 0.001 g into Teflon tubes equipped with a pressure relief mechanism. This was done in triplicates for each soil sample.
2. 9 ± 0.1 mL concentrated nitric acid was added to each vessel in a fume hood using a positive displacement pipette.
3. Spikes were prepared by adding 1ml of lead, zinc stock solutions and 200µl of cadmium, copper and mercury stock solutions into a vessel and adding 9 ± 0.1 mL concentrated nitric acid. This was done in triplicates.
4. Method blanks were also added in triplicates to separate vessels to be digested along with the soil samples. This was also done in triplicates. (Method blanks were simply vessels containing same acid mixtures and concentrations as the samples)
5. All the vessels were sealed according to manufacturer's instructions, safely loaded into the microwave and appropriate temperature and pressure sensors were connected to the vessels according to manufacturer's instructions.
6. Samples, spikes, and blanks were digested in the Ethos Up High-Performance Microwave Digestion System using a pre-installed EPA Method 3051A.
7. After the completion of the digestion the digestion, vessels were vented in a fume cupboard according to manufacturer's instruction and allowed to cool. Sample extracts were filtered into 100 mL volumetric flasks using Whatman No.41 filter paper and made to mark with distilled water. The final sample was refrigerated until analysis by ICP – OES.

3.5.2 Plant Extraction for Heavy Metal Analysis

0.5g of dried, homogenized plant materials were weighed and transferred into Teflon tubes and processed using the same methods as described in **3.5.1.1** above.

3.5.3 Plant And Soil Analysis for Heavy Metal Using ICP-OES

- The calibration blanks were prepared by adding 9 ± 0.1 mL concentrated nitric acid and 3 ± 0.1 mL concentrated hydrofluoric acid accurately measured into 100ml volumetric flasks and diluting to volume with reagent water.
- A calibration curve was prepared daily with a minimum of a calibration blank, and four standards and the curve had a correlation coefficient of 0.998.
- All the heavy metal (Pb) concentrations that had been extracted for plants and soils were analysed using the Optima 5300 DV Optical Emission Spectrometer (Perkin Elmer) following a 4-point (0.5, 2, 5 and 10 mg L^{-1}) calibration with Pb, Zn, Cd, and Cu mixed calibration standard solution.
- A Continuing Calibration Verification (CCV) and a Continuing Calibration Blank (CCB) was analysed after the analysis of every 10 samples and after every analysis batch.
- The system was rinsed with the calibration blanks before analysing each sample.

3.5.4 Soil Extraction for TPH And PAH Analysis

The extraction and analysis of soil samples for TPH and PAH analysis was outsourced to ELab (MCERTS, UKAS 2683). They performed PAH extractions using Solvent Extraction which involved shaking of the samples in extraction solvent (DCM) prior to chromatographic analysis.

3.5.5 Soil TPH Analysis by GC-FID

The extraction and analysis of soil samples for TPH analysis was outsourced to a ELab (MCERTS, UKAS 2683), and Total Extractable Petroleum Hydrocarbons (TPH) were extracted from the sample matrix by shaking with hexane containing iso-octane as an internal standard. The extract was then filtered and subjected to examination by high resolution gas chromatography with flame ionisation detection (GCFID).

3.5.6 Soil PAH Analysis by GC-MS

The extraction and analysis of soil samples for PAH analysis was outsourced to a ELab (MCERTS, UKAS 2683). They performed PAH extractions using Solvent Extraction which involved shaking of the samples in extraction solvent (DCM) prior to a high-resolution gas chromatographic analysis.

The sample extracts were analysed using an Agilent 6890N gas chromatograph with a 5975-mass spectrometer detector. The typical operational conditions were Column 20m x 0.18mm ID x 0.30µm df 5% diphenyl/ 95% dimethyl polysiloxane. Carrier gas helium 1.1ml / min. Injector 300^{o}C. Oven programme 45 degrees for 2 minutes, 5^{o}C /min to 300^{o}C, held 0 mins, 50^{o}C/ min to 320^{o}C, held for 2 minutes.

3.6 EXPERIMENTAL DESIGN

The experimental design features 3 greenhouse experiments accompanied by laboratory analyses which were carried out at the beginning and at the end of the greenhouse studies. The three greenhouse experiments independently set out to investigate the effect of SEO concentrations on germination, effects on growth and effects of SEO and mining soil co-contamination on growth and phytoremediation abilities of *Helianthus annuus* and *Brassica* *juncea* in line with the objectives of the study. The details of the design of these experiments are covered in sections **3.6.1** to **3.6.3** of this chapter.

3.6.1 Germination experiment

This experiment was conducted to investigate the effects of various concentrations of SEO on the germination of *Helianthus annuus* and *Brassica juncea*. This was conducted in a completely randomized design in the greenhouse at the Centre for Agroecology, Water and Resilience (CAWR). Large quantities of SEO were sourced from a local mechanic workshop and stored at room temperature. The planting soil used was clean (unpolluted) topsoil sourced directly from Ryton gardens, homogenized, and air-dried in the greenhouse. Seedling trays and seeds of *Helianthus annuus* and *Brassica juncea* used for the experiment were purchased from the local B&Q in Coventry, United Kingdom. Mixing trays came from the greenhouse.

The experiment was composed of soils from 0% SEO to 6% SEO w/w polluted at 0.2% increments from 0 to 6% to give a total of 30 SEO concentrations and this was done in triplicates. The choice to use a 6% maximum SEO concentration was informed by the experiment in section **3.6.2** where germination was completely inhibited at 9.2% SEO concentration, so the aim was to be able to establish the maximum SEO dose that could support germination for the chosen species. The soils were artificially polluted with SEO by thoroughly mixing soils with appropriate volumes of SEO in a mixing tray with positive displacement pipettes and mixed by hand to achieve a homogenized mixture. These were subsequently transferred into the seeding trays which were labelled appropriately.

The treated soils were kept in the greenhouse for a week before sowing 5 seeds of both plants in all the treatments. This was done to mimic real-world conditions as contaminated soils would have aged for variable periods of time before remedial actions commence, and sowing into freshly contaminated sites would be largely unrealistic. Keeping it to 14 days before sowing would have been more ideal from a consistency standpoint, however, time constraints necessitated the tweaking of this part of the study. The treatments were watered with once each week and germination data were collected over a 3-week period by counting the number of germinations in each treatment. Percentage germination was calculated as shown below.

Percentage Germination = (Total Germination ÷ Total Seeds Sown) X 100

3.6.2 Plant Growth in SEO Contaminated Soil Experiments

This experiment was conducted to investigate the effects of various concentrations of SEO on the growth and survival of *Helianthus annuus* and *Brassica juncea*. This was conducted in a completely randomized design in the greenhouse at the Centre for Agroecology, Water and Resilience (CAWR) in planting pots. The planting pots with saucers, seedlings of *Helianthus annuus* and *Brassica juncea* were purchased from a local B&Q store in Coventry, United Kingdom. The process for the collection and processing of the mixing trays, clean soil and SEO used for this experiment are described in Error! Reference source not found. above.

The experiment was composed of unpolluted soils, soils polluted with 4.6% w/w and 9.2% w/w, all in triplicates. The soils were artificially polluted with SEO by thoroughly mixing soils with appropriate volumes of SEO in a mixing tray with positive displacement pipettes and mixed by hand to achieve a homogenized mixture. These were then transferred to well labelled planting pots and allowed to sit for 14 days before planting. This was done to mimic real-world conditions as contaminated soils would have aged for variable periods of time before remedial actions commence, and sowing into freshly contaminated sites would be largely unrealistic. Four seedlings of each plant species were sown in each pot and grown until both plants reached flowering stage (a period of 124 days).

Throughout the experiment duration, plant height, number of leaves, laminar leaf area and total dry biomass were measured as described in Error! Reference source not found..

3.6.3 SEO and Mine-Spoils Co-Contamination Experiment

This experiment was conducted to investigate the effects of SEO and mine-spoils co-contamination on the growth and survival of *Helianthus annuus* and *Brassica juncea*, and their phytoremediation abilities in the uptake of Pb and in the dissipation of Pb, TPH and Total PAHs in co-contaminated soils. This was conducted in a completely randomized design (shown in **Figure *3.4*** below) in the greenhouse at the Centre for Agroecology, Water and Resilience (CAWR) in planting pots. The planting pots with saucers, seedlings of *Helianthus annuus* and *Brassica juncea* were purchased from a local B&Q store in Coventry, United Kingdom. The process for the collection and processing of the mixing trays, clean soil and SEO used for this experiment are described in Error! Reference source not found. above.

The experiment was composed of unpolluted soils, soils polluted at 0.8% SEO + 10% mine-spoils and 1.6% SEO + 10% mine-spoils w/w planted separately with *Helianthus annuus* and *Brassica juncea*, and unplanted soils polluted at 0.8% SEO + 10% mine-spoils and 1.6% SEO + 10% mine-spoils w/w. This gave a total of 8 treatments in triplicates.

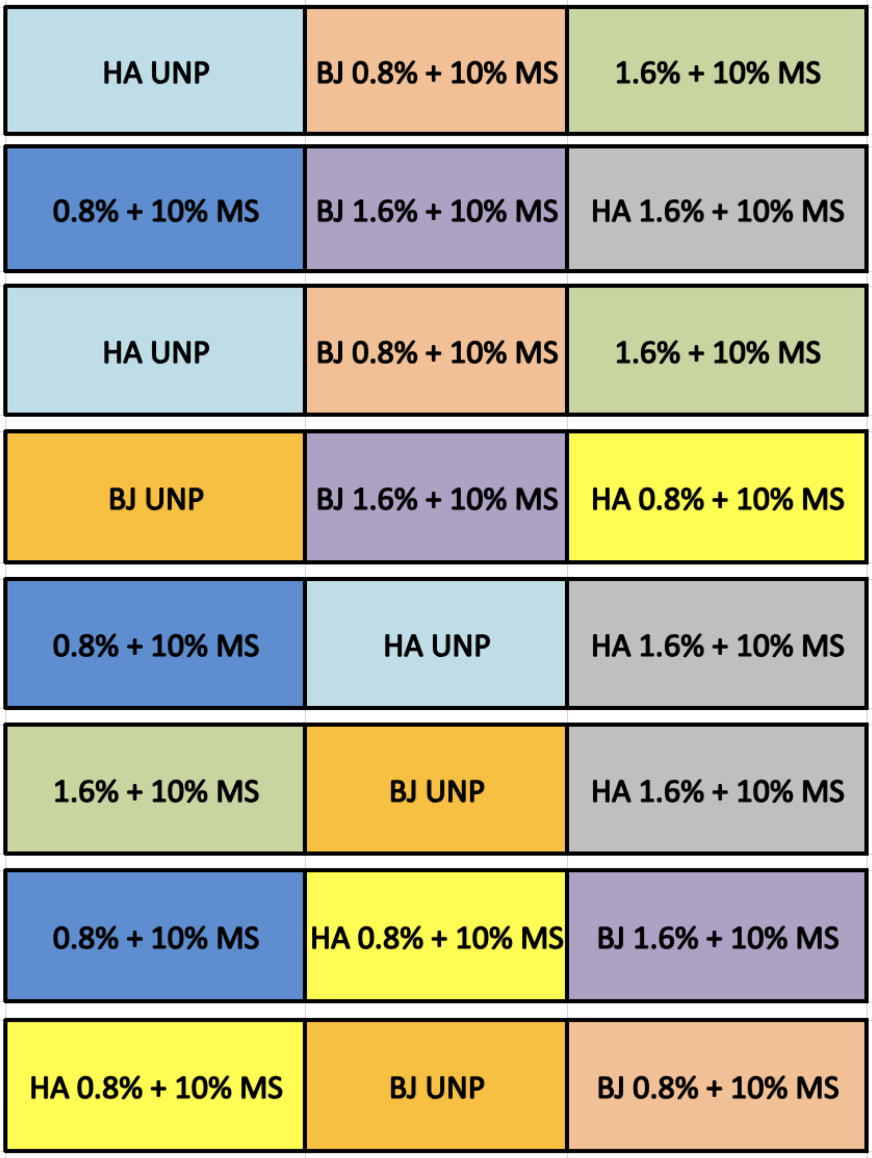

Figure 3.4 SEO and Mine-Spoils Co-Contamination Experiment in a Completely Randomized Design (HA = *Helianthus annuus*, = *Brassica juncea*, UNP = Unpolluted treatment, MS = mine spoils and the boxes not containing letters represent co-contaminated unplanted treatments)

The procedures for the preparation of the co-contaminated soils are elucidated below.

- 4kg clean soil was weighed into a mixing tray, and 400mg of homogenized mine-spoils were weighed out and added to the mixing tray to yield a 10% w/w mine-spoil concentration in the mixture.
- This was followed by a thorough homogenization in the mixing tray until a homogenous mixture was achieved.

- The second level of contamination was achieved by pipetting SEO volumes to give concentrations of 0.8% and 1.6% w/w for the appropriate treatments and thoroughly mixing by hand in the mixing tray until a homogenized mixture was achieved.

The percentage of mine-spoils (10%) used for the co-contamination yielded a Pb concentration of 303.6 mg/kg soil. This was done to reflect real life scenarios of heavy metals and SEO co-contamination found in a lot of automobile garages found in Nigeria. This has been demonstrated in various studies (Ololade and Ololade 2014; Olajumoke Abidemi 2011; Jolaoso *et al*. 2019; Ifeanyi and Agwu 2014; Shola Caleb and Adedotun Onoyinka 2020) which showed Pb concentrations ranging from 210 mg/kg to 482.2 mg/kg in the soils of automobile garages in various parts of the country, often co-contaminated with SEO and heavy metals. Thus, the chosen percentage of mine-spoils used was chosen to fall within this range.

While there is limited literature on mine spoils and SEO co-contamination, subpar waste management practices, and mismanagement SEO during equipment maintenance on metal mining sites can lead to leaks and spills resulting in SEO and heavy metal co-contamination. Although this form of contamination may not be as widespread as other types due to limited metal mining activities globally, this pollution profile which features organic (TPH & PAHs) and inorganic (heavy metals) co-contamination remains a significant concern with widespread applicability worldwide (more details/context already provided in **Chapter 1:**). The probability of the occurrence of organic and inorganic co-contamination in the UK has been extensively reported. For instance, UK industry profiles for Metal Manufacturing, refining and finishing works, power stations (excluding nuclear power stations), and road vehicle fuelling, service and repair have all been reported to be potentially possess multi-contamination containing organic contaminants (like fuels, spent engine oils, PAHs, PCBs etc) and heavy metals (like Pb, Cr, Zn, Cu, etc) in areas relating to material storage, process areas, waste disposal, and fuel storage (Department of Environment 1995).

To investigate the effects of nutrient supplementation on plant growth and phytoremediation abilities of both plant species under mixed contaminant stress, treatments containing struvite and NPK fertilizers were also prepared at 0.8% and 1.6% w/w SEO concentrations. Unplanted treatments containing the same concentrations of mixed contamination were also prepared to investigate the role of natural attenuation. These were then transferred into well labelled planting pots and allowed to sit for 14 days before planting. Four seedlings of each plant species were sown in each pot and grown for a period of 114 days. Due to time limitations linked to impending laboratory renovations and the possibility of delays, the experiments had to be expedited to prevent any associated setbacks from affecting the timely completion of the study. This resulted in a lack of consistency in experiment duration with the experiment in section **3.6.2**.

Throughout the experiment duration, plant height, number of leaves, laminar leaf area and total dry biomass were measured as described in Error! Reference source not found..

3.7 STATISTICAL ANALYSIS

A two-factor ANOVA with replications at a 95% confidence level and 0.05 alpha level was used for analysing the data from all the studies. This was carried out using Microsoft Excel in the data analysis tool tab.

Chapter 4: RESULTS

4.1 Introduction

This chapter covers the presentation of the results from all the experiments carried out to investigate the phytoremediation abilities of *Helianthus annuus* and *Brassica juncea* in line with all the objectives of this study.

It begins in section **0** by presenting the results of the germination experiment in line with the first objective of the study which was the germination response of *Helianthus annuus* and *Brassica juncea* to SEO concentrations as an indication of their suitability for the phytoremediation of SEO polluted soils.

Section **4.4** of this chapter features the presentation of results of the effects of SEO on the growth of both species and the potential for mixed cropping to enhance the growth parameters of *Helianthus annuus* and *Brassica juncea* at higher SEO concentrations. This was in line with the second objective of the study which was to investigate the potential for mixed cropping on reducing the impacts of high SEO concentrations (4.6 and 9.2% w/w) on the growth of *Helianthus annuus* and *Brassica juncea*.

This was followed by presenting the results of the effects of SEO and mine-spoils co-contamination on the growth parameters of *Helianthus annuus* and *Brassica juncea* (section **4.5**) in line with the third objective of the study which was to determine the effect of mine-spoils and SEO co-contamination on the growth parameters of *Helianthus annuus* and *Brassica juncea*. Sections **4.7** to **4.9** of this chapter covered the presentation of results of the reduction of TPH and Total PAHs in *Helianthus annuus* and *Brassica juncea* soils co-contaminated with SEO and mine-spoils. These were in line with the fourth objective of this study which was to investigate the abilities of *Helianthus annuus* and *Brassica juncea* to reduce TPH and PAH concentrations from SEO and mine-spoils co-contaminated soils. Section **4.11** covered the presentation of results on the reduction of Pb in *Helianthus annuus* and *Brassica juncea* soils co-contaminated with SEO and mine-spoils. These were in line with the fifth objective of this study which was to investigate the abilities of *Helianthus annuus* and *Brassica juncea* to reduce Pb concentrations from SEO and mine-spoils co-contaminated soils. The results of the potential for struvite and NPK amendments to increase the reduction Pb in *Helianthus annuus* and *Brassica juncea* soils co-contaminated with SEO and mine-spoils were also presented in these sections in line with the sixth objective of the which sought to evaluate and compare the potential for an industrial waste (struvite) in enhancing the growth and phytoremediation abilities of both species in comparison with NPK fertilizer.

Section **4.12** covered the presentation of results on the uptake of Pb by *Helianthus annuus* and *Brassica juncea* in soils co-contaminated with SEO and mine-spoils. These were in line with the fifth objective of this study which was to investigate the abilities of *Helianthus annuus* and *Brassica juncea* to uptake Pb from SEO and mine-spoils co-contaminated soils. The results of the potential for struvite and NPK amendments to increase the uptake of Pb in *Helianthus annuus* and *Brassica juncea* in soils co-contaminated with SEO and mine-spoils were also presented in these sections in line with the sixth objective of the study which sought to evaluate and compare the potential for an industrial waste (struvite) in enhancing the growth and phytoremediation abilities of both species in comparison with NPK fertilizer.

Section **4.6** covered the presentation of results of the potential for struvite amendment to improve the growth parameters of both plant species which was in line with the sixth objective of the study which sought to evaluate and compare the potential for an industrial waste (struvite) in enhancing the growth and phytoremediation abilities of both species in comparison with NPK fertilizer.

The results of the potential for struvite and NPK amendments to increase the reduction of TPH and PAHs in *Helianthus annuus* and *Brassica juncea* soils co-contaminated with SEO and mine-spoils were covered in section **4.10** in line with the sixth objective of the study already referenced above.

All the summary tables of the statistical analysis have been moved to the **Appendices** on the advice of the subject expert at my PRP.

4.1.1 Summary of Abbreviations

The abbreviations used in this chapter are summarized in **Table *4.1*** below.

Table 4.1. Summary of Abbreviations

Acronym	Definition
BJ	*Brassica juncea*
HA	*Helianthus annuus*
SEO	Spent Engine Oil
STRV	Struvite
LLA	Laminar Leaf Area
H	Height
NL	Number of Leaves
TPH	Total Petroleum Hydrocarbons
PAH	Polycyclic Aromatic Hydrocarbons

4.2 Soil Characterization

The results of the soil characterization are displayed in **Table *4.2*** and **Table *4.3*** below. The results showed that moisture content, total carbon and total nitrogen were significantly higher in the Ryton soil when compared to the Frongoch Mine soil. There were no notable differences between the pH for both soils.

Table 4.2. Mean pH, Moisture Content, Total Nitrogen and Total Carbon Concentrations in Ryton Soil and Frongoch Mine Soil

Soil	pH	Moisture Content (%)	Total Carbon (%)	Total Nitrogen (%)
Ryton Soil	7.32	17.5	4.57	0.37
Frongoch Mine Spoils	7.54	9.1	0.35	0.06

In terms of heavy metal concentrations, the Ryton soil had negligible concentrations for Cu, Pb, Ni and Zn while Cd and Hg were not detected, indicating the absence of heavy metal pollution. Frongoch Mine soil however, had high concentrations of Pb and Zn, Cu and Ni concentrations were very low, and Cd and Hg were not detected. In the SEO and Mining soil co-contaminated soil, Pb concentrations were high while all the other metals were undetected, and this accounts for why Pb was the only metal studied in later sections of this chapter.

Table 4.3. Mean Heavy Metal Concentrations in Ryton Soil, Frongoch Mine Soil and Co-Contaminated Soil

Soil	Cd (mg/Kg)	Cu (mg/Kg)	Hg (mg/Kg)	Pb (mg/Kg)	Ni (mg/Kg)	Zn (mg/Kg)

Ryton Soil	≤ 0	0.03	≤ 0	0.03	0.001	0.06
Frongoch Mine Spoils	≤ 0	47.30	≤ 0	9184.82	2.94	324.91
Co-Contaminated Soil	≤ 0	≤ 0	≤ 0	303.6	≤ 0	≤ 0
LOD (mg/kg)	0.01	0.04	0.1	0.1	0.05	0.02

4.3 Effects of SEO Concentrations on Percentage Germination of *Helianthus annuus* and *Brassica juncea*

This experiment set out to investigate the effects of various SEO concentrations on the germination of *Helianthus annuus* and *Brassica juncea* as a measure of their suitability for phytoremediation. It also investigated if there was any difference in the germination response of both plant species to various SEO concentrations. This was done in line with the first objective of the study which was the germination response of *Helianthus annuus* and *Brassica juncea* to SEO concentrations as an indication of their suitability for the phytoremediation of SEO polluted soils.

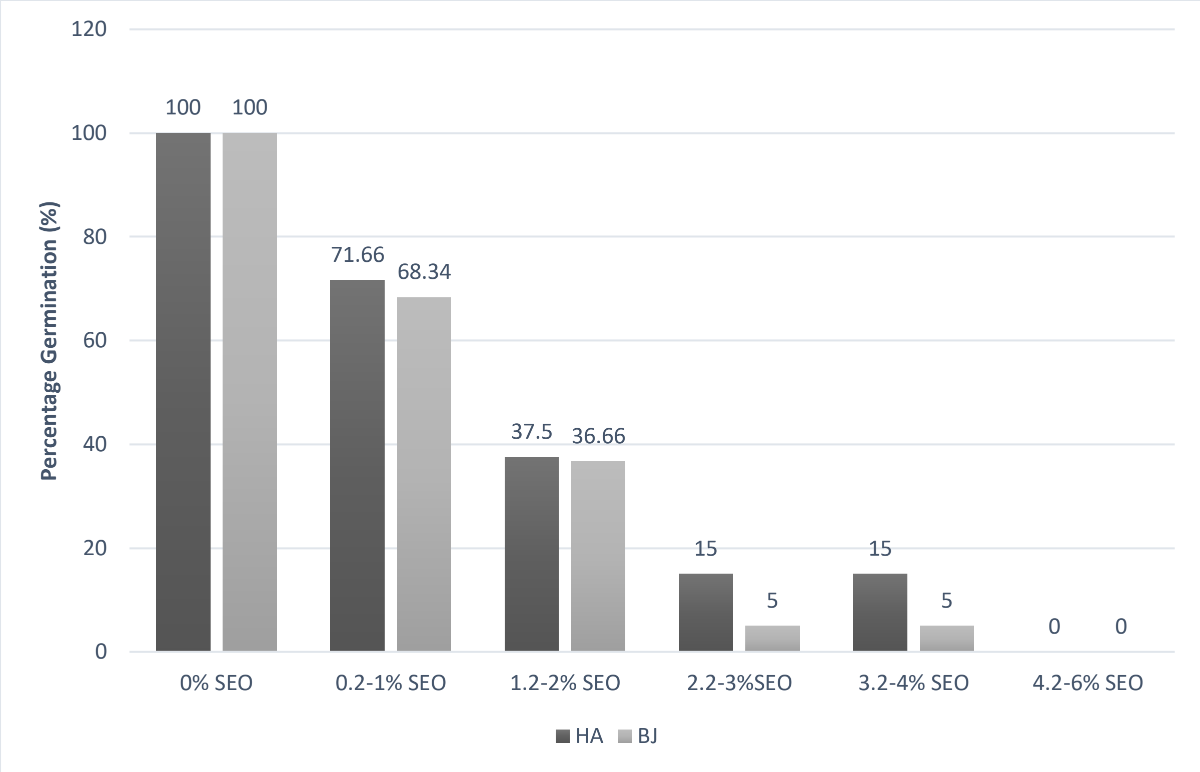

Figure 4.1. Effects of SEO Concentrations on the Mean Percentage Germination of *Helianthus annuus* & *Brassica juncea*

As seen in **Figure *4.1***, the results showed a general decline in the percentage germination as the concentration of SEO increased for both plants. The rate of decline for both plants showed no marked differences up until 2.2% -4% SEO concentration where *Brassica juncea* showed notable decrease in percentage germination when compared to *Helianthus annuus*. For both plant species, germination was completely inhibited beyond 4% SEO concentration.

4.4 Effects of SEO on the Growth Parameters of *Helianthus annuus* and *Brassica juncea*

This experiment aimed to investigate the effects of 4.6% and 9.2% w/w SEO concentrations on the growth parameters of *Helianthus annuus* and *Brassica juncea* as an indication of their suitability for the phytoremediation of SEO contaminated soils. This was in line with the second objective of the study which was to investigate the potential for mixed cropping on reducing the impacts of high SEO concentrations (4.6 and 9.2% w/w) on the growth of *Helianthus annuus* and *Brassica juncea*.

The results shown in subsequent sections do not show data at 9.2% SEO because both plants were unable to germinate and grow at that pollution concentration.

The mean growth parameters of *Helianthus annuus* and *Brassica juncea* in polluted and unpolluted soils are captured in **Figure *4.2***, **Figure *4.3*** and **Figure *4.4*** above. The results showed a 48.6%, 92.2% and 34.6% decrease in height, laminar leaf area and number of leaves for *Helianthus annuus* in the 4.6% w/w SEO treated soils when compared to the 0% SEO soils. Similarly, a 91%, 97.6% and 72.7% reduction in height, laminar leaf area and number of leaves was observed for *Brassica juncea* in the 4.6% w/w SEO soils when compared to the 0% SEOsoils. These decreased in the studied growth parameters for both species were statistically significant at P= 1.0029E-07, 3.4911E-14 and 5.191E-07 for plant height, laminar leaf area and number of leaves respectively.

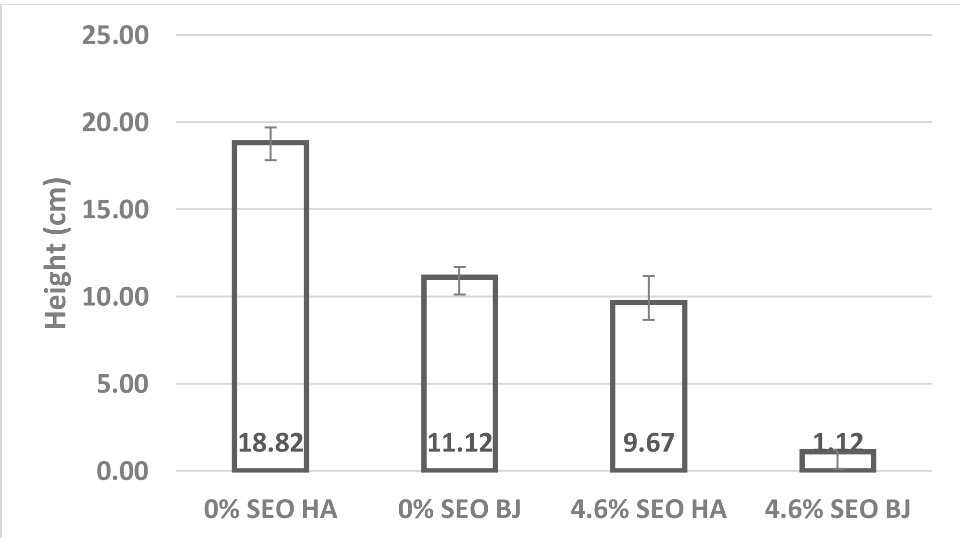

Figure 4.2. Effects of SEO on the Mean Height of *Helianthus annuus* and *Brassica juncea*

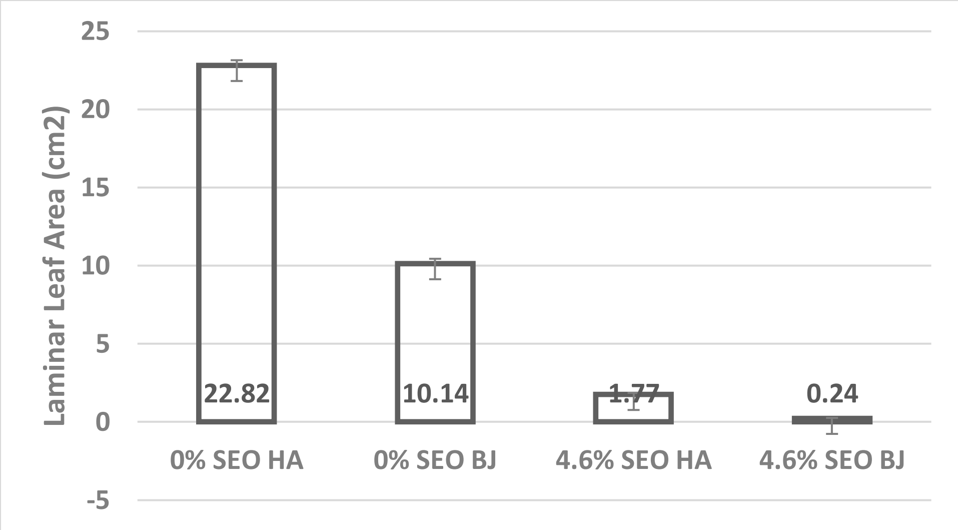

Figure 4.3 Effects of SEO on the Mean Laminar Leaf Area of *Helianthus annuus* and *Brassica juncea*

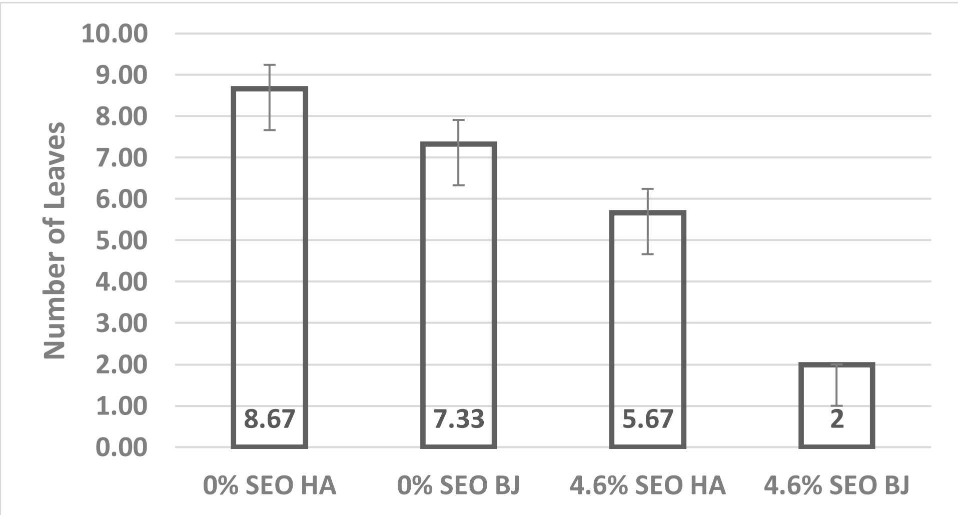

Figure 4.4 Effects of SEO on the Mean Number of Leaves of *Helianthus annuus* and *Brassica juncea*

4.4.1 Effects of Mixed Cropping on the Growth Parameters of *Helianthus annuus* and *Brassica juncea* in SEO Contaminated Soils

This experiment was aimed at investigating the potential for mixed cropping to improve the growth parameters of *Helianthus annuus* and *Brassica juncea* in soils polluted with SEO.

The mean growth parameters of *Helianthus annuus* and *Brassica juncea* in mixed and mono-cropping treatments in 0% SEO and 4.6% SEO soils are summarized in **Figure *4.5***, **Figure *4.6***, and **Figure *4.7*** below (see **Table *4.1*** for abbreviations). The results showed that for *Helianthus annuus*, the plant height was significantly higher (p=0.0005) by 8.2% and 47.2% with mixed cropping in 0% SEO and 4.6% SEO w/w polluted treatments respectively when compared to mono-cropping treatments. Laminar leaf area was significantly higher (p=1.6523E-06) by 53.4% and 13.6% in mono-cropping treatments in 0% SEO and 4.6% SEO w/w treatments respectively when compared to mixed cropping treatments. However, there were no notable difference in the number of leaves between mixed cropping and unmixed cropping treatments.

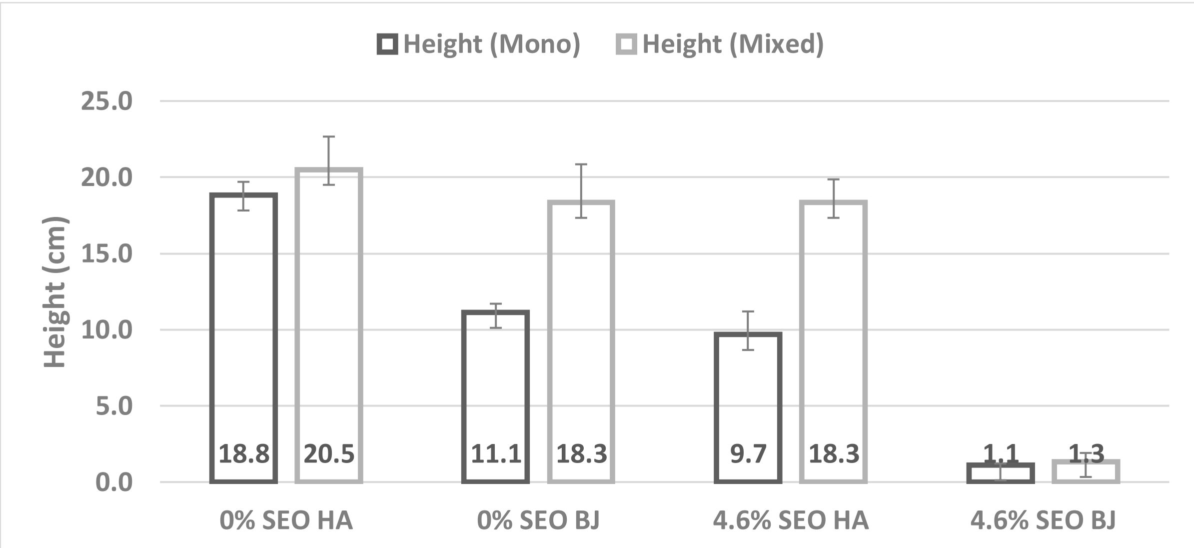

Figure 4.5. Effects of SEO on the Mean Height of *Helianthus annuus* and *Brassica juncea* in Mixed and Mono Cropping Treatments

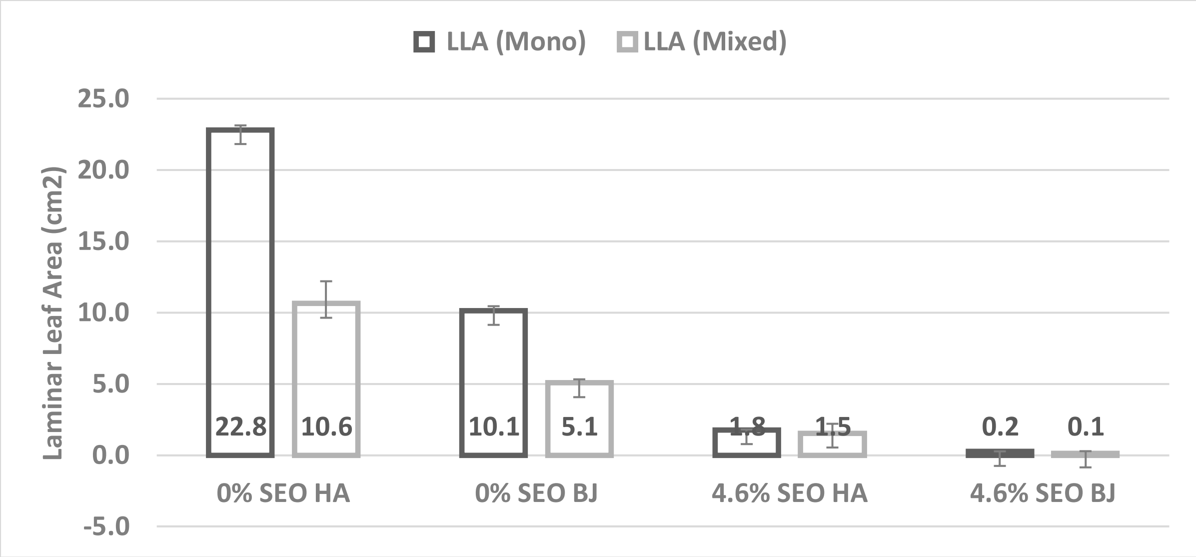

Figure 4.6 Effects of SEO on the Mean Laminar Leaf Area of *Helianthus annuus* and *Brassica juncea* in Mixed and Mono cropping Treatments

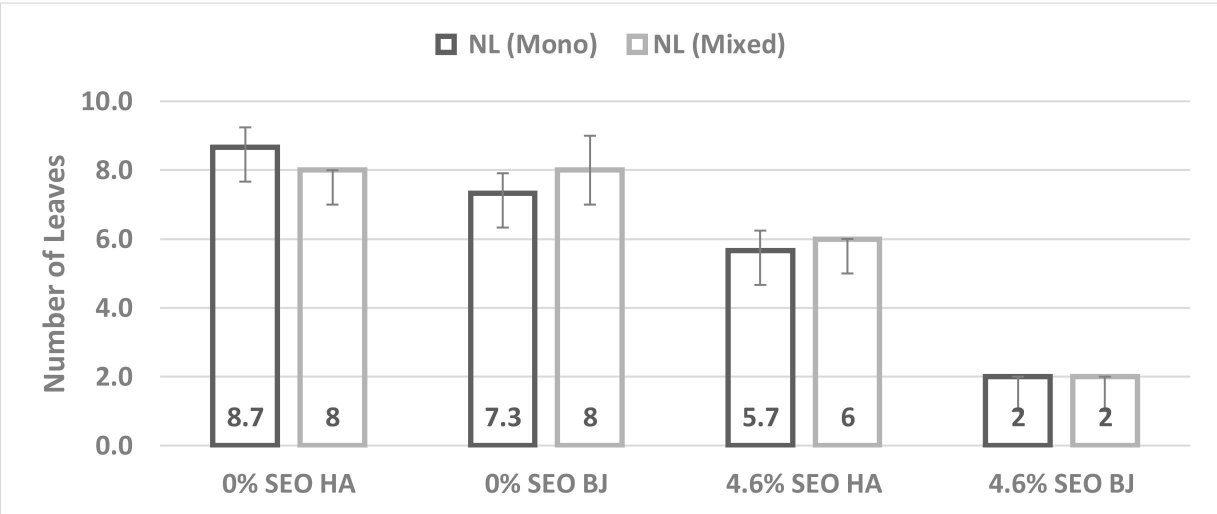

Figure 4.7Effects of SEO on the Mean Number of Leaves of *Helianthus annuus* and *Brassica juncea* in Mixed and Mono cropping Treatments

In the case of *Brassica juncea*, plant height was significantly higher (p=0.0013) by 39.3% and 15.4% in mixed cropping treatments at 0% SEO and 4.6% SEO w/w respectively when compared to mono-cropping treatments. Laminar leaf area was significantly lower (p=2.5791E-08) by 49.5% and 50% in mixed cropping treatments at 0% SEO and 4.6% SEO w/w respectively when compared with mono-cropping treatments. However, there was no notable difference in the number of leaves between mixed and mono-cropping treatments in polluted and unpolluted soils.

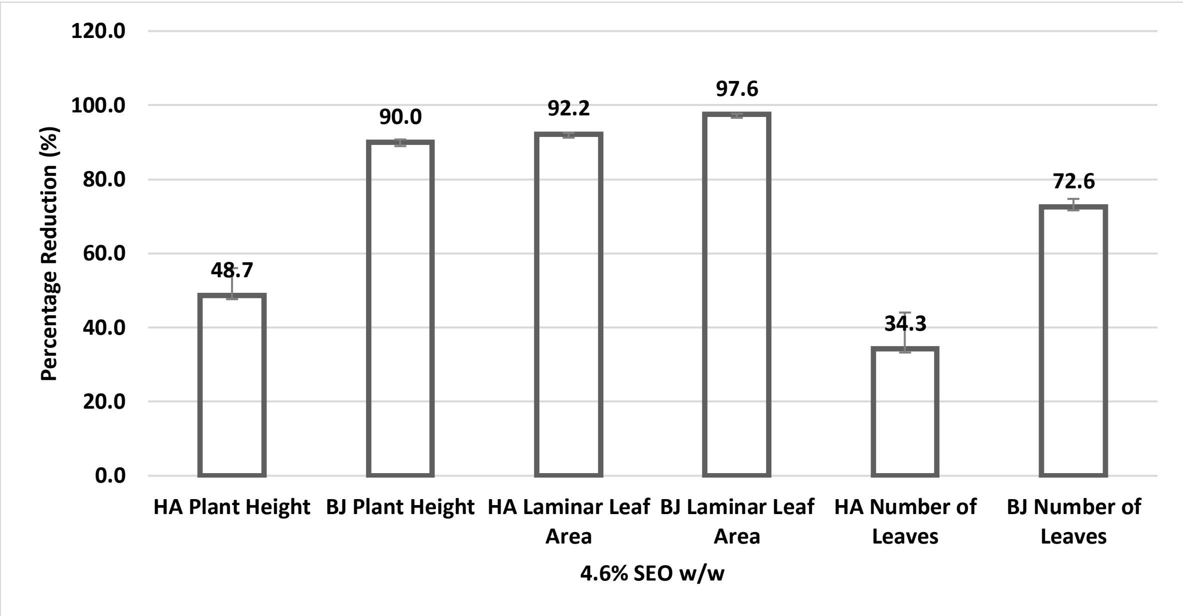

Figure 4.8. Percentage Reduction Mean Growth Parameters *Helianthus annuus* and *Brassica juncea* in Mixed Cropping at 4.6% SEO

The percentage reduction in mean growth parameters of *Helianthus annuus* and *Brassica juncea* are summarized in **Figure *4.8*** above. For all the growth parameters, the percentage reduction in mean growth parameters were generally higher in *Brassica juncea* when compared to *helianthus* annus especially in terms of plant height and number of leaves.

4.5 Effects of SEO and Mine-Spoils Co-Contamination on the Growth Parameters of *Helianthus annuus* and *Brassica juncea*

This experiment aimed to investigate the effects of SEO and mine-spoils co-contamination on the growth parameters of *Helianthus annuus* and *Brassica juncea*. This was done in line with the third objective of the study which was to determine the effect of mine-spoils and SEO co-contamination on the growth parameters of *Helianthus annuus* and *Brassica juncea*. The SEO concentrations were adjusted based on the outcome of the earlier experiment (*See section **4.4***) in which the SEO concentrations used proved to be too toxic to support plant growth especially at 9.2% SEO w/w so this experiment used lower SEO concentrations at 0.8% and 1.6% w/w respectively.

The plant growth parameters examined include plant height, number of leaves and laminar leaf area. The effect of SEO and Pb co-contamination on the dry biomass of both plants was also covered in this experiment.

A visual observation of the effect of SEO and mine-spoils co-contamination on *Helianthus annuus* and *Brassica juncea* can be seen in **Figure *4.9*** and **Figure *4.10*** below. As seen in the images below, a marked decrease in plant growth was observed with the addition of 0.8% SEO + 10% mine-spoils. The addition of an extra 0.8% SEO to the co-contamination can be seen to exert a marked further reduction in the growth of both species as can be seen in the pots on the far right.

Figure 4.9. Effects of SEO and Mine-Spoils Co-Contamination on the Growth of *Helianthus annuus* (left to right shows pots with 0% pollution, 0.8% SEO + 10% Mine-Spoils and 1.6% SEO + 10% Mine-Spoils respectively)

Figure 4.10. Effects of SEO and Mine-Spoils Co-Contamination on the Growth of *Brassica juncea* (left to right shows pots with 0% pollution, 0.8% SEO + 10% Mine-Spoils and 1.6% SEO + 10% Mine-Spoils respectively).

The results of the effects of SEO and mine-spoils co-contamination on all the growth parameters studied for are summarized in **Figure *4.11***, **Figure *4.12***, and **Figure *4.13*** below. The results for *Helianthus annuus* showed an observed reduction of up to 30.9%, 23.3% and 63.8% for plant height, number of leaves and laminar leaf area respectively when compared to the unpolluted treatments at a co-contaminant concentration of 0.8% SEO + 10% mine-spoils. Doubling the SEO concentration in the co-contamination led to reductions reaching 69.5%, 40% and 92.4% for plant height, number of leaves and laminar leaf area respectively when compared to the unpolluted counterparts.

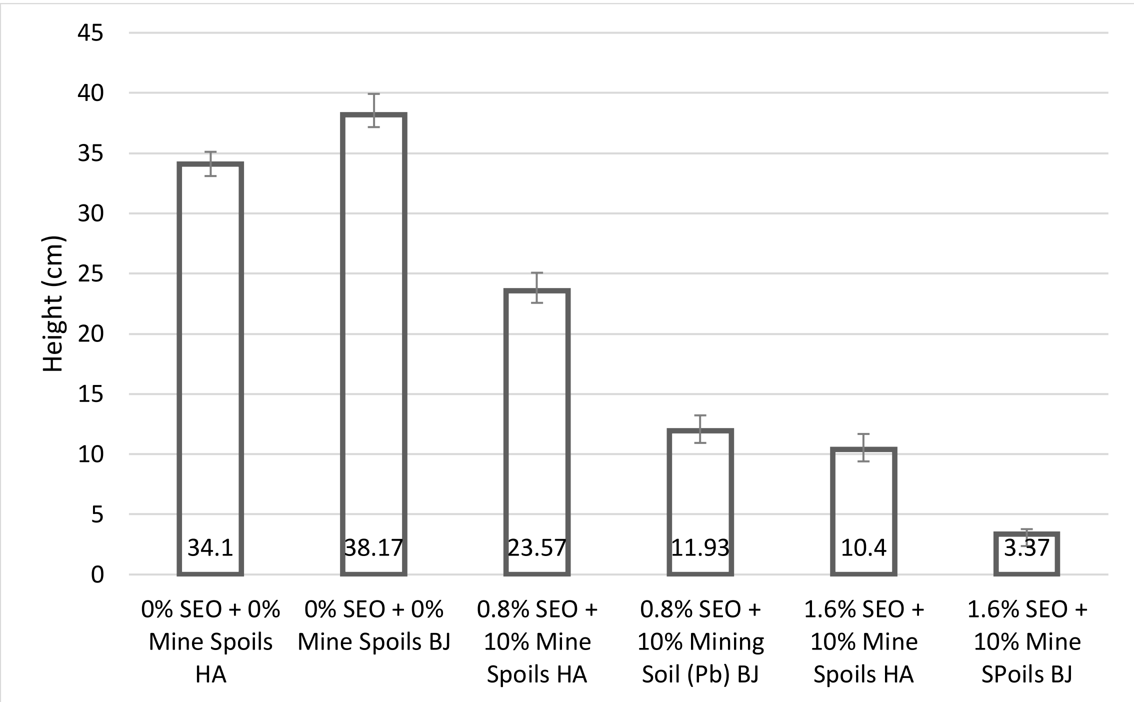

Figure 4.11. Effects of SEO and Mine-Spoils Co-Contamination on the Mean Height of *Helianthus annuus* and *Brassica juncea*

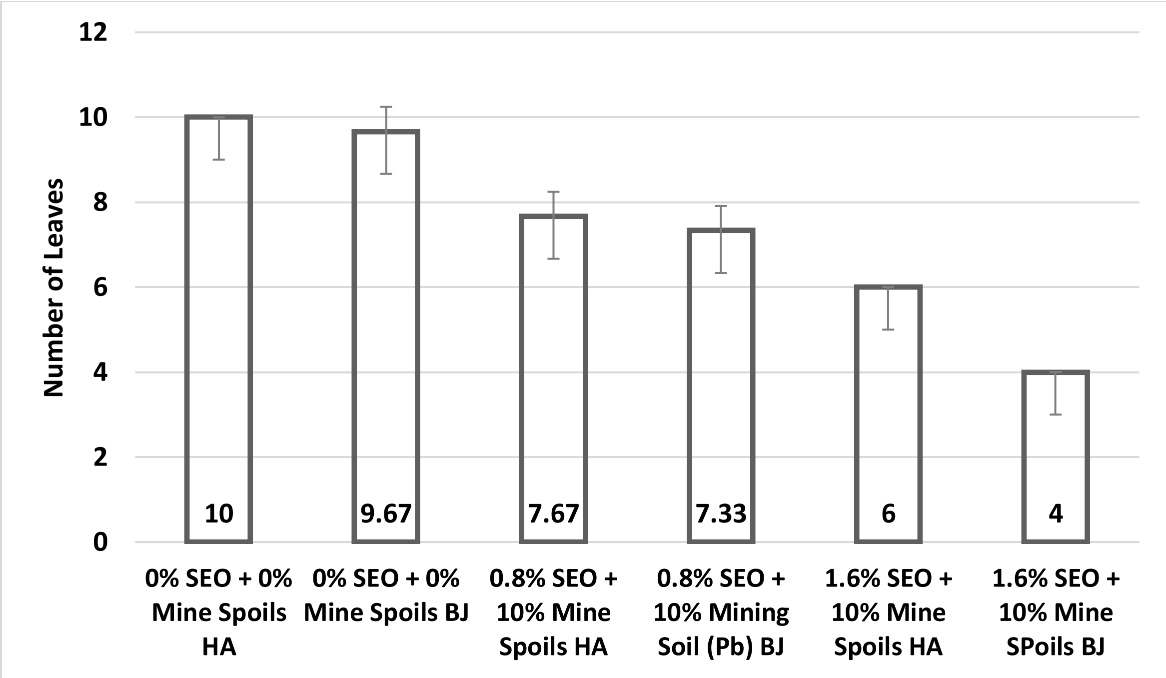

Figure 4.12 The Effects of SEO and Mine-Spoils Co-Contamination on the Mean Number of Leaves of *Helianthus annuus* and *Brassica juncea*

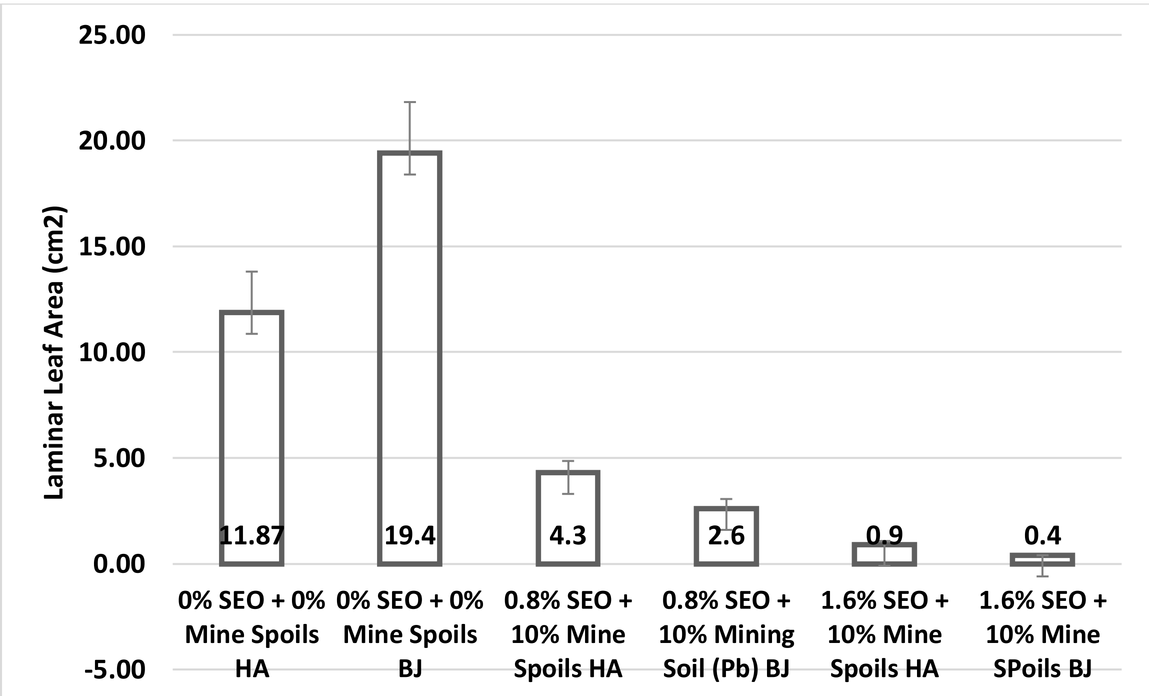

Figure 4.13 Effects of SEO and Mine-Spoils Co-Contamination on the Mean Laminar Leaf Area of *Helianthus annuus* and *Brassica juncea*

Similar trends were observed for *Brassica juncea* with 68.7%, 23.8% and 86.6 for plant height, number of leaves and laminar leaf area respectively when compared to the unpolluted treatments at a co-contaminant concentration of 0.8% SEO + 10% mine-spoils. Doubling the SEO concentration in the co-contamination led to reductions reaching 91.1%, 58.6% and 97.9% for plant height, number of leaves and laminar leaf area respectively when compared to the unpolluted counterparts.

The reduction in growth parameters for both species were statistically significant at p=1.698E-13, p=4.1156E-10 and p=3.159E-10 for plant height, number of leaves and laminar leaf area respectively.

4.5.1 Differences Between the Reduction in Growth Parameters of *Helianthus annuus* and *Brassica juncea* in SEO and Mine-Spoils Co-Contaminated Soils

The differences between the mean percentage reduction in the growth parameters of *Helianthus annuus* and *Brassica juncea* are displayed in **Figure *4.14*** below. For all the growth parameters studied, *Brassica juncea* showed a higher percentage reduction under SEO and mine-spoils co-contamination stress. This demonstrated that *Brassica juncea* was significantly more affected with the contamination than *Helianthus annuus* (p=3.999E-08, p=0.002 and p=0.0015 for height, number of leaves and laminar leaf area respectively).

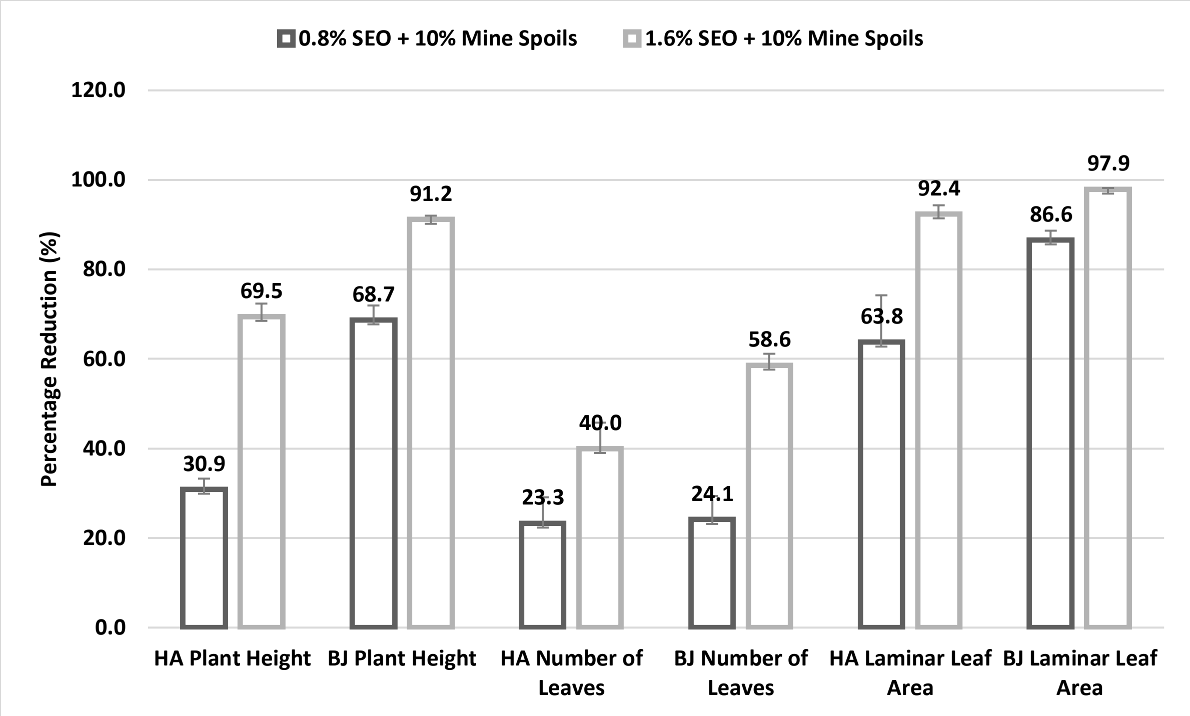

Figure 4.14. Mean Percentage Reduction Growth Parameters for *Helianthus annuus* and *Brassica juncea* in SEO and Mine-Spoils Co-Contaminated Soils

4.5.2 Effects of SEO and Mine-Spoils Co-Contamination on the Dry Biomass of *Helianthus annuus* and *Brassica juncea*

The effects of SEO and Pb co-contamination on the mean dry biomass of *Helianthus annuus* and *Brassica juncea* are summarized in **Figure *4.15*** below. Mean dry biomass of both species significantly declined (p=3.322E-11) in co-contaminated soils with reductions reaching 55.2% and 70.3% for *Helianthus annuus* and *Brassica juncea* respectively at 0.8% SEO + 10% mine-spoils co-contamination level when compared with the unpolluted treatments. A more severe reduction in dry biomass reaching 87.6% and 94.6% for *Helianthus annuus* and *Brassica juncea* respectively were recorded at 1.6% SEO + 10% mine-spoils when compared with the unpolluted treatments. For both plant species, a marked decline was observed at 0.8% SEO + 10% mine-spoils when compared with the unpolluted treatments. When comparing mean dry biomass for both species, *Helianthus annuus* mean dry biomass was significantly higher (p=6.5828E-10) than *Brassica juncea* in polluted and unpolluted treatments.

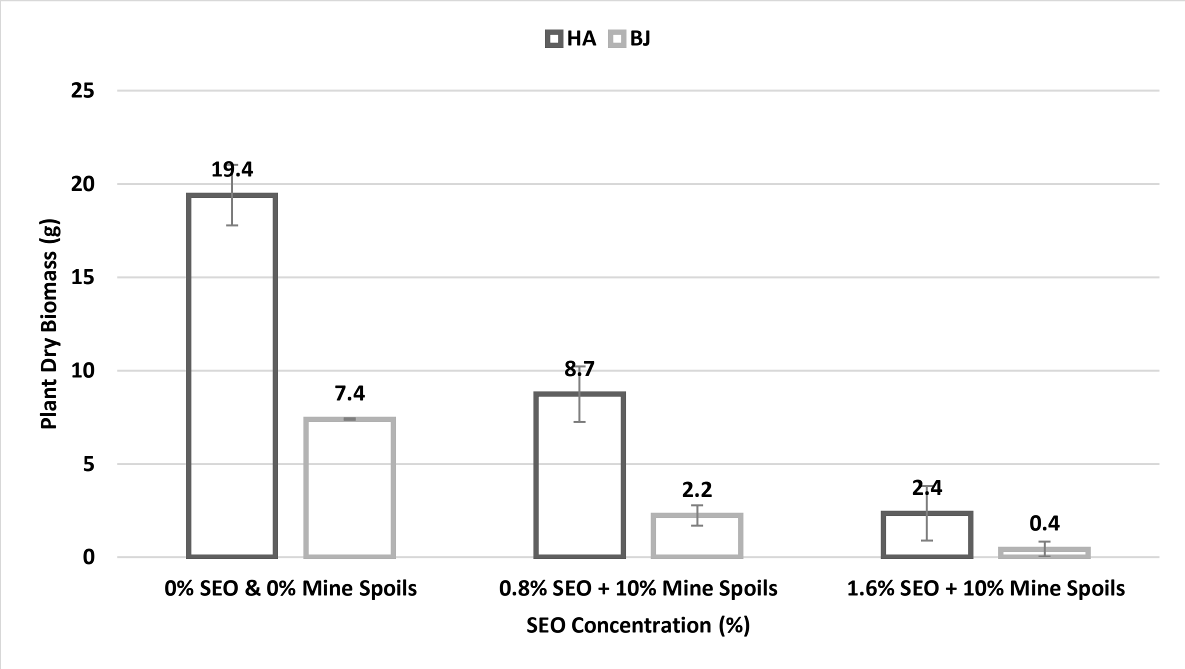

Figure 4.15. Effects of SEO and Mine Spoils Co-Contamination on Mean Dry Biomass of *Helianthus annuus* and *Brassica juncea*

4.6 Effects of Struvite Amendment on the Growth Parameters of *Helianthus annuus* and *Brassica juncea* in SEO and Mine-Spoils Co-Contaminated Soils.

This experiment was aimed at investigating the potential for using struvite to improve on the growth parameters of *Helianthus annuus* and *Brassica juncea* in soils co-contaminated with SEO concentrations and mine-spoils. This was in line with the sixth objective of the study which sought to evaluate and compare the potential for an industrial waste (struvite) in enhancing the growth and phytoremediation abilities of both species in comparison with NPK fertilizer. The plant growth parameters examined include plant height, number of leaves and laminar leaf area. The effect of struvite amendment on the dry biomass of both plants was also covered in this experiment.

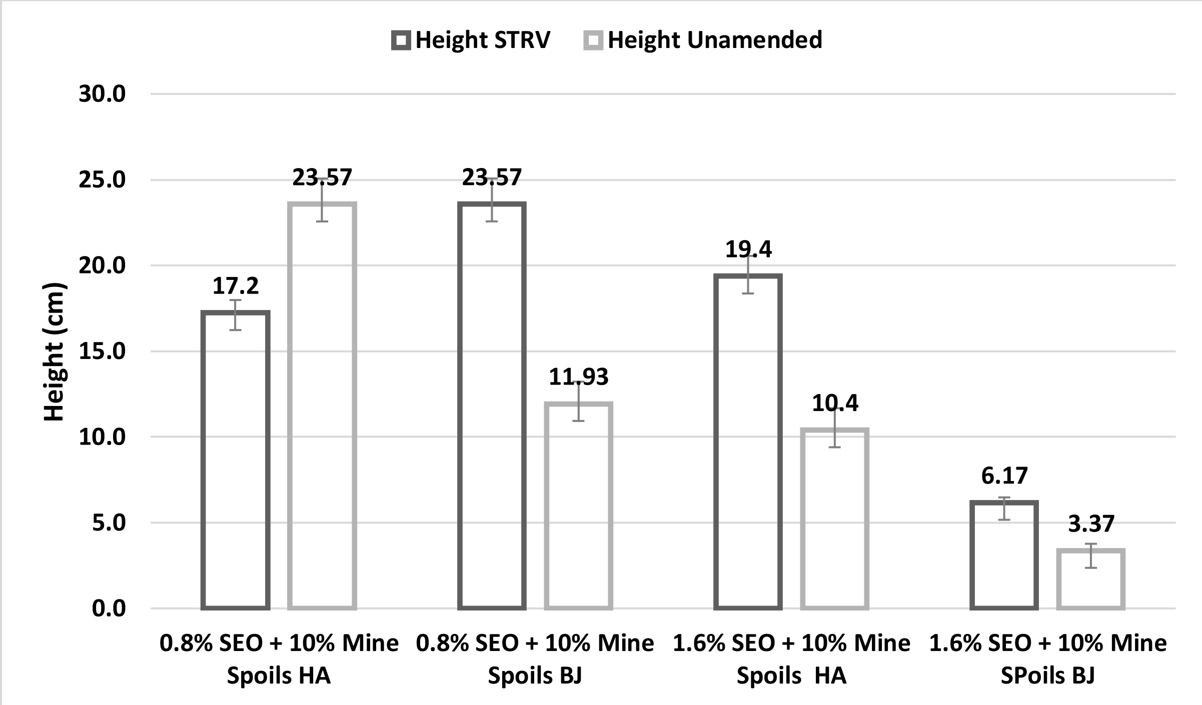

Figure 4.16. Effects of Struvite Supplementation on the Mean Height of *Helianthus annuus* and *Brassica jucea* in SEO and Mine Spoils Co-Contaminated Soils

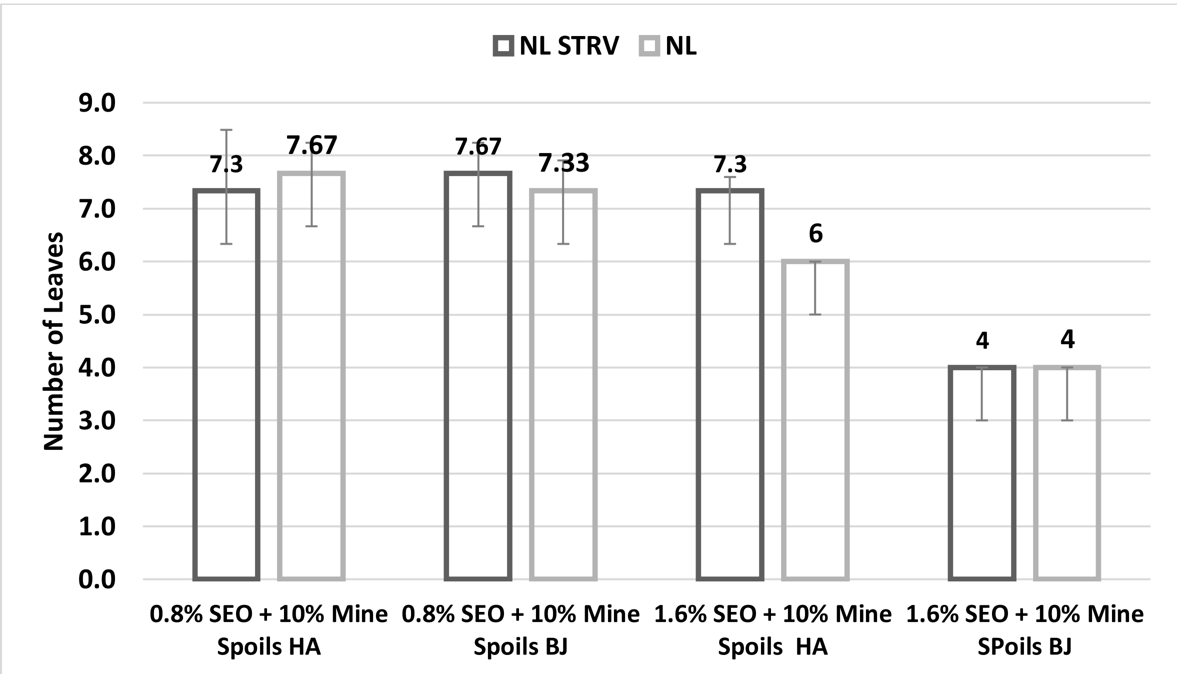

Figure 4.17 Effects of Struvite Supplementation on the Mean Number of Leaves of *Helianthus annuus* and *Brassica jucea* in SEO and Mine Spoils Co-Contaminated Soils

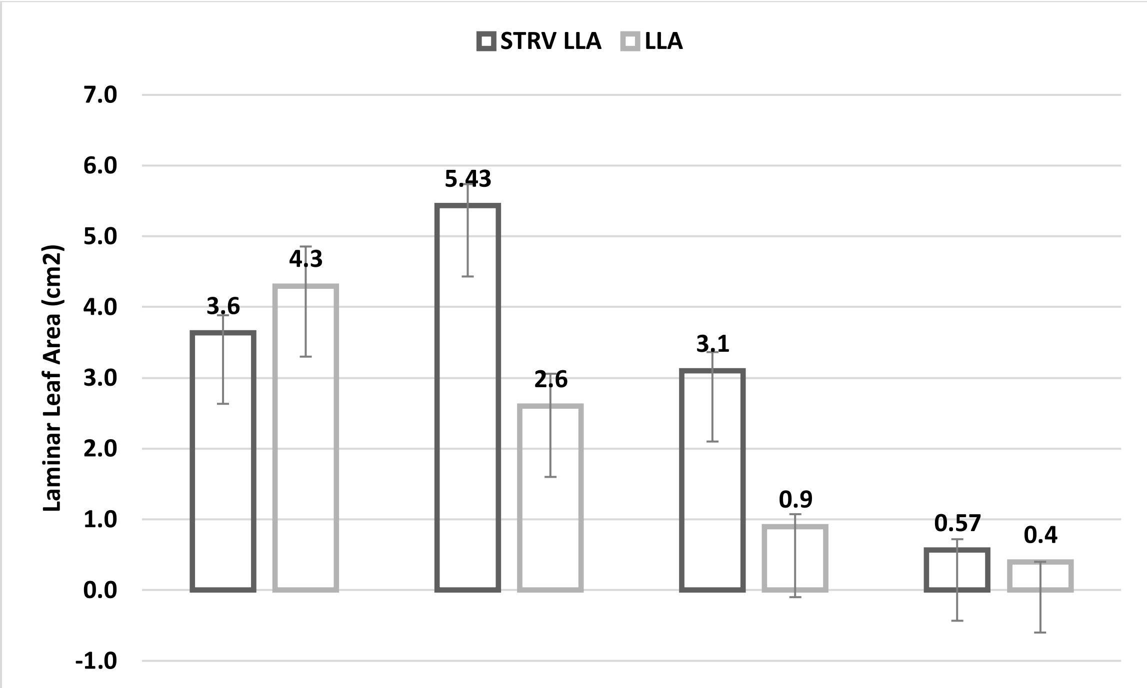

Figure 4.18 Effects of Struvite Supplementation on the Mean Laminar Leaf Area of *Helianthus annuus* and *Brassica jucea* in SEO and Mine Spoils Co-Contaminated Soils

The effects of struvite amendment on the mean growth parameters of *Helianthus annuus* and *Brassica juncea* are captured in **Figure *4.16***, **Figure *4.17***, and **Figure *4.18*** above (see **Table *4.1*** for abbreviations). As seen above, struvite amendments had a mix of positive and negative effects on the growth parameters. For *Helianthus annuus*, struvite amendment reduced plant height, number of leaves and laminar leaf area by 27%, 4.8% and 16.3% respectively at 0.8% SEO + 10% mine-spoils while it increased plant height, number of leaves and laminar leaf area by 46.4%, 17.8% and 71% respectively at 1.6% SEO + 10% mine-spoils when compared with unamended treatments. The effects of struvite on the growth parameters of *Helianthus annuus* at both contaminant levels was statistically significant only for laminar leaf area (p=0.005). For *Brassica juncea*, plant height and laminar leaf area were significantly higher in struvite treatments (p=1.8634E-06 and p=1.7204E-05 respectively) by 49.4% and 52.1%

respectively at 0.8% SEO + 10% mine-spoils and by 45.4% and 17.5% respectively at 1.6% SEO + 10% mine-spoils when compared to the unamended treatments. There were no significant differences in number of leaves between struvite amended and unamended treatments.

4.6.1 The Effects of Struvite Amendment on the Dry Biomass of *Helianthus annuus* and *Brassica juncea* in SEO and **Mine-Spoils** Co-Contaminated Soils

The mean dry biomass of *Helianthus annuus* in struvite amended and unamended treatments are summarized in **Figure *4.19*** below. A 6.9% reduction in dry biomass was observed at 0.8% SEO + 10% mine-spoils in the struvite amended soils when compared to the unamended treatments. However, dry biomass was significantly higher (p=0.002) in struvite amended treatments by up to 68% at 1.6% SEO + 10% mine-spoils when compared with the unamended treatments.

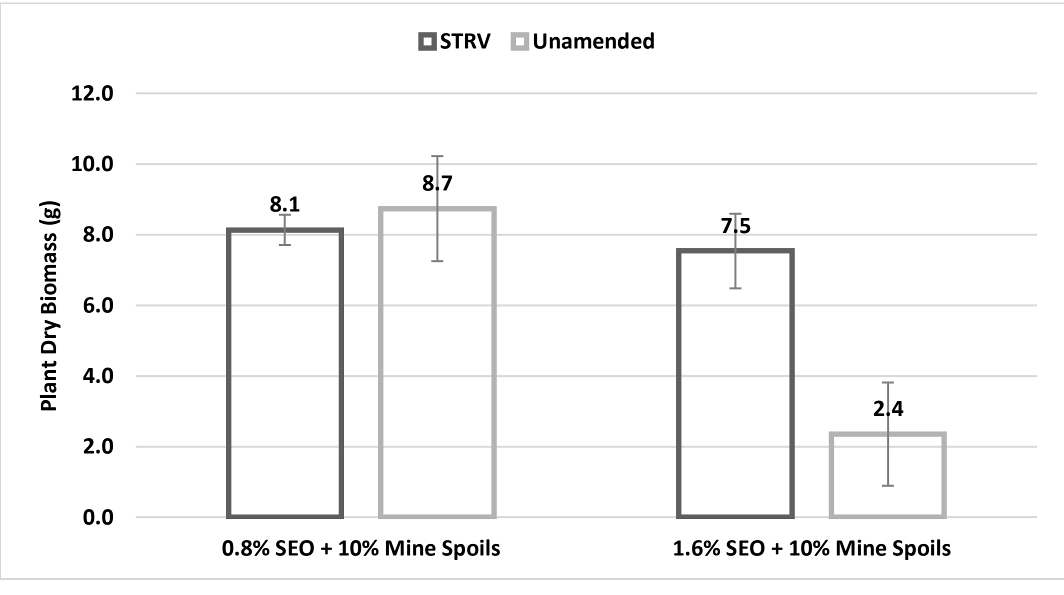

Figure 4.19. Mean Dry Biomass of *Helianthus annuus* in Struvite Amended and Unamended Soils

The mean dry biomass of *Brassica juncea* in struvite amended and unamended treatments are summarized in **Figure *4.20*** below. It was observed that the mean plant dry biomass was significantly higher (p=0.00078) in struvite amended soils when compared to the unamended treatments especially at 0.8% SEO + 10% mine-spoils where the dry biomass was 51.1% higher in struvite amended treatments when compared to the unamended treatments.

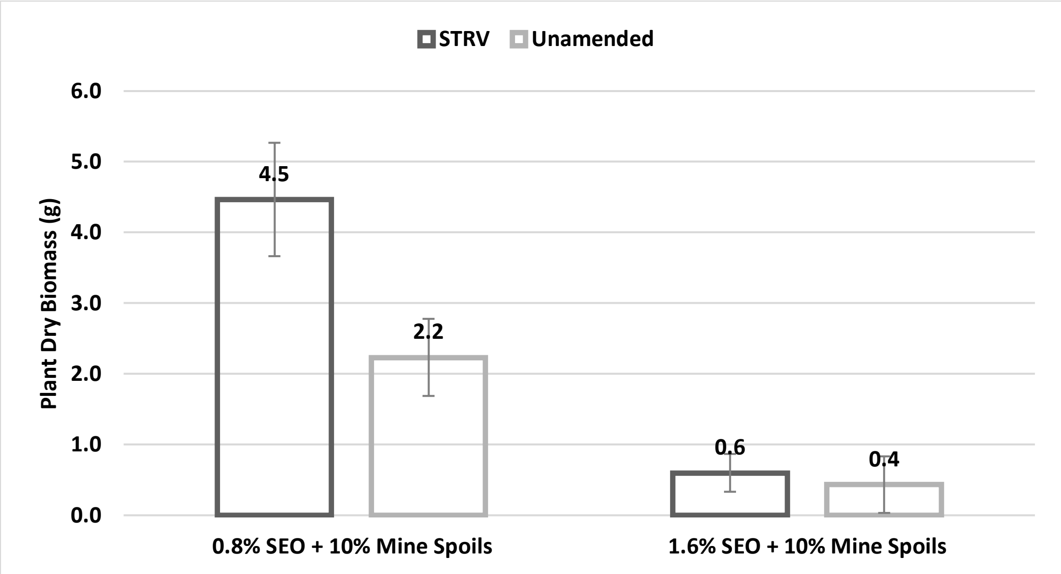

Figure 4.20. Mean Dry Biomass of *Brassica juncea* in Struvite Amended and Unamended Soils

4.6.2 Comparing the Effects of Struvite and NPK Fertilizer Amendments on the Growth Parameters of *Helianthus annuus* and *Brassica juncea* in SEO and **Mine-Spoils** Co-Contaminated Soils.

The mean growth parameters of *Helianthus annuus* amended with struvite and NPK fertilizer in SEO and mine-spoils co-contaminated soils are shown in **Figure *4.21***, **Figure *4.22***, and **Figure *4.23*** below (see **Table *4.1*** for abbreviations). The results showed mixed responses of various growth parameters at the two contaminant levels. For *Helianthus annuus*, plant height was 9.5% higher in NPK treatments compared to struvite treatments at 0.8% SEO + 10% mine-spoils, whereas plant height was 11.3% higher in struvite treatments when compared to NPK treatments at 1.6% SEO + 10% mine-spoils. Laminar leaf area was 64.7% higher in NPK treatments compared to struvite treatments at 0.8% SEO + 10% mine-spoils whereas struvite treatments showed 6.5% higher laminar leaf area compared to NPK treatments at 1.6% SEO + 10% mine-spoils. These differences were only statistically significant in the case of laminar leaf area (p=3.2026E-08).

For *Brassica juncea*, the results showed that all growth parameters were notably higher in the NPK treatments by 46.7%, 14.8% and 15.2% for plant height, number of leaves and laminar leaf area respectively at 0.8% SEO + 10% mine-spoils and 84.3%, 39.4% and 95.6% for plant height, number of leaves and laminar leaf area respectively at 1.6% SEO + 10% mine-spoils when compared with the struvite treatments. These were all statistically significant at p=8.8761E-07, p=1.0836E-08 and p=0.0028 for plant height, laminar leaf area and number of leaves respectively.

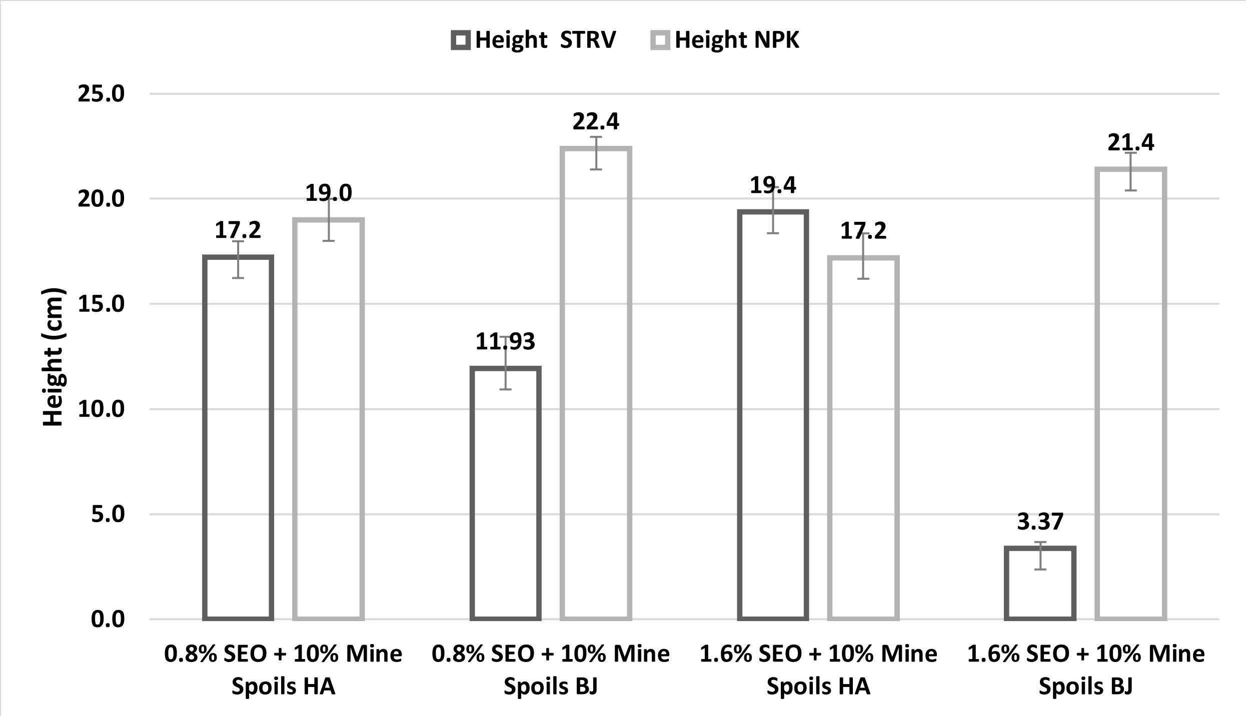

Figure 4.21. Mean Height of *Helianthus annuus* and *Brassica juncea* in Struvite and NPK Amended Soils Co-Contaminated with SEO and Mine Spoils

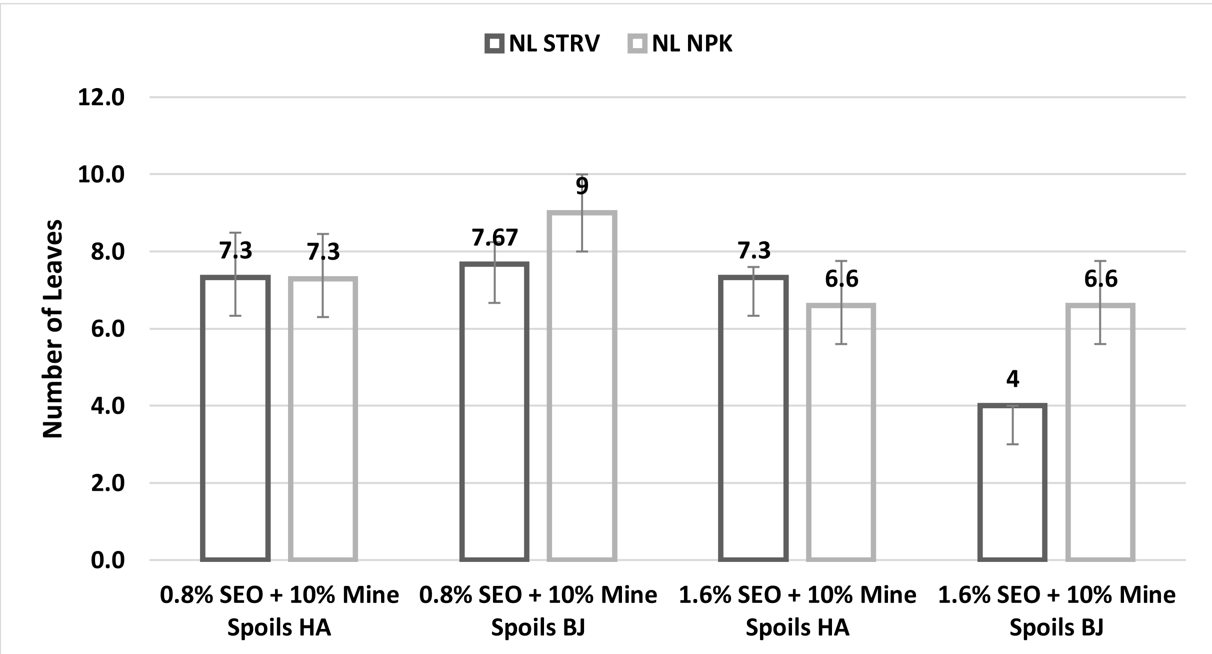

Figure 4.22 Mean Number of Leaves of *Helianthus annuus* and *Brassica juncea* in Struvite and NPK Amended Soils Co-Contaminated with SEO and Mine Spoils

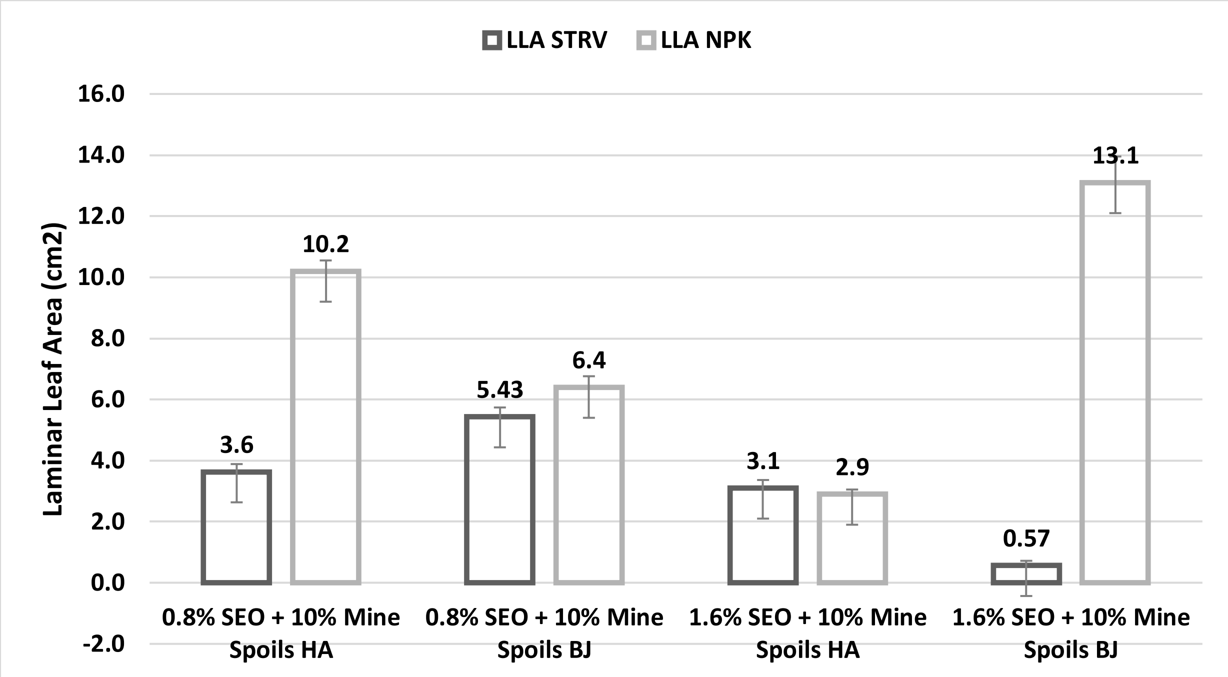

Figure 4.23 Mean Laminar Leaf Area of *Helianthus annuus* and *Brassica juncea* in Struvite and NPK Amended Soils Co-Contaminated with SEO and Mine Spoils

4.6.3 Comparing the Effects of Struvite and NPK Amendments on the Dry Biomass of *Helianthus annuus* and *Brassica juncea*.

The mean dry biomass of *Helianthus annuus* in struvite and NPK amended soils co-contaminated with SEO concentrations and mine-spoils are summarized in **Figure *4.24*** below. The mean dry biomass of *Helianthus annuus* was significantly higher (p=0.00027) by 12.5% and 25.3% at 0.8% SEO + 10% mine-spoils and 1.6% SEO + 10% mine-spoils respectively in struvite amended treatments when compared to NPK treatments.

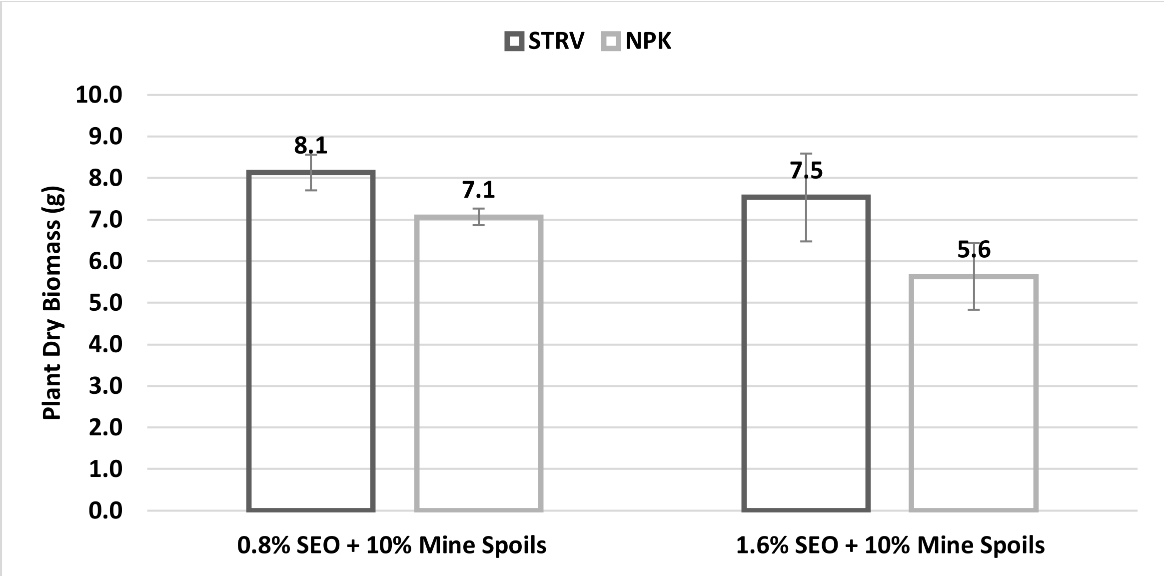

Figure 4.24. Mean Dry Biomass of *Helianthus annuus* in Struvite and NPK Amended Soils Co-Contaminated with SEO and Mine Spoils

The mean dry biomass of *Brassica juncea* in struvite and NPK amended soils co-contaminated with SEO and mine-spoils are shown in **Figure *4.25*** below. It was observed that mean dry biomass was 24.4% higher in struvite treatments at 0.8% SEO + 10% mine-spoils when compared to NPK treatments. On the other hand, mean dry biomass was 86.4% higher in NPK treatments at 1.6% SEO + 10% mine-spoils when compared with struvite treatments. The observed differences were statistically significant (p=9.7039E-05).

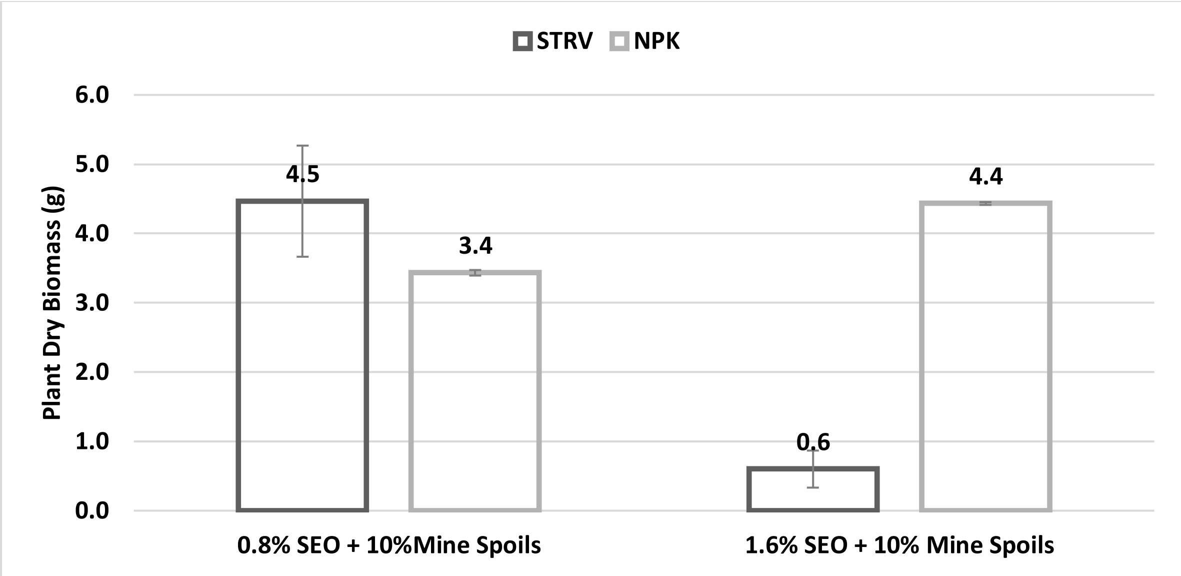

Figure 4.25. Mean Dry Biomass of *Brassica juncea* in Struvite and NPK Amended Soils Co-Contaminated with SEO and Mine Spoils

4.7 TPH Reduction in *Helianthus annuus* and *Brassica juncea* Soils Co-Contaminated with SEO and Mine-Spoils

This experiment aimed to investigate the potential for *Helianthus annuus* and *Brassica juncea* to reduce the TPH concentrations in soils co-contaminated with 0.8% SEO + 10% mine-spoils and 1.6% SEO + 10% mine-spoils w/w. This done was in line with the fourth objective of this study which was to investigate the abilities of *Helianthus annuus* and *Brassica juncea* to reduce TPH and PAH concentrations from SEO and mine-spoils co-contaminated soils.

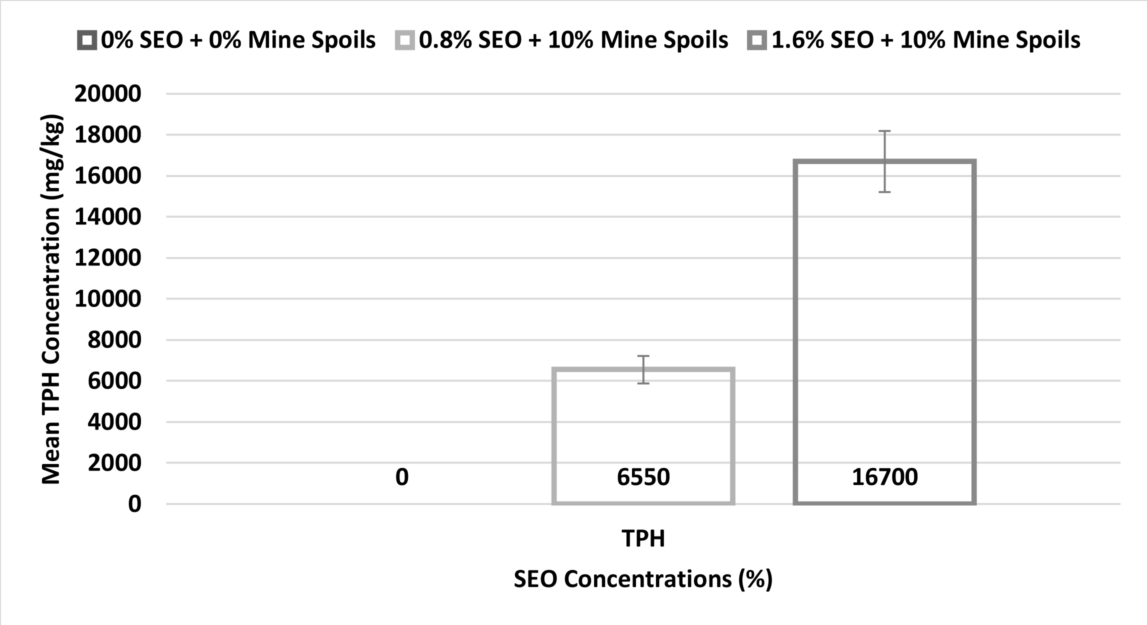

Figure 4.26. Mean TPH Concentrations in Soils Co-Contaminated with SEO and Mine Spoils before Planting with *Helianthus annuus* and *Brassica juncea* Soils

The mean concentrations of TPH in unpolluted soils and in soils co-contaminated with 0.8% SEO + 10% mine-spoils and 1.6% SEO + 10 mine-spoils are summarized in **Figure *4.26*** above. The TPH concentrations increased as the concentration of SEO increased in the SEO and mine-spoils co-contaminated soils.

4.7.1 Differences in TPH Reductions Between *Helianthus annuus*, *Brassica juncea* and Unplanted Soils Co-Contaminated with SEO and Mine-Spoils

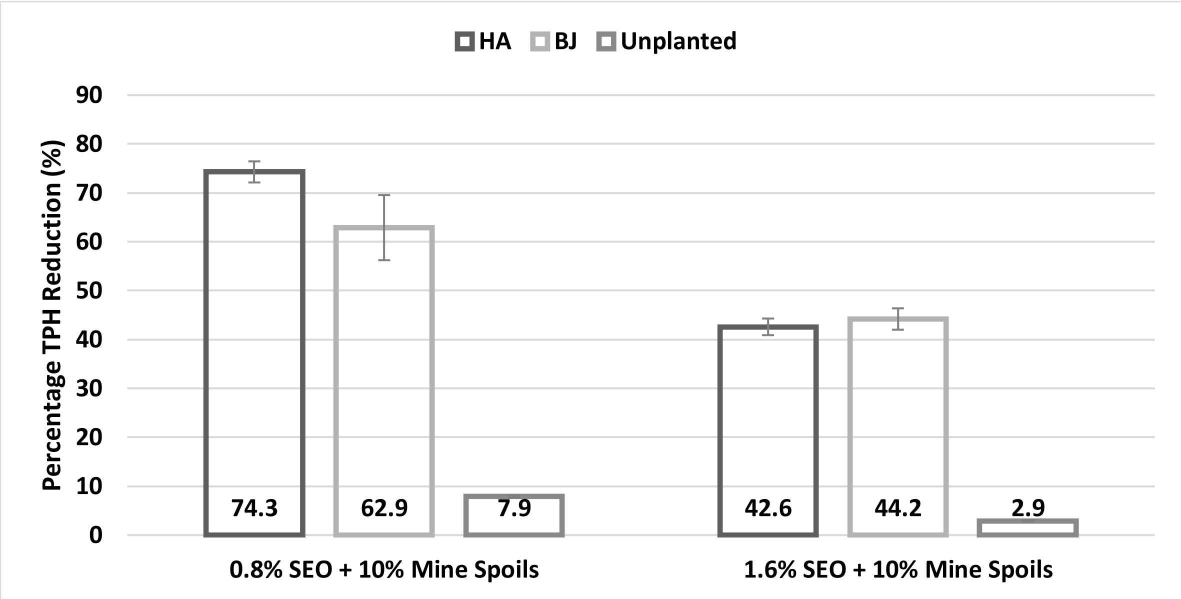

Figure 4.27. Mean Percentage TPH Reduction in *Helianthus annuus*, *Brassica juncea* & Unplanted Soils Co-Contaminated with SEO and Mine Spoils

The mean reduction in TPH concentration in *Helianthus annuus*, *Brassica juncea*, and unplanted soils co-contaminated with SEO and mine-spoils are summarized in **Figure *4.27*** above. Percentage TPH reduction was significantly higher (p=2.9071E-12) in both planted treatments when compared with the unplanted treatments with *Helianthus annuus* soils showing the highest percentage TPH reduction. It was also observed that for both planted and unplanted treatments, percentage TPH reduction reduced at 1.6% SEO + 10% mine-spoils when compared to 0.8% SEO + 10% mine-spoils.

When TPH reductions in *Helianthus annuus* and *Brassica juncea* soils were compared, TPH reductions were significantly higher (p=0.05) in *Helianthus annuus* planted soils.

4.8 Effects of Struvite Amendment on the Reduction of TPH in *Helianthus annuus* and *Brassica juncea* Soils Co-Contaminated with SEO and Mine-Spoils

This experiment aimed to investigate the potential for struvite amendment to increase the reduction of TPH *Helianthus annuus* and *Brassica juncea* soils co-contaminated with SEO concentrations and mine-spoils. It also compared the effectiveness of struvite with NPK fertilizer in line with the sixth objective of the study which was to evaluate and compare the potential for an industrial waste (struvite) in enhancing the growth and phytoremediation abilities of both species in comparison to NPK fertilizer.

The percentage TPH reduction in struvite amended and unamended soils planted with *Helianthus annuus* and *Brassica juncea* in SEO and mine-spoils co-contaminated soils are summarized in **Figure *4.28*** below.

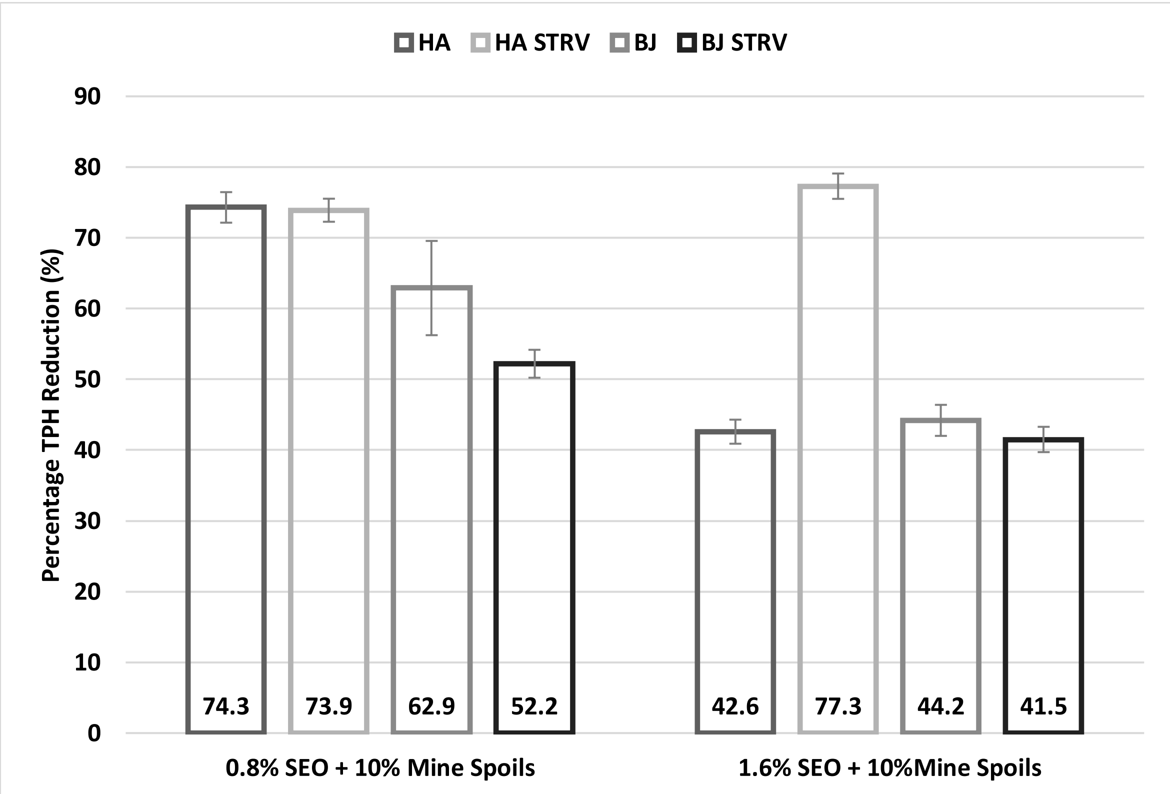

Figure 4.28. Percentage TPH Reduction in *Helianthus annuus* and *Brassica juncea* Soils with and without Struvite Amendment Soils

Struvite amendment had significantly higher (p=2.1113E-07) percentage TPH reduction of 34.7% for *Helianthus annuus* planted soils at 1.6% SEO + 10% mine-spoils when compared with the unamended counterpart, whereas percentage TPH reduction was significantly lower (p=0.02) for *Brassica juncea* with struvite amendment at both pollution levels.

4.8.1 Comparing the Effects of Struvite and NPK Fertilizer Amendments on the Reduction of TPH in *Helianthus annuus* and *Brassica juncea* Soils Co-Contaminated with SEO and Mine-Spoils

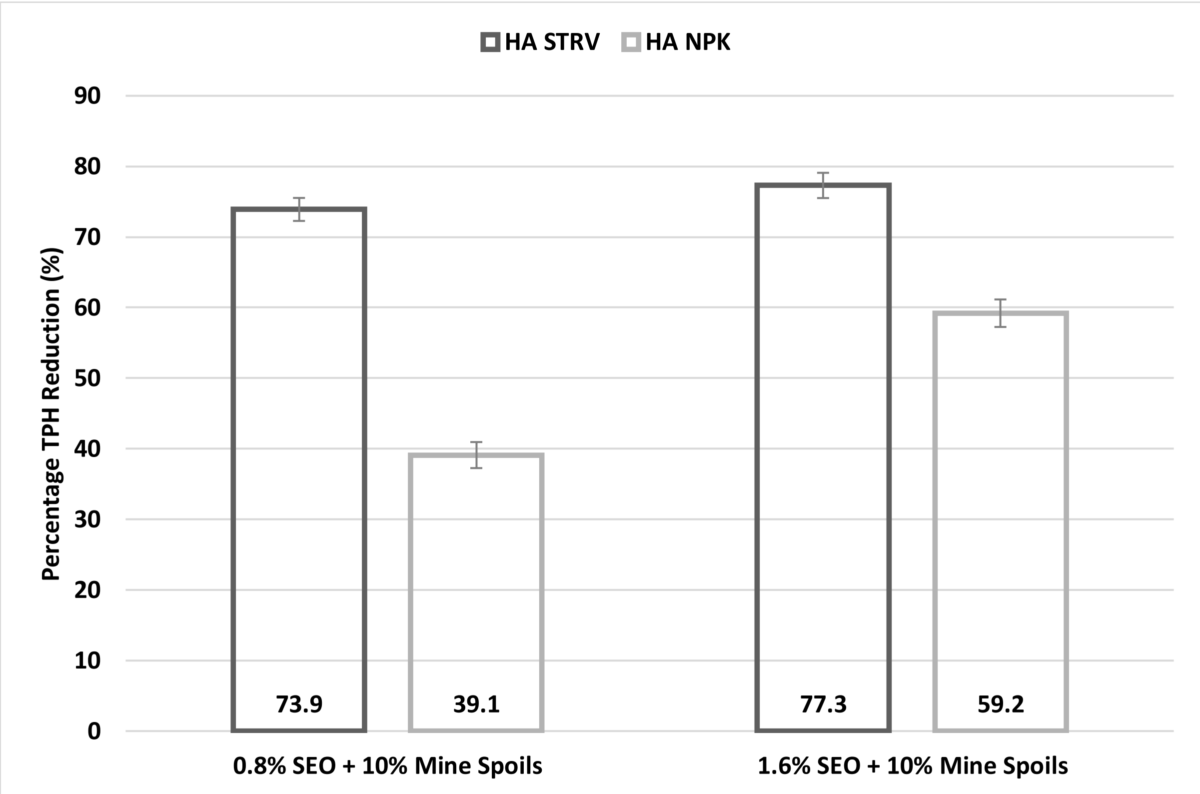

Figure 4.29. Mean Percentage Reduction of TPH in *Helianthus annuus* with Struvite and NPK Amendment in Soils Co-Contaminated with SEO and Mine Spoils

This experiment was aimed at comparing the efficacy of struvite and NPK fertilizer supplementation on the reduction of TPH concentrations by *Helianthus annuus* and *Brassica juncea* in SEO and mine-spoils co-contaminated soils. This was carried out in line with the sixth objective of the study which sought to evaluate and compare the potential for an industrial waste (struvite) in enhancing the growth and phytoremediation abilities of both species in comparison with NPK fertilizer. The mean percentage reduction of TPH in *Helianthus annuus* with struvite and NPK amendments in soils co-contaminated with SEO mine-spoils are summarized in **Figure *4.29*** above. It was observed that the percentage TPH reduction was significantly higher (p=6.4066E-09) by 34.8% and 18.1% for *Helianthus annuus* with struvite amendment when compared with the NPK amendment counterpart at 0.8% SEO + 10% mine-spoils and 1.6% SEO + 10% mine-spoils respectively.

The mean percentage reduction of TPH in *Brassica juncea* with struvite and NPK amendments in soils co-contaminated with SEO mine-spoils are summarized in **Figure *4.30*** below. As seen below, there was no notable differences in the percentage TPH reduction for both struvite and NPK treatments except at 1.6% SEO + 10% mine-spoils where the percentage TPH reduction was significantly lower (p=0.0005) by 11.2% in struvite treatments when compared with NPK treatments .

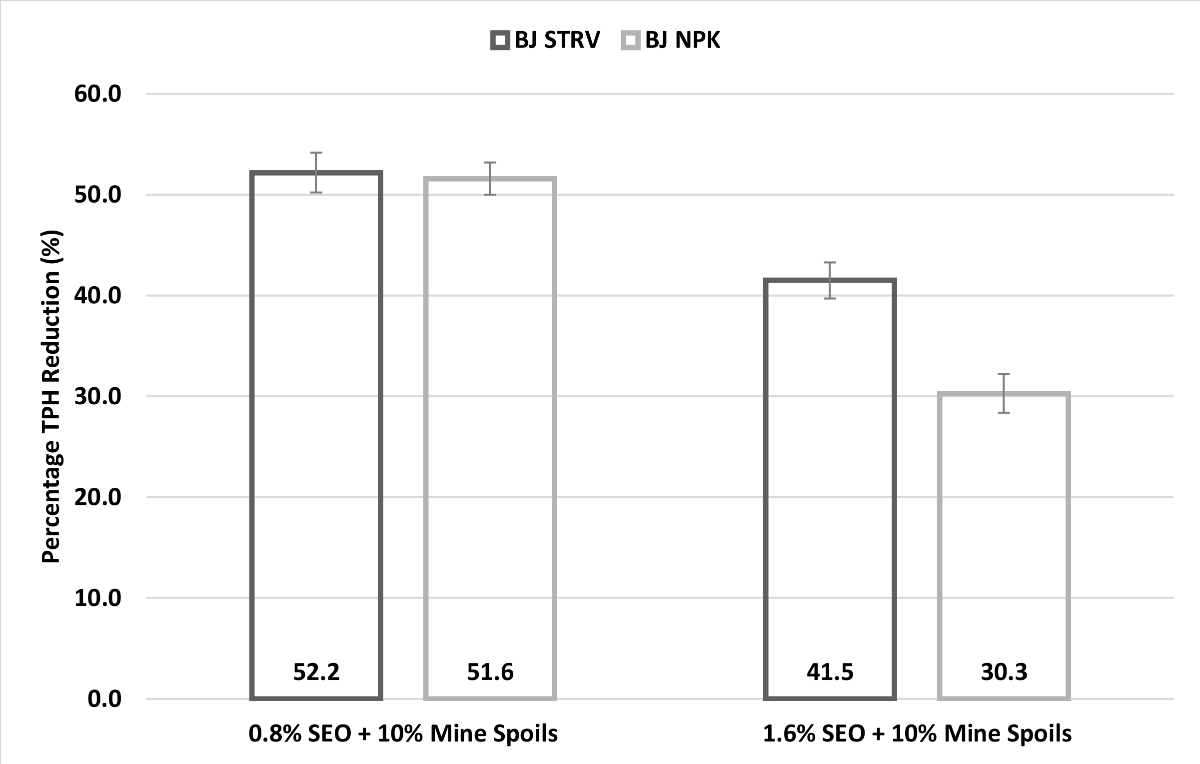

Figure 4.30. Mean Percentage Reduction of TPH in *Brassica juncea* with Struvite and NPK Amendment in Soils Co-Contaminated with SEO and Mine Spoils

4.9 Total PAHs Reduction in *Helianthus annuus* and *Brassica juncea* Soils Co-Contaminated with SEO and Mine-Spoils

This experiment aimed to investigate the potential for *Helianthus annuus* and *Brassica juncea* to reduce the Total PAHs concentrations in soils co-contaminated with 0.8% SEO + 10% mine-spoils and 1.6% SEO + 10% mine-spoils w/w. This was in line with the fourth objective of this study which was to investigate the abilities of *Helianthus annuus* and *Brassica juncea* to reduce TPH and PAH concentrations from SEO and mine-spoils co-contaminated soils.

The Mean Total PAH concentrations in SEO and Pb co-contaminated soils are summarized in **Figure *4.31Figure 4.31*** below. The Total PAHs concentrations increased as the concentration of SEO increased in the SEO and mine-spoils co-contaminated soils.

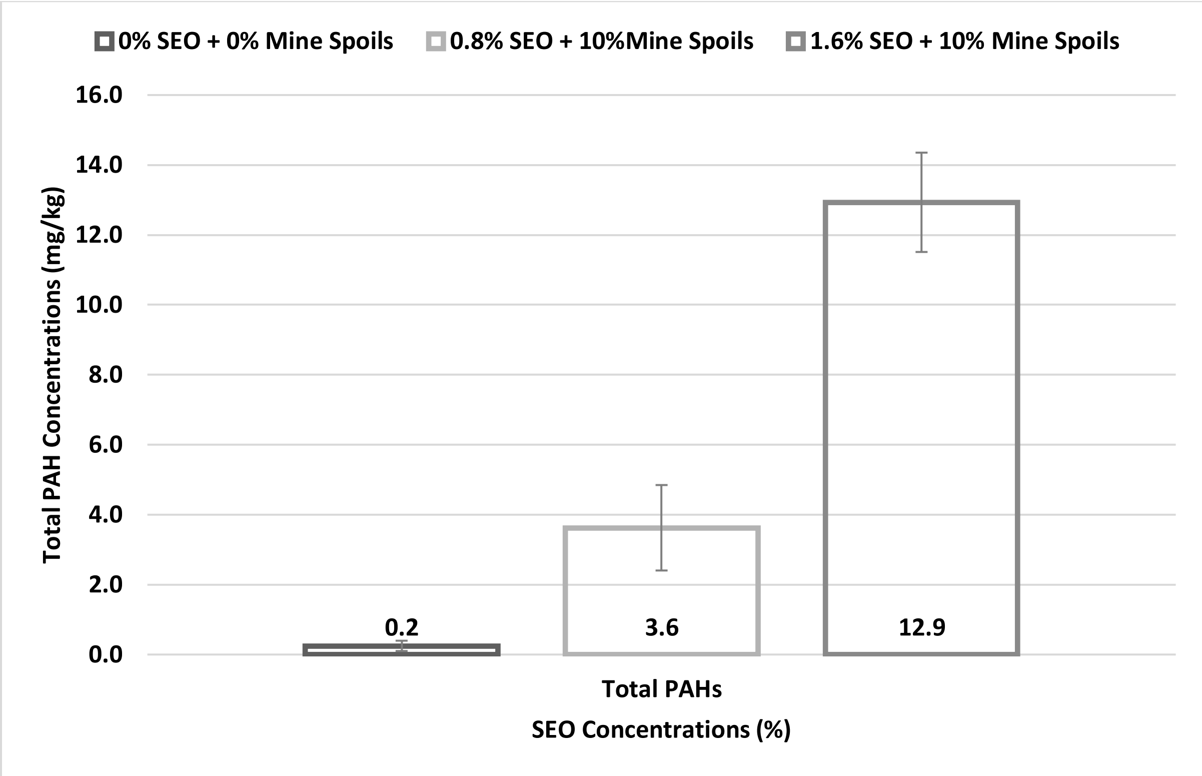

Figure 4.31. Mean Total PAH Concentrations in Soils Co-Contaminated with SEO and Mine Spoils before Planting

4.9.1 Differences in Total PAH Reductions Between *Helianthus annuus*, *Brassica juncea* and Unplanted Soils Co-Contaminated with SEO and Mine-Spoils

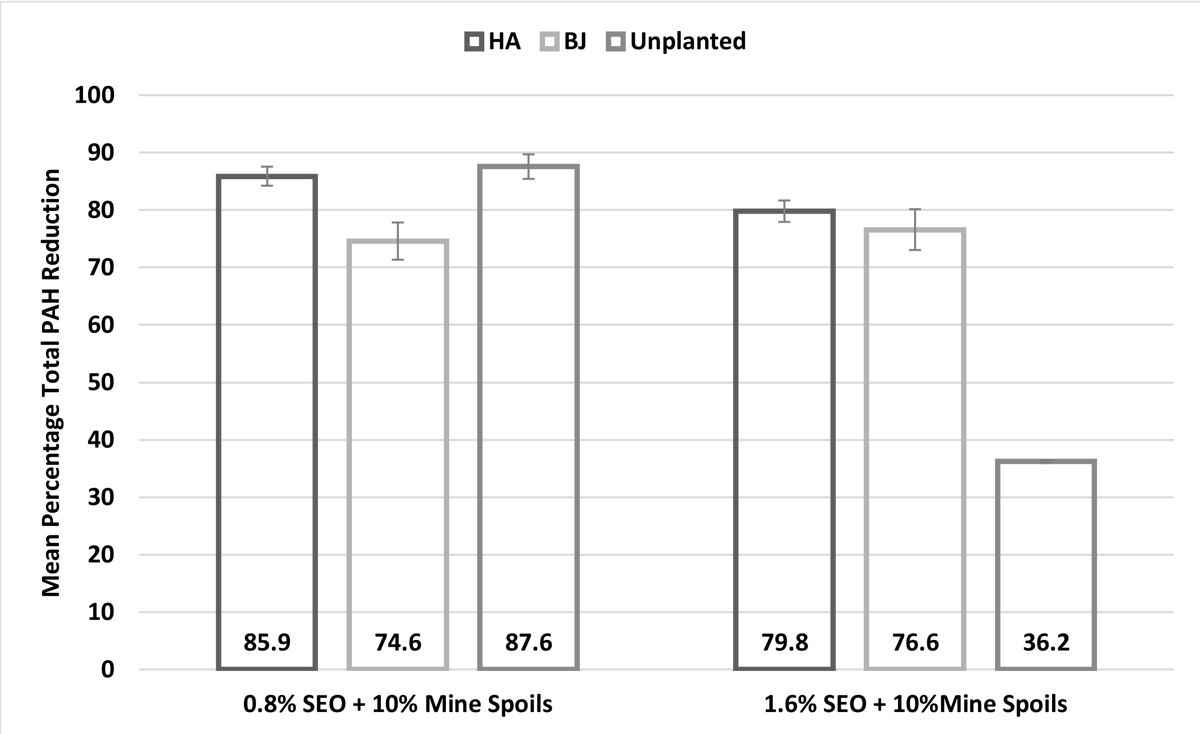

Figure 4.32. Mean Percentage Total PAH Reduction in *Helianthus annuus*, *Brassica juncea* & Unplanted Soils Co-Contaminated with SEO and Mine Spoils

The mean reductions in Total PAHs concentration in *Helianthus annuus*, *Brassica juncea*, and unplanted soils co-contaminated with SEO and mine-spoils are summarized in **Figure *4.32*** above. The above shows that the percentage Total PAHs reduction was significantly higher (p=1.8888E-08 and p=1.8956E-05) by 43.6% and 40.4% in *Helianthus annuus* and *Brassica juncea* treatments respectively at 1.6% SEO + 10% mine-spoils when compared with the unplanted treatments. However, there were no notable differences at 0.8% SEO + 10% mine-spoils between *Helianthus annuus* and unplanted treatments. On the other hand, the percentage Total PAHs reductions was 13% higher at 0.8% SEO + 10% mine-spoils in the unplanted when compared with *Brassica juncea*. Percentage Total PAH reductions were generally higher in *Helianthus annuus* treatments when compared with *Brassica juncea* treatments.

When comparing the percentage total PAH reduction in *Helianthus annuus* and *Brassica juncea* soils, total PAH reductions were significantly higher (p=0.001) for *Helianthus annuus* planted soils at 0.8% SEO + 10% mine-spoils when compared with *Brassica juncea*. However, the differences in total PAH reductions at 1.6% SEO + 10% mine-spoils were not significant when both species were compared.

4.10 Effects of Struvite Amendment on the Reduction of Total PAHs in *Helianthus annuus* and *Brassica juncea* Soils Co-Contaminated with SEO and Mine-Spoils

This experiment aimed to investigate the potential for struvite amendment to increase the reduction of Total PAHs *Helianthus annuus* and *Brassica juncea* soils co-contaminated with SEO concentrations and mine-spoils. It also compared the effectiveness of struvite with NPK fertilizer. These were carried out in line with the sixth objective of the study which sought to evaluate and compare the potential for an industrial waste (struvite) in enhancing the growth and phytoremediation abilities of both species in comparison with NPK fertilizer.

The percentage Total PAH reductions in struvite amended and unamended soils planted with *Helianthus annuus* and *Brassica juncea* in SEO and mine-spoils co-contaminated soils are summarized in **Figure *4.33*** below. The percentage Total PAH reduction was significantly higher (p=0.003) for *Helianthus annus* treatments by 11% in struvite amended treatments when compared with unamended treatments at 1.6% SEO + 10% mine-spoils. However, the differences between amended and unamended treatments in all other instances were not significant.

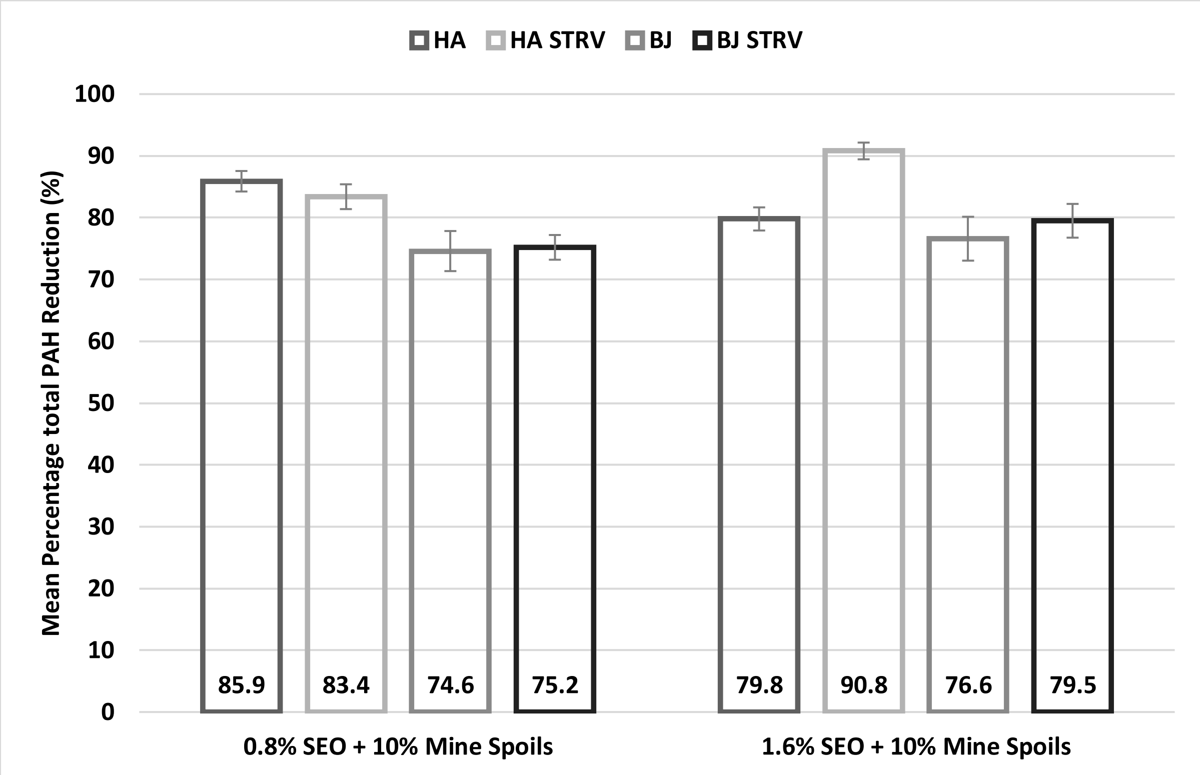

Figure 4.33. Percentage Total PAH Reduction in *Helianthus annuus* and *Brassica juncea* Soils with and without Struvite Amendment Soils

4.10.1 Comparing the Effects of Struvite and NPK Fertilizer Amendments on the Reduction of Total PAHs in *Helianthus annuus* and *Brassica juncea* Soils Co-Contaminated with SEO and Mine-Spoils

This experiment was aimed at comparing the efficacy of struvite and NPK fertilizer supplementation on the reduction of total PAHs concentrations by *Helianthus annuus* and *Brassica juncea* in SEO and mine-spoils co-contaminated soils. This was carried out in line with the sixth objective of the study which sought to evaluate and compare the potential for an industrial waste (struvite) in enhancing the growth and phytoremediation abilities of both species in comparison with NPK fertilizer. The mean percentage reduction of TPH in *Helianthus annuus* with struvite and NPK amendments in soils co-contaminated with SEO mine-spoils are summarized in **Figure *4.34*** below. The results showed that the mean percentage Total PAH reductions were significantly higher (p=2.1037E-09) by 16.5% and 34.8% in the struvite amended treatments when compared with the NPK treatments at 0.8% SEO + 10% mine-spoils and 1.6% SEO + 10% mine-spoils respectively. It was also observed that the mean percentage Total PAH reductions were higher at 1.6% SEO + 10% mine-spoils in the struvite treatments while the opposite was observed in the NPK treatments.

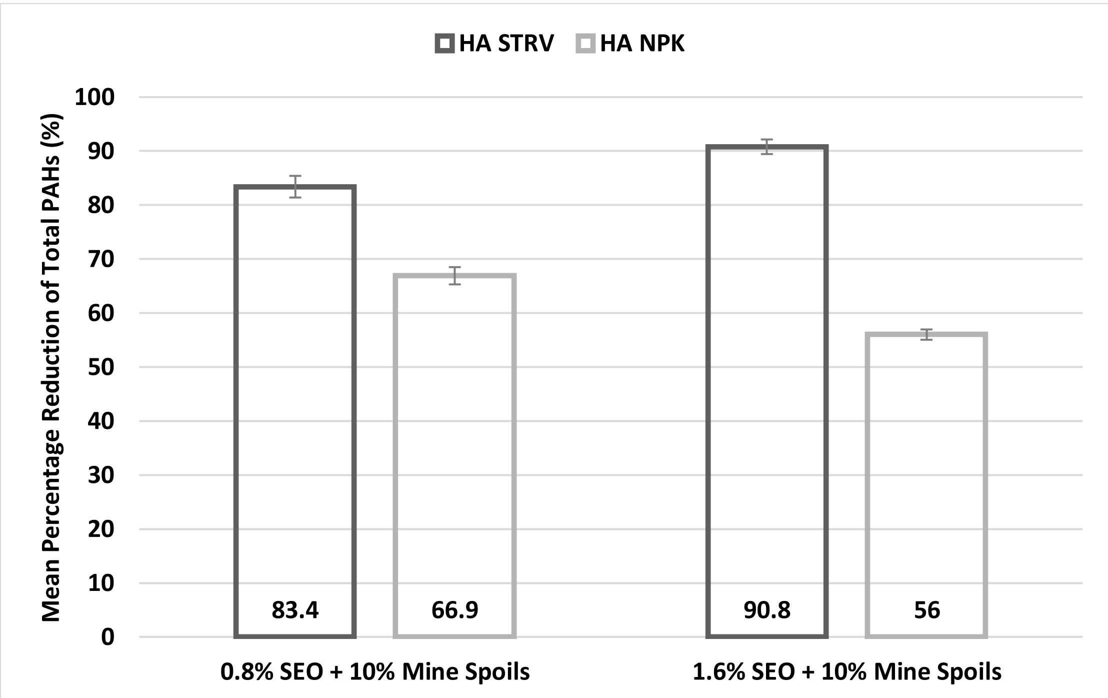

Figure 4.34. Mean Percentage Reduction of Total PAH in *Helianthus annuus* with Struvite and NPK Amendment in Soils Co-Contaminated with SEO and Mine Spoils

The mean percentage reduction of TPH in *Brassica juncea* with struvite and NPK amendments in soils co-contaminated with SEO mine-spoils are summarized in **Figure *4.35*** below. It was observed that the mean percentage Total PAH reductions were slightly higher in the NPK treatments when compared with the struvite treatments although the differences were not statistically significant. It was also observed that the mean percentage Total PAH reductions were higher at 1.6% SEO + 10% mine-spoils for both struvite and NPK amended treatments.

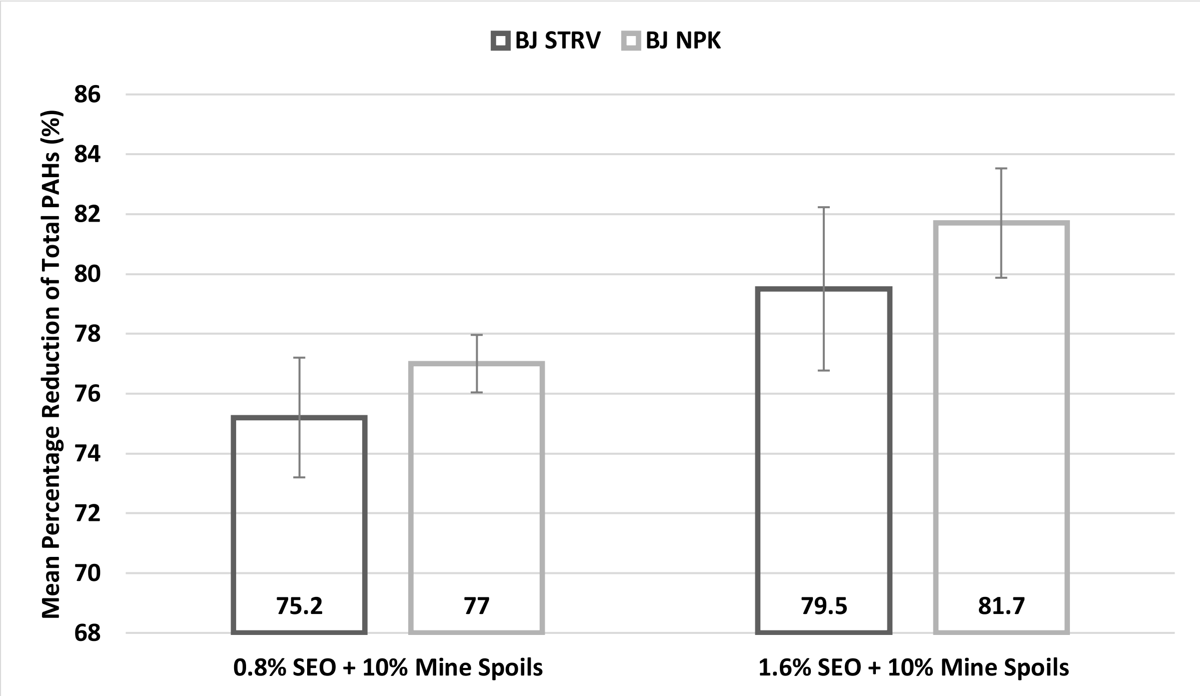

Figure 4.35. Mean Percentage Reduction of Total PAH in *Brassica juncea* with Struvite and NPK Amendment in Soils Co-Contaminated with SEO and Mine Spoils

4.11 Reduction of Pb Concentrations in *Helianthus annuus* and *Brassica juncea* Soils Co-Contaminated with SEO and Mine-Spoils

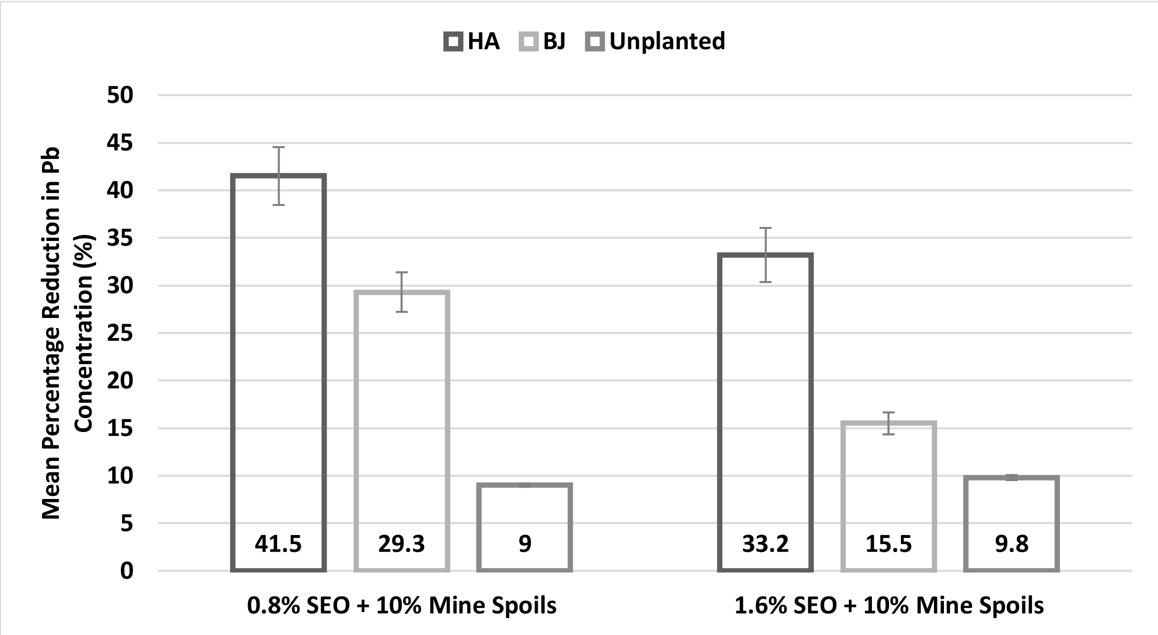

Figure 4.36. Mean Percentage Reduction of Pb in *Helianthus annuus*, *Brassica juncea* and Unplanted Soils Co-Contaminated with SEO and Mine Spoils

This experiment aimed to investigate the reduction of Pb concentration effected by *Helianthus annuus* and *Brassica juncea* in soils co-contaminated with SEO and mine-spoils in line with the fifth objective of this study which was to investigate the abilities of *Helianthus annuus* and *Brassica juncea* to reduce Pb concentrations from SEO and mine-spoils co-contaminated soils. The mean percentage reduction of Pb in *Helianthus annuus*, *Brassica juncea* and unplanted soils are displayed in **Figure *4.36*** above. It was observed that the mean percentage Pb reduction was significantly higher (p=1.2683E-08) in planted treatments by 32.5% - 23.4% for *Helianthus annuus* and 20.3% - 5.7% for *Brassica juncea* at 0.8% SEO + 10% mine-spoils and 1.6% SEO + 10% mine-spoils respectively when compared with the unplanted treatments. It was also noted that the mean percentage Pb reduction in planted soils was significantly lower (p=0.01) at 1.6% SEO + 10% mine-spoils when compared to 0.8% SEO + 10% mine-spoils.

When the differences in percentage Pb reduction were compared between the planted treatments, the results showed that the mean percentage Pb reduction was significantly higher (p=1.8593E-06) in *Helianthus annuus* soils by 12.2% and 17.7% at 0.8% SEO + 10% mine-spoils and 1.6% SEO + 10% mine-spoils respectively when compared to *Brassica juncea* soils.

4.11.1 Effects of Struvite Amendment on the Reduction of Pb Concentrations in *Helianthus annuus* and *Brassica juncea* Soils Co-Contaminated with SEO and Mine-Spoils

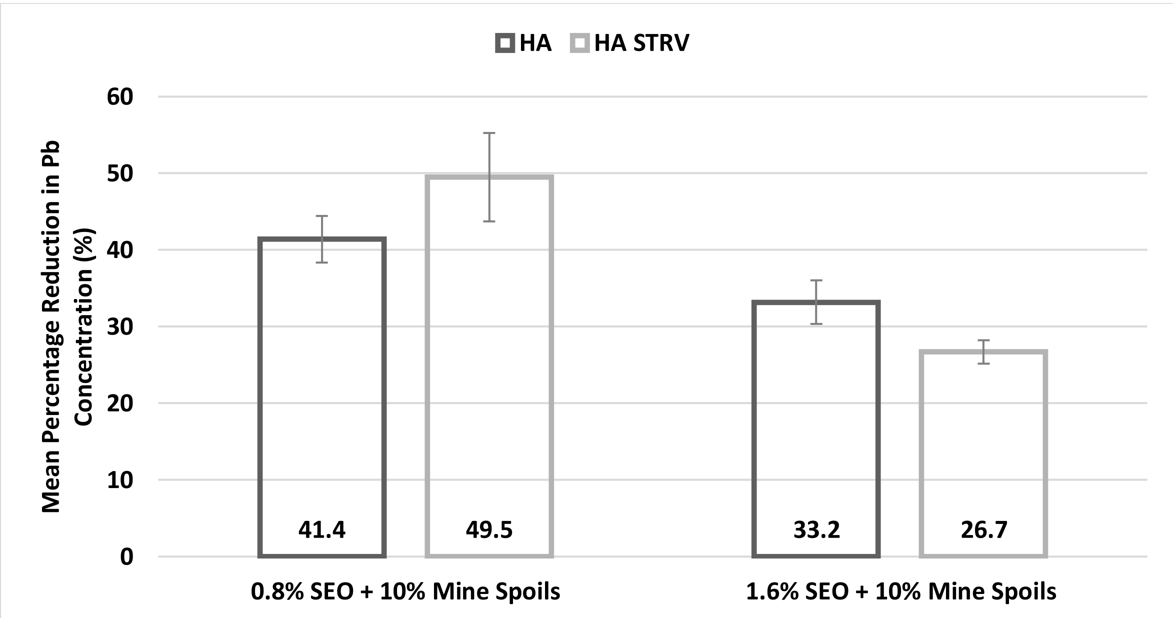

Figure 4.37. Mean Percentage Reduction of Pb in Struvite Amended and Unamended *Helianthus annuus* Soils Co-Contaminated with SEO and Mine Spoils

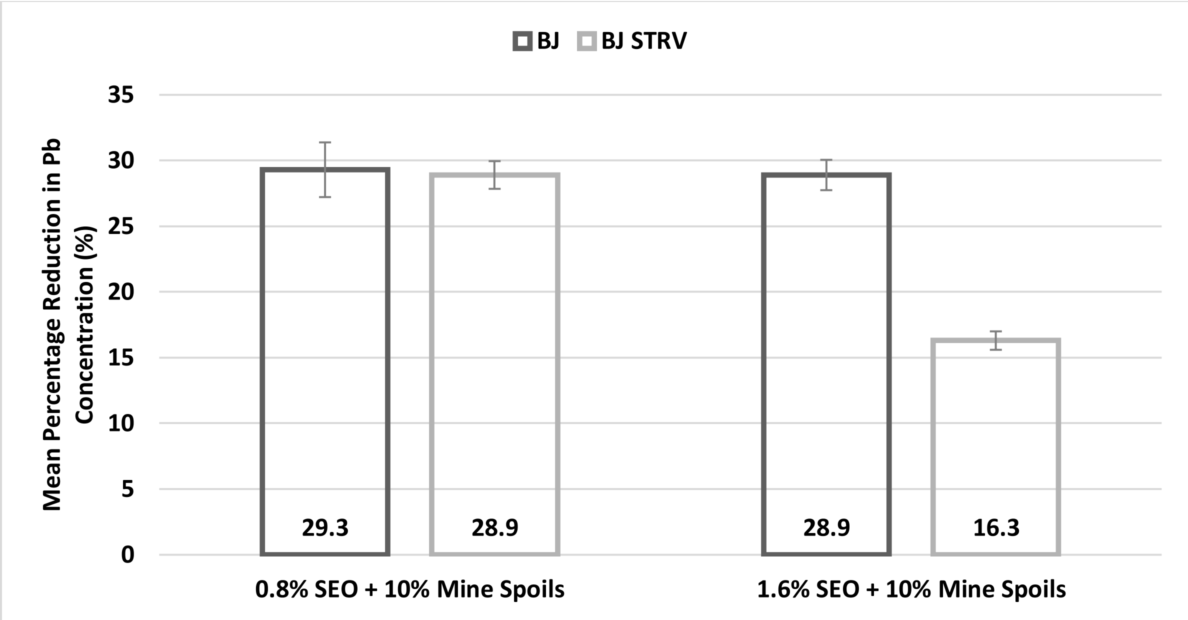

Figure 4.38. Mean Percentage Reduction of Pb in Struvite Amended and Unamended *Brassica juncea* Soils Co-Contaminated with SEO and Mine Spoils

This experiment was aimed at investigating the effects of struvite supplementation on the reduction of Pb concentrations in SEO and mine-spoils co-contaminated soils planted with *Helianthus annuus* and *Brassica juncea.* This was carried out in line with the sixth objective of the study which sought to evaluate and compare the potential for an industrial waste (struvite) in enhancing the growth and phytoremediation abilities of both species in comparison with NPK fertilizer. The mean percentage reduction of Pb in struvite amended and unamended soils are displayed in **Figure *4.37*** and **Figure *4.38*** above. It was observed that for *Brassica juncea* soils, there was no notable difference in the mean percentage reduction of Pb between struvite amended and unamended soils at 0.8% SEO + 10% mine-spoils, whereas mean Pb reduction was 43.6% higher in struvite amended treatments at 1.6% SEO + 10% mine-spoils when compared with unamended treatments. On the other hand, it was observed that in *Helianthus annuus* soils, mean percentage reduction was higher in struvite amended treatments at 0.8% SEO + 10% mine-spoils and lower at 1.6% SEO + 10% mine-spoils when compared with unamended treatments.

4.11.2 Comparing Reduction of Pb Concentrations in *Helianthus annuus* and *Brassica juncea* with Struvite and NPK Amendments in Soils Co-Contaminated with SEO and Mine-Spoils.

This experiment was aimed at comparing the efficacy of struvite and NPK fertilizer supplementation on the reduction of Pb concentrations by *Helianthus annuus* and *Brassica juncea* in SEO and mine-spoils co-contaminated soils. This was carried out in line with the sixth objective of the study which sought to evaluate and compare the potential for an industrial waste (struvite) in enhancing the growth and phytoremediation abilities of both species in comparison with NPK fertilizer. The mean percentage reduction of Pb concentrations in *Helianthus annuus* and *Brassica juncea* with struvite and NPK amendments in soils co-contaminated with SEO and mine-spoils are displayed in **Figure *4.39*** and **Figure *4.40*** below. For the two plant species, it was observed that the mean percentage Pb reductions were significantly higher (p=1.268E-05 and p=3.1573E-06 for *Helianthus annuus* and *Brassica juncea* respectively) in struvite amended soils when compared with NPK treatments. Also, it was observed that *Helianthus annuus* soils had higher mean percentage Pb reductions in both soil amendments when compared with *Brassica juncea*.

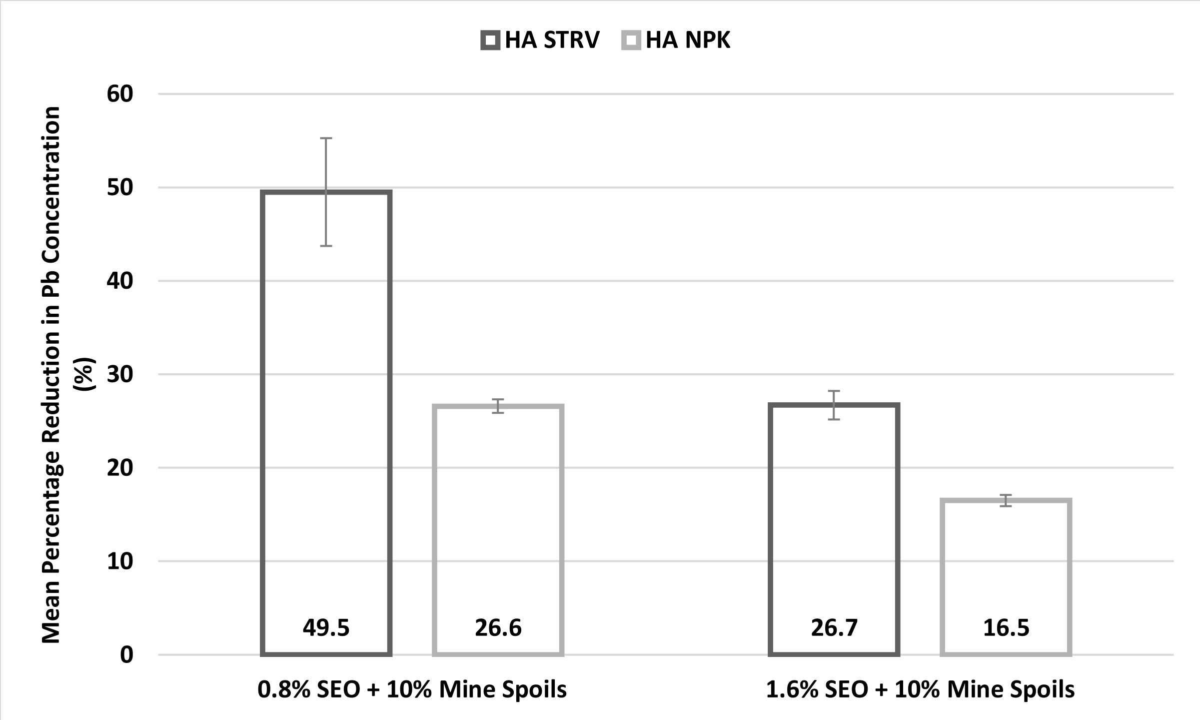

Figure 4.39. Mean Percentage Reduction of Pb Concentrations in *Helianthus annuus* with Struvite and NPK Amendments in Soils Co-Contaminated with SEO and Mine Spoils

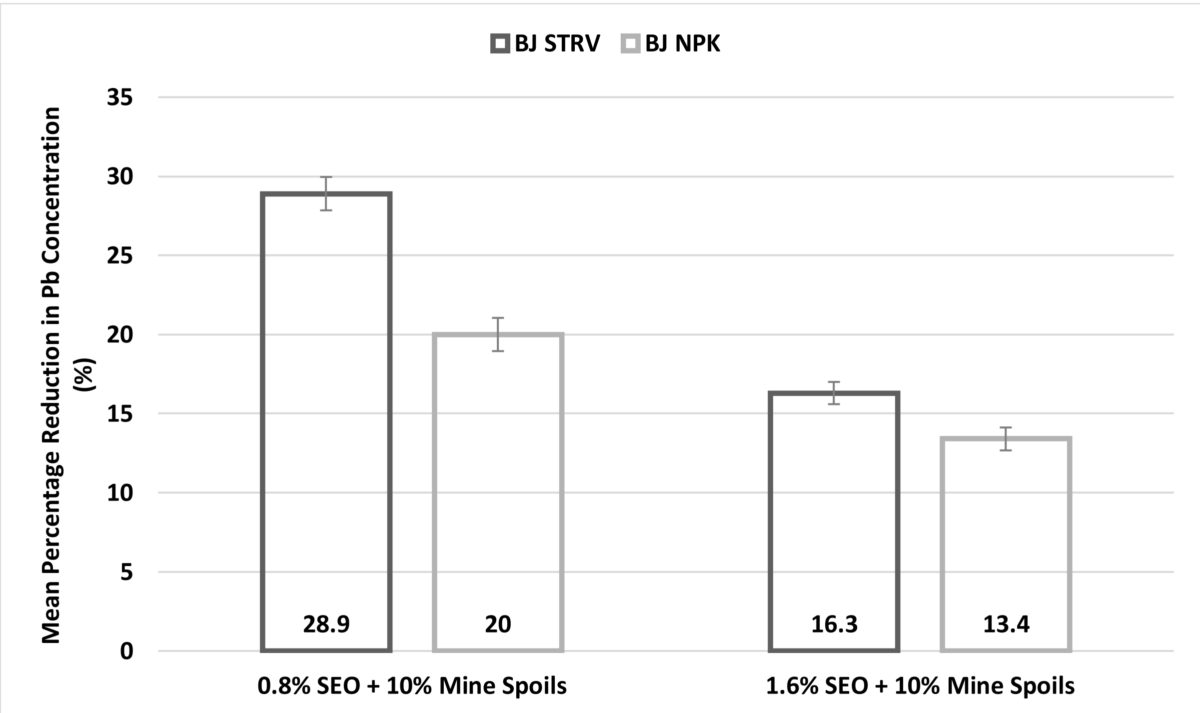

Figure 4.40. Mean Percentage Reduction of Pb Concentrations in *Brassica juncea* with Struvite and NPK Amendments in Soils Co-Contaminated with SEO and Mine Spoils

Table 4.4. Summary of the Mean Reductions of Pb in Amended and Unamended *Helianthus annuus* and *Brassica juncea* Soils Co-Contaminated with SEO and Mine Spoils

Mean Reduction of Pb in HA and BJ Soils Co-Contaminated with SEO and Pb						
Treatment	**0% SEO + 0% Mine Spoils**		**0.8% SEO + 10% Mine Spoils**		**1.6% SEO + 10% Mine Spoils**	
	Reduction (mg/kg)	**Pb Percentage Reduction**	**Reduction (mg/kg)**	**Pb Percentage Reduction**	**Reduction (mg/kg)**	**Pb Percentage Reduction**
HA	27.5	89.6	92	41.5	74.8	33.17
BJ	21.6	86.5	68.6	29.33	37.7	15.67
Unplanted			40.3	8.97	32.9	9.8
HA STRV			106.1	49.5	70.8	26.7
BJ STRV			62.7	28.9	34.4	16.27
HA NPK			65	26.6	47.9	16.5
BJ NPK			48.7	20.0	46.3	13.4

4.12 Total Uptake of Pb by *Helianthus annuus* and *Brassica juncea* in Soils Co-Contaminated with SEO and Mine-Spoils

This experiment aimed to investigate and compare the potential for *Helianthus annuus* and *Brassica* to uptake Pb in soils co-contaminated with SEO and mine-spoils in line with the fifth objective of this study which was to investigate the abilities of *Helianthus annuus* and *Brassica juncea* to reduce Pb concentrations from SEO and mine-spoils co-contaminated soils. There was no data for *Brassica juncea* at 1.6% SEO + 10% mine-spoils as the plant material was too small to analyse. It also sought to investigate the effects of struvite and NPK amendments on the Pb uptake of *Helianthus annuus* and *Brassica juncea*.

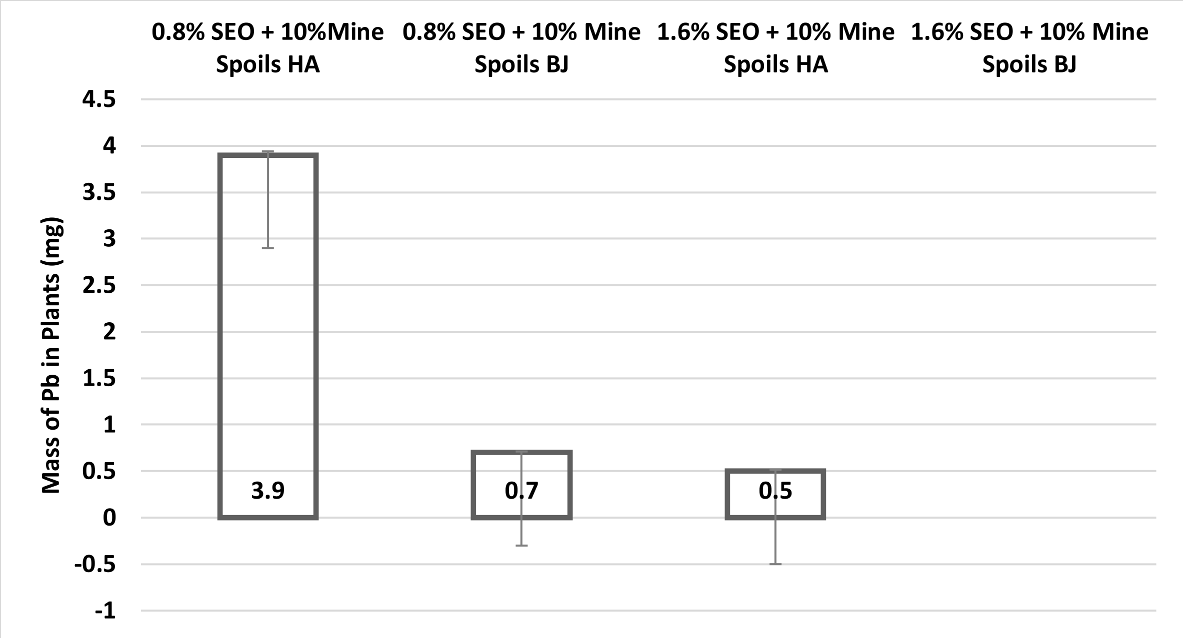

Figure 4.41. Mean Uptake of Pb by *Helianthus annuus* and *Brassica juncea* in SEO and Mine Spoils Co-Contaminated Soils

The mean uptake of Pb by *Helianthus annuus* and *Brassica juncea* in SEO and mine-spoils co-contaminated soils are displayed in **Figure *4.41*** above. It was observed that the mass of Pb in *Helianthus annuus* at 0.8% SEO + 10% mine-spoils was significantly higher (p=4.0353E-17) by 82.1% when compared with *Brassica juncea* treatments. The mass of Pb in *Helianthus annuus* was significantly higher (p=1.7876E-17) by 87.2% at 0.8% SEO + 10% mine-spoils when compared with 1.6% SEO + 10% mine-spoils.

4.12.1 Effects of Struvite Amendment on the Total Uptake of Pb by *Helianthus annuus* and *Brassica juncea* in Soils Co-Contaminated with SEO and Mine-Spoils

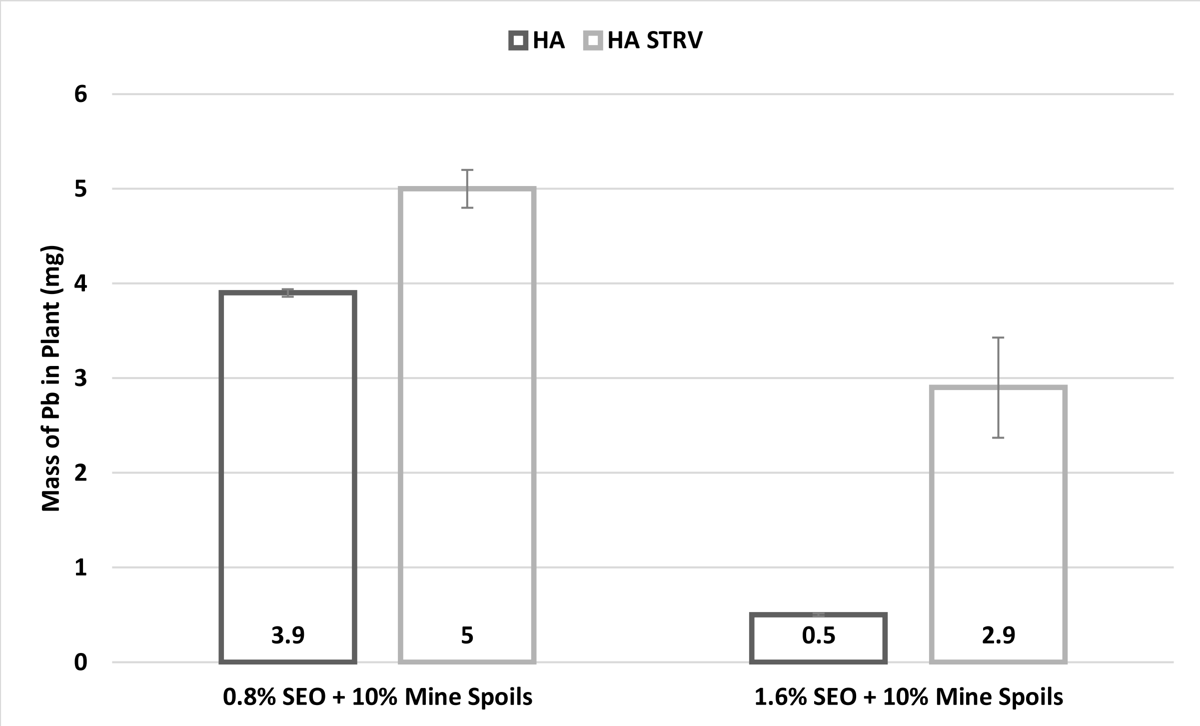

Figure 4.42. Mean Uptake of Pb by *Helianthus annuus* with and without Struvite Amendments in SEO and Mine Spoils Co-Contaminated Soils

This experiment was aimed at investigating the effects of struvite supplementation on the uptake of Pb by *Helianthus annuus* and *Brassica juncea* in SEO and mine-spoils co-contaminated soils. This was carried out in line with the sixth objective of the study which sought to evaluate and compare the potential for an industrial waste (struvite) in enhancing the growth and phytoremediation abilities of both species in comparison with NPK fertilizer. The mean uptake of Pb by *Helianthus annuus* with and without struvite amendments in SEO and mine-spoils co-contaminated soils are displayed in **Figure *4.42*** above. The results showed that the mean Pb uptake was significantly higher (p=5.3658E-06) by 22% and 82.6% at 0.8% SEO + 10% mine-spoils and 1.6% SEO + 10% mine-spoils respectively when compared with the unamended treatments. It was also noted that the total Pb uptake were significantly lower (p=1.6789E-07) in amended and unamended treatments at the higher pollution level.

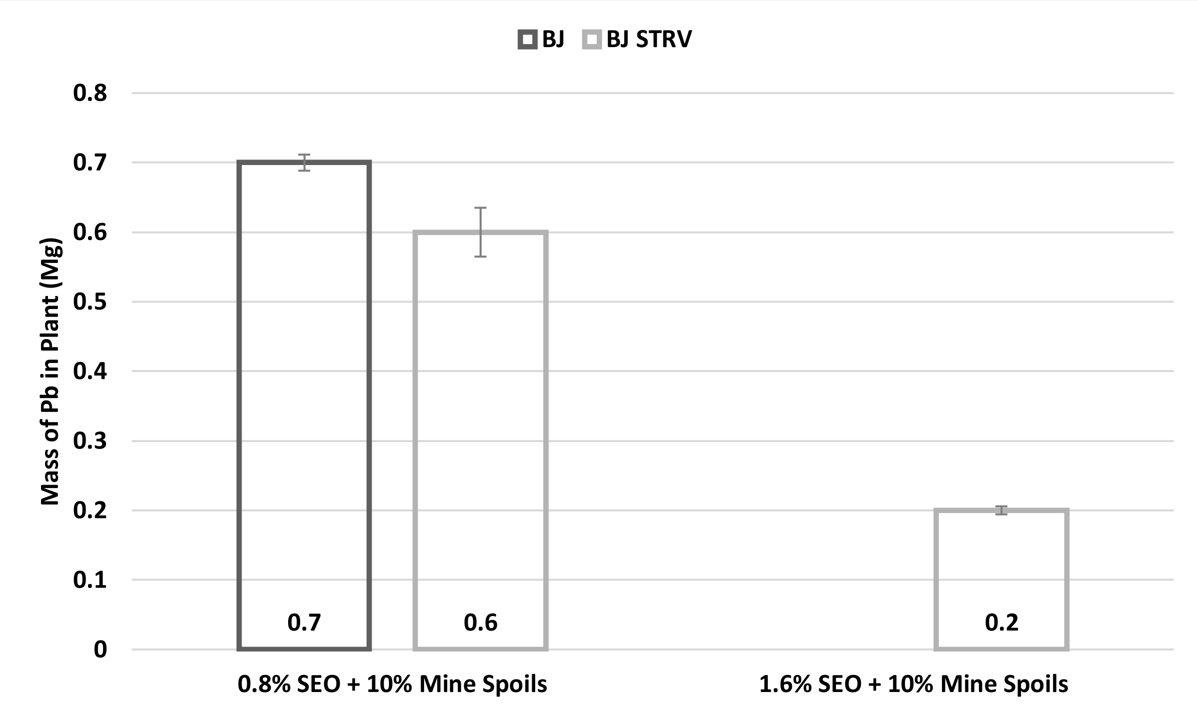

Figure 4.43. Mean Uptake of Pb by *Brassica juncea* with and without Struvite Amendments in SEO and Mine Spoils Co-Contaminated Soils

The mean uptake of Pb by *Brassica juncea* with and without struvite amendments in SEO and mine-spoils co-contaminated soils are displayed in **Figure *4.43*** above. It was observed that the Pb uptake was significantly lower (p=0.003) in struvite amended treatments by 14.3% when compared with the unamended treatment. The total Pb uptake was significantly lower (p=4.3446E-09) at the higher pollution level in the struvite amended treatments.

4.12.2 Comparing the Total Pb Uptake of *Helianthus annuus* and *Brassica juncea* with Struvite and NPK Amendments in Soils Co-Contaminated with SEO and Mine-Spoils

This experiment was aimed at comparing the efficacy of struvite and NPK fertilizer supplementation on the uptake of Pb by *Helianthus annuus* and *Brassica juncea* in SEO and mine-spoils co-contaminated soils. This was carried out in line with the sixth objective of the study which sought to evaluate and compare the potential for an industrial waste (struvite) in enhancing the growth and phytoremediation abilities of both species in comparison with NPK fertilizer. The mean total Pb uptake by *Helianthus annuus* and *Brassica juncea* with struvite and NPK amendments in soils co-contaminated with SEO and mine-spoils are summarized in **Figure *4.44*** and **Figure *4.45*** below. For *Helianthus annuus*, the mean total Pb uptake was significantly higher (p=3.2426E-05) in the struvite amended treatments when compared with the NPK treatments. On the other hand, mean Pb uptake was significantly higher for *Brassica juncea* (p=0.00013) in the NPK treatments compared to the struvite treatments. For the two plants in struvite and NPK treatments, the mean Pb uptake was generally higher at 0.8% SEO + 10% mine-spoils except for *Brassica juncea* in the NPK treatment where the mean Pb uptake was much higher at 1.6% SEO + 10% mine-spoils.

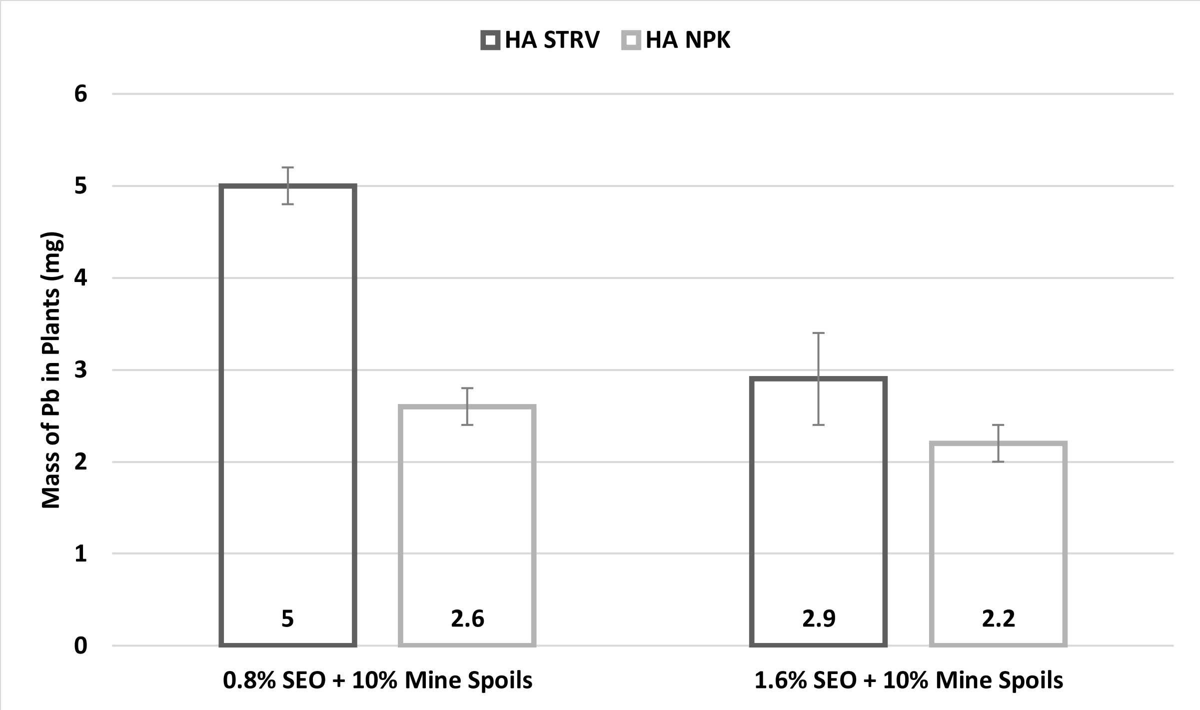

Figure 4.44. Mean Pb Uptake by *Helianthus annuus* with Struvite and NPK Amendments in SEO and Mine Spoils Co-Contaminated Soils

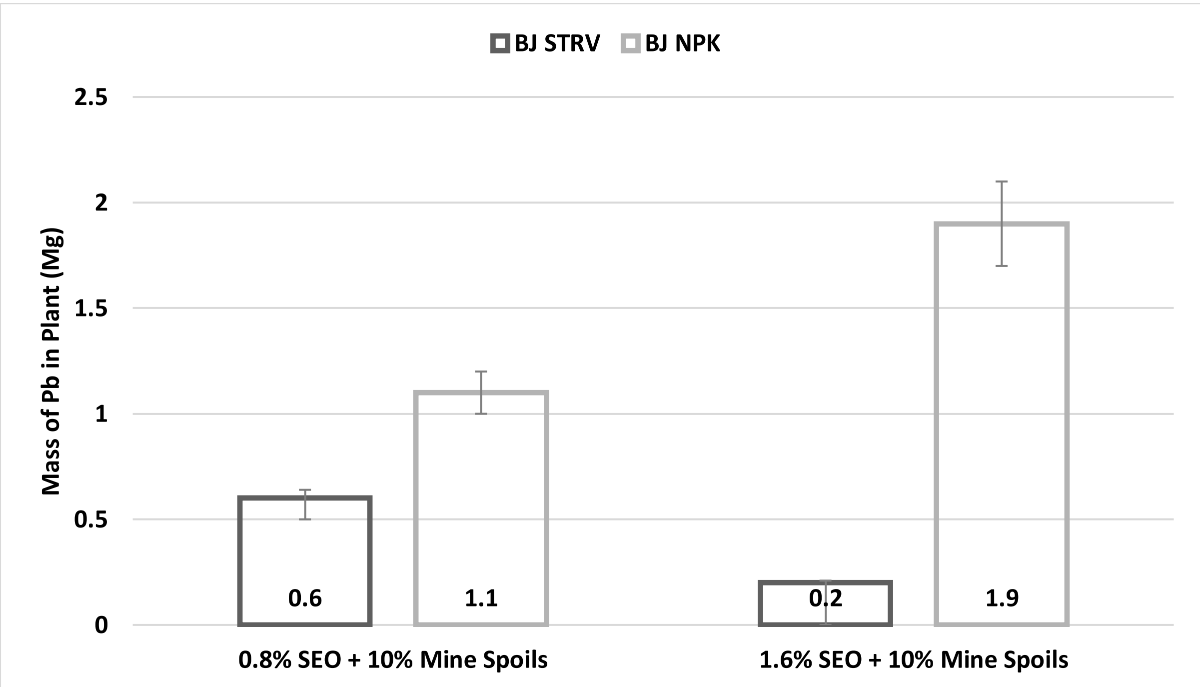

Figure 4.45. Mean Pb Uptake by *Brassica juncea* with Struvite and NPK Amendments in SEO and Mine Spoils Co-Contaminated Soils

4.13 Summary of Findings

The results of the various experiments provided insight to the germination and growth response of *Helianthus annuus* and *Brassica juncea* to SEO concentrations, and SEO & mine-spoils co-contamination. Both species displayed a dose-dependent decline in germination, height, laminar leaf area, and dry biomass production, with more acute responses manifested by *Brassica juncea*. The results of the mixed cropping experiment revealed negative responses for all the growth metrics studied except for plant height which yielded improvement for both species when compared with the mono-cropping counterparts. Amendment with struvite yielded improvement in growth metrics for both species with *Brassica juncea* displaying positive responses to struvite at both concentration doses in the co-contamination mixture, while the growth improvements with *Helianthus annuus* only being apparent at the higher co-contamination doses. When the effects of struvite and NPK fertilizers on the growth metrics of both species were compared, NPK fertilizer yielded significantly higher improvements for *Brassica juncea*, struvite yielded better results for *Helianthus annuus* at higher co-contamination doses in terms of plant height and number of leaves, and NPK yielded superior results at both co-contaminant doses for the laminar leaf area of *Helianthus annuus*.

In terms of the assessment of the phytoremediation efficacy of both species in SEO and mine-spoils co-contaminated soils, the results revealed significantly higher reductions in TPH concentrations in planted soils when compared to the unplanted controls, with a dose-dependent decline in TPH reduction observed for both species. Struvite and NPK fertilizer amendments generally had negative effects on TPH reduction for both species. Total PAH reductions in soil were higher in planted treatments at the 1.6% SEO + 10% mine-spoils co-contamination level when compared with the unplanted counterparts. Struvite amendments yielded positive enhancement of total PAH reductions for both species at the 1.6% SEO + 10% mine-spoils co-contamination level when compared with the unamended treatments. Comparing struvite and NPK supplementation, NPK yielded better results for *Brassica juncea* and struvite yielded better results for *Helianthus annuus* in a total PAH reduction context. Pb reduction in co-contaminated soils were significantly higher in planted pots when compared to the unplanted controls. Pb uptake was higher in *Helianthus annuus* compared to *Brassica juncea* and there was a dose dependent decline in Pb uptake as contaminant doses increased, and the best Pb reduction and Pb uptake was observed in *Helianthus annuus* treatments supplemented with struvite fertilizer.

Chapter 5: DISCUSSION

This chapter covers the discussion of the results of this research as presented in **Chapter 4** and the order these are discussed represents the order of plant lifecycle from germination all the way to the harvest of the plants. It begins in section **5.1** with discussing the results on the effects of SEO concentrations on the percentage germination of *Helianthus annuus* and *Brassica juncea*. This is followed by the evaluation of the results of the effects of SEO contamination on the growth parameters of the chosen species in section **5.2**. This section (**5.2.1**) also covers the results on the effects of mixed cropping on the growth of both species under SEO stress. This is followed by section **5.3** which covers the effects of SEO and mine-spoils co-contamination on the growth of the studied species. This section also covers the effects of struvite fertilizer on the growth of the studied species under co-contamination stress and compares the effects to the impacts of NPK fertilizers on the growth of the studied species (section **5.3.1**). Finally, the results of the phytoremediation abilities of the studied species in terms of TPH, total PAHs and heavy metal reductions in soils and the effects of struvite and NPK fertilizers on contaminant reductions in soils is discussed in sections **5.4**, **5.5** and **5.6**. The results on Pb uptake in SEO and mine-spoils co-contaminated soils by the studied species and the influence of NPK and struvite fertilizer supplementation on Pb uptake are discussed in sections **5.6** and **5.6.1**.

5.1 Effects of SEO Concentrations on Percentage Germination of *Helianthus annuus* and *Brassica juncea*

Germination is an important factor when considering the phytoremediation abilities of both species for the chosen contaminants as it is a fundamental step in evaluating the tolerance of phytoremediation species for specific contaminants in soils. The results of the germination experiment (**section 0**) showed that percentage germination achieved showed a significant reduction in both species as the concentration of SEO increased in the soil (**Figure *4.1***). This corresponds with the findings of various previous studies (Agbogidi and Ilondu 2013, Anoliefo and Vwioko 1994, Hussain *et al*. 2019, Sharifi, Sadeghi, and Akbarpour 2007, Atagana 2011, Oluwanisola and Abdulrahaman 2018) where a dose-dependent decrease in the percentage germination in SEO contaminated soils were also observed. However, the extent of manifestation of germination inhibition varies between plant species as a demonstration of plant tolerance to SEO contamination. For instance, the maximum impact of SEO concentration on germination in this study was observed at 4% SEO concentration with 15% and 5% germination for *Helianthus annuus* and *Brassica juncea* respectively. This clearly shows a significant difference in the germination response to SEO doses for both species with *Helianthus annuus* demonstrating superior tolerance when compared to *Brassica juncea*. Similarly, a study by Onwusiri, Aguoru, and Akomolafe (2017) reported a germination of 41.67% for *Telfairia occidentalis* at 4% SEO concentration. This was significantly higher than the results obtained in my study. To take it a step further, my study recorded no germination beyond 4% SEO for the two species studied, unlike the results reported for *Telfairia occidentalis* that showed 8% germination at 5% SEO in the study by Onwusiri, Aguoru, and Akomolafe (2017), indicating that *Telfairia occidentalis* could be more efficacious species for SEO treatment from a germination and overall tolerance superiority standpoint. *Sorghum saccharatum* has demonstrated impressive tolerance in a study by Ezenwa, Adieze, and Aririatu (2017) with a reported 90% germination at 2% SEO compared to the 71.66% germination recorded in my study for *Helianthus annuus* at 2% SEO. However, *Helianthus annus* and *Brassica juncea* in my study demonstrated better tolerance for 2% SEO compared to *Solanum lycopersium* in the study by Ezenwa, Adieze, and Aririatu (2017) as germination percentages of 37.5% and 36.7% were recorded for *Helianthus annuus* and *Brassica juncea* respectively when compared to the 25% germination reported for *Solanum lycopersium* at 2% SEO. Overall, the dose dependent decline in germination phenomenon has been attributed to the hydrophobic properties of SEO which creates unsatisfactory soil conditions like reducing water penetration in soils from above, reduction in soil aeration via clogging of soil pore spaces and waterlogging of soils after watering which all have concomitant effects on the overall seed viability (Agbogidi and Ilondu 2013, Anoliefo and Vwioko 1994, Hussain *et al*. 2019). This was particularly demonstrated by Hussain *et al*. (2019) who showed that vegetable oil amendment significantly reduced germination of Italian ryegrass, indicating that germination reduction was due to the physical changes in the soil imposed by the hydrophobic properties of oil. This situation could have implications for phytoremediation especially in soils with severe SEO pollution, indicating that transplantation of healthy pre-germinated phytoremediation species from unpolluted soils might be necessary for exploring their phytoremediation potentials at elevated SEO levels as this could potentially help bypass the germination constraints. This further shows why, as recently proposed by Walakulu Gamage *et al*. (2020) seedling germination tests in polluted environments is a crucial screening step in determining the suitability of plants as phytoremediation species.

5.2 Effects of SEO on the Growth Parameters of *Helianthus annuus* and *Brassica juncea*

The ability of the species to survive and grow in contaminated environments is not only a measure of its tolerance to that specific contaminant but the extent of tolerance for contaminant concentrations could have implications for the overall effectiveness of the phytoremediation project. The results in **section 4.4** showed that 4.6% SEO concentration significantly stunted the growth of both species which was reflected in the marked decline in height, laminar leaf area and number of leaves were observed in the SEO treatments when compared with the unpolluted counterparts. *Brassica juncea* particularly showed acute stunting in growth with an observed 91%, 97.6% and 72.7% reduction in height, laminar leaf area and number of leaves respectively. The severity of the growth inhibition observed for *Brassica juncea* shows a very low tolerance for SEO contamination and could be indicative of a low suitability for the phytoremediation of SEO contaminated soils. This negative effect of SEO on plant growth has been observed in several studies [Njoku 2012, Donald, Henrietta, and Francis (2016), Kayode, Olowoyo, and Oyedeji (2009), Odjegba and Sadiq (2002), Okonokhua, B.O., Ikhajiagbe, B., Anoliefo, G.O. and Emede (2007), Nwoko *et al*. (2007), Eremrena and Mensah (2017), Lum and Chikoye (2018), Walakulu Gamage *et al*. (2020)]. For instance, the study by Lum and Chikoye (2018) reported that SEO concentrations reduced root and shoot biomass by 51.9% - 90.6% and 58.1% - 89.5% respectively for *Kyllinga erecta S.* and reduced root and shoot biomass for *Cyperus rotundus L.* by 57.3% - 92.0% and 55.9% - 92.8% respectively. Kayode, Olowoyo, and Oyedeji (2009) also reported reductions in height of *Vigna uniguiculata* and *Zea mays* reaching 53.9% and 64.9% respectively at 250ml SEO/kg soil. This study by Kayode, Olowoyo, and Oyedeji (2009) showed a more acute height response to SEO contamination compared to my study which recorded a 48.8% height reduction to SEO contamination. This analogy could potentially be flawed as Kayode, Olowoyo, and Oyedeji (2009) did not report the concentration of SEO in the soil so it creates the difficulty to put the total volume of SEO used (250ml) into a comparable context with my study (110ml which translates to 4.6% SEO w/w). Similarly, the 48.6% reduction in the height of *helianthus annuus* at 4.6% SEO reported in my study showed significantly higher tolerance when compared to the 73.9% reduction in the height of *Telfaira occidentalis* at 4% SEO concentration, which is a lower SEO concentration than that of my study (4.6%). *Brassica juncea* had significantly less tolerance when compared to *Telfaira occidentalis*. This further reinforces the fact that *Helianthus annuus* although significantly impacted by SEO within a growth response context, still demonstrates resilience under SEO stress when compared to other species under similar growth conditions. The stunted growth in SEO treatments could be a consequence of the SEO-imposed unsatisfactory soil conditions which altered soil physical properties, resulting in reduced nutrient availability, reduction in plant-water relations resulting in physiological drought, interference with gaseous exchange and reduction in soil aeration (Kayode, Olowoyo, and Oyedeji 2009, Okonokhua, B.O., Ikhajiagbe, B., Anoliefo, G.O. and Emede 2007, Walakulu Gamage *et al*. 2020).

The severity of these effects on growth parameters could also be linked to time of exposure as younger plants could be more vulnerable to toxicity effects of SEO as opposed to plants that have attained a certain degree of maturity prior to SEO exposure. This corresponds with a study by Njoku (2012) where it was observed that *Zea mays* plants showed more severe stunted growth when exposed to SEO at an earlier stage when compared to counterparts that were exposed to SEO at a more advanced stage of plant development. This could mean that transplanting mature phytoremediation species into SEO contaminated sites might prove advantageous compared to sowing directly into contaminated soils. As much as adopting this approach could have implications for the environment in terms of increased emissions as this might require more transportation of mature plants to site, it offers a higher success potential for an environment friendly technology for the remediation of contaminated land.

The results of the effects of SEO on the growth parameters of *Helianthus annuus* and *Brassica juncea* showed disparities in the extent of the antagonistic effects of SEO on both species. Where the growth of both species was significantly reduced with SEO pollution, *Brassica juncea* showed a more acute growth response to SEO exposure when compared to *Helianthus annuus*. This agrees with a study by Donald, Henrietta, and Francis (2016) where disparities were also observed in the growth response of *Capsicum frutescens, Capsicum chinense* and *Capsicum annum* to SEO pollution. However, worthy of note is that the two species utilized in my study, despite the significant impacts of 4.6% SEO demonstrated significantly higher tolerance for SEO contamination when compared to *Capsicum frutescens, Capsicum chinense* and *Capsicum annum* in the study by Donald, Henrietta, and Francis (2016), as their study reported no plant growth beyond 1% SEO. Furthermore, the growth response metrics reported in their study at 1% SEO contamination was comparable to the results from my study at 4.6% SEO, which was 3.6 times higher than that of their study especially in the leaf area of *Capsicum chinense* which reduced by 80% at 1% SEO compared to the 92.2% reduction in leaf area of *Helianthus annuus* at 4.6% SEO. A similar analogy can be made for the study by Walakulu Gamage *et al*. (2020) where the tolerance of *Helianthus annuus* at 4.6% SEO as seen in my study was higher than that of *Crotalaria retusa L.* and *Impatiens balsamina L*. This was evidenced in the higher reductions in growth metrics particularly plant height recorded for Crotalaria *retusa L.* and *Impatiens balsamina L*. (over 50%) compared to the 48.6% reported for *Helianthus annuus* in my study. This shows that various plant species have different tolerance levels for different contaminants, and in this case, *Helianthus annuus* demonstrated more resilience to SEO contamination, suggesting that it could be a viable option for the phytoremediation of SEO contaminated soils.

5.2.1 Effects of Mixed Cropping on the Growth Parameters of *Helianthus annuus* and *Brassica juncea* in SEO Contaminated Soils

Mixed cropping is an agronomic practice that has been used to enhance crop yield, and thus, exploring the potential for the combination of the chosen species to improve plant growth under SEO stress could provide new insights on the prospect of employing this agronomic practice in phytoremediation projects. The results of the mixed cropping experiment (see **section 4.4.1**) showed varying responses in terms of the effects of mixed cropping on the growth parameters of *Helianthus annuus* and *Brassica juncea* in SEO contaminated soils and in uncontaminated soils. For both plant species, mixed cropping showed inhibitory effects on laminar leaf area and number of leaves in unpolluted treatments when compared to the mono-cropping counterparts except in the case of plant height. This corresponds with Gill, Abid, and Azam (2009) who also observed inhibition of root proliferation, total biomass and grain yield of chickpea when grown in mixture with wheat when compared to chickpea grown in isolation. However, very positive results were reported for wheat in their study with a 58.3% increase in total biomass after mixed cropping when compared to the monocropping treatment. Although this might not be a fair analogy as their study was not in a contaminated soil, similar findings have been reported in the literature where mixed cropping yielded a boost in growth metrics in a pollution context. For instance, a study by Vergara Cid, Pignata, and Rodriguez (2020) showed growth inhibitory effects on soybean monocropping treatments where 1500 mg/kg Pb concentration hindered growth progression beyond the first and second fully developed trifoliate leaf. However, mixed cropping with *Tagetes minuta* led to growth progression manifested through grain production, improved biomass production and grain quality. Similar findings have been reported in the literature (Bian *et al.*

2021, Cui *et al.* 2022, Samudro and Mangkoedihardjo 2020) where mixed cropping enhanced plant growth metrics in heavy metal contaminated soils. Although the argument can be made that the soil conditions in these studies differ from that in mine especially as it relates to the effects of the physical property alterations like hydrophobicity and soil aeration depletion imposed by SEO, the significant potential demonstrated in their results is perhaps indicative of a poor choice of plant combinations in my study, and hence, necessitates further experimentation of optimal combinations for *Helianthus annuus* and *Brassica juncea* for the attenuation of SEO induced growth antagonization.

In my study, slightly different results were observed for both species in mixed cropping with SEO contamination. For *Helianthus annuus*, mixed cropping with *Brassica juncea* showed a significant increase in plant height when compared to mono-cropping treatments in SEO contaminated soils whereas there were no notable differences in laminar leaf area and number of leaves when comparing mixed and unmixed treatments. On the other hand, *Brassica juncea* showed no notable differences in growth parameters between mixed and mono-cropping treatments under SEO stress. Comparing the effects of mixed cropping in unpolluted and SEO polluted soils, mixed cropping showed no negative effects on either species under SEO stress. In fact, it was significantly beneficial to *Helianthus annuus* in terms of height (see **Figure *4.5***). This indicates that the effects of mixed cropping could differ under plant stress and various soil conditions. It could also be said that the effects of mixed cropping on plant growth in unpolluted soils might not always present a clear picture of performance metrics in polluted soils as seen in this study where the negative effects of combining *Helianthus annuus* and *Brassica juncea* were notably less severe under SEO stress when compared to unpolluted treatments. This means that although it is imperative to experiment on the compatibility of various phytoremediation species in unpolluted soils, it is equally important to test the same combinations under various contaminant stresses ahead of a phytoremediation project as the response could potentially vary from one contaminant to another.

5.3 Effects of SEO and Mine-Spoils Co-Contamination on the Growth Parameters of *Helianthus annuus* and *Brassica juncea*

Oil and heavy metal co-contamination are often prevalent at sites with SEO contamination such as mechanic workshops and machinery service points, thus, an investigation of the effects of this type of co-contamination on the growth of the studied phytoremediation species could help provide insight on real world scenarios. The results of the effects of SEO and mine-spoils co-contamination on the growth parameters of *Helianthus annuus* and *Brassica juncea* (section **4.5**) showed a decline in plant height, laminar leaf area, number of leaves and dry biomass for both plant species when compared to their counterparts in unpolluted soils. This could be a result of the combined effects of SEO and Pb contamination as both are known to negatively impact the growth and development of plants. This is supported by Balakhnina and Nadezhkina (2017) which showed a 36% and 29% reduction in plant height and fresh weight respectively for *Triticum aestivum L*. when exposed to 100 mg/kg Pb. In comparison to my study, *Helianthus annuus* demonstrated significantly higher tolerance for Pb concentrations (30.9% reduction in plant height) that were three times that of the referenced study (303 mg/kg) in addition to being under 0.8% SEO stress. Although *Brassica juncea* exhibited more acute effects (69.5% height reduction) of Pb and SEO co-contamination from a growth aspect in my study, it can be argued that the growth response was comparable if not superior to that of *Triticum aestivum L*. in the referenced study when considering the fact that Pb concentrations were three times higher in my study and the co-contamination must have exacerbated the growth retardation manifested in *Brassica juncea*. The observed growth retardation under Pb induced stress could be a result of photosynthetic dysfunction and induced oxidative stressed imposed by Pb concentrations in soil (Balakhnina and Nadezhkina 2017).

Several studies have also observed the deleterious effects of SEO on plant growth (see **5.2**) and the dose dependent effect of SEO concentrations on plant height, number of leaves, laminar leaf area and dry biomass was demonstrated in this study. This was evidenced in the further decline in all the growth parameters for both species in treatments with higher doses of SEO (10% mine-spoils + 1.6% SEO) when compared with plants in treatments with lower doses of SEO (10% mine-spoils + 0.8% SEO), with *Brassica juncea* exhibiting subpar tolerance when compared to *Helianthus annuus*. This agrees with Walakulu Gamage *et al*. (2020) which observed a dose dependent decline in shoot length, root length, shoot and root wet and dry biomass of *Impatiens balsamina L*. with inhibitions exceeding 50% when compared to the unpolluted controls. Lum and Chikoye (2018) reported similar findings of an SEO dose dependent reduction in root and shoot biomass for *Kyllinga erecta Schumach* and *Cyperus rotundus Linn* by up to 90% in soils with 20-60ml/kg (1.6% - 5%) SEO concentrations. The growth response of the species used in their study (89.3% and 88.2% biomass reductions at 1.6% SEO for *Kyllinga erecta Schumach* and *Cyperus rotundus Linn* respectively) was comparable to that of our study (87.6% and 94.6% biomass reduction for *Helianthus annuus* and *Brassica juncea* respectively). However, when considering the added effects of 303 mg/kg Pb in the co-contamination in my study, it can be argued that *Helianthus annuus* and *Brassica juncea* has a significantly higher contaminant tolerance than the species used in the referenced study. The SEO induced growth retardation could be attributed to the reduction in availability and uptake of water due to the hydrophobic conditions imposed by SEO with concomitant reduction in biomass (Lum and Chikoye 2018).

Although *Helianthus annuus* and *Brassica juncea* showed significant growth inhibitions in SEO and Pb co-contaminated soils, the former showed a significantly higher tolerance for the said co-contamination than the later. This makes it a more promising specie for phytoremediation of this contaminant combination especially at lower concentrations. *Brassica* juncea, on the other hand, showed a very high sensitivity for the contaminant combination studied, even at the lower concentration range, and this could make its suitability for the phytoremediation of this type of co-contamination questionable.

5.3.1 Effects of Struvite and NPK Supplementation on the Growth of *Helianthus annuus* and *Brassica juncea* in SEO and Mine-Spoils Co-Contaminated Soils.

Struvite supplementation had varying effects on the height, number of leaves, plant dry biomass and laminar leaf area of *Helianthus annuus* and *Brassica juncea* at the two contaminant levels (0.8% SEO + 10% mine-spoils and 1.6% SEO + 10% mine-spoils) when compared with the unamended treatments (section **4.6**). Struvite amended treatments improved all the growth parameters studied for *Brassica juncea* at both contaminant levels, while for *Helianthus annuus*, the growth parameters reduced with struvite supplementation at the lower contaminant level but were higher at the higher contaminant levels when compared with the unamended treatments. Similar observations were made by González-Alejandre *et al*. (2018) where they observed up to 30-40% reversal of the inhibitory effects of Cr on *Zea mays* after P and Fe supplementation. While the results from the referenced study are notable, the results of my study show even more impressive reversal of inhibitory effects of 51% -68% for *Brassica juncea* and *Helianthus annuus* respectively after struvite supplementation under even more drastic conditions (303 mg/kg Pb and up to 1.6% SEO co-contamination in my study compared to 194 mg/kg Cr in the referenced study). This provides insights into the potential for struvite utilization as an amendment for the attenuation of the inhibitory effects of high Pb doses and SEO co-contaminated soils, and has significant sustainability implications from a circular economy standpoint. Wei *et al*. (2010) reported similar results with urea and chicken manure significantly increasing shoot dry weight of *Solanum nigrum L*. at 50 mg/kg Cadmium concentration. This increase in plant growth after nutrient supplementation could be due to an increase in nutrient availability which is usually subject to interference by various contaminant concentrations in soil (Walakulu Gamage *et al*. 2020).

Helianthus annuus and *Brassica juncea* displayed affinity for struvite and NPK supplementation at the various contaminant levels with NPK performing better generally at both contaminant levels for *Brassica juncea* and struvite performing better at 1.6% SEO + 10% mine-spoils for *Helianthus annuus*. These differences in specie response to amendment types under contaminant stress was also reported in Bryson and Barker (2007) which showed highest plant biomass production for Fescue in urea amended treatments when compared to calcium nitrate, manure and compost treatments in zinc polluted soils. Plant selectivity for different amendments was reported by Jidere, Akamigbo, and Ugwuanyi (2012) where cowpea had the highest yield when amended with 4 t/ha Poultry Droppings + 4 t/ha Cassava Peels + 8 t/ha NPK fertilizer, and maize on the other hand, had the highest yield when amended with 8 t/ha Poultry Droppings + 0 t/ha Cassava Peels + 4 t/ha NPK fertilizer in crude oil contaminated soils. This variation and affinity for various amendments could be related to the nutrient bioavailability of the nutrients in the various amendments and the ability of the plants to assimilate these nutrients in various soil conditions imposed by contaminant doses. This shows that there is no universal amendment that would generate the same results for all plant types in all contaminated soil situations, hence, the need to consider this while screening amendments for phytoremediation purposes to ensure that compatibility exists between plant, amendment, and contaminant type/concentration.

Finally, the results reported in the study by Jidere, Akamigbo, and Ugwuanyi (2012) which demonstrated the highest attenuation of inhibitory effects using a combination of nutrient amendments could be indicative of potential benefits of combining various amendment types, which warrants experimenting a combination of struvite fertilizers with other organic amendment types to explore the possibility to optimize the attenuation of inhibitory effects while maximizing the sustainability and circular economic benefits.

5.4 TPH Reductions in *Helianthus annuus* and *Brassica juncea* Soils Co-Contaminated with SEO and Mine-Spoils

TPH reductions of 66.4% and 55% at 0.8% SEO (containing 6, 550 mg/kg TPH) + 10% mine-spoils, and 39.7% - 41.3% recorded at 1.6% SEO (containing 16, 700 mg/kg TPH) + 10% mine-spoils were recorded for *Helianthus annuus* and *Brassica juncea* treatments respectively after 114 days in (**Figure *4.27***). This was significantly higher than the results reported by Nero (2021) who reported a 16.2% and 10.3% TPH reduction in soils containing 22, 666 mg/kg TPH treated with *Jatropha curcas* and *Vetiveria zizanioides* respectively after 112 days. Worthy of note is the fact that although the remediation duration was comparable, the TPH concentrations in the soil of the reference study was significantly higher than the highest TPH concentration in my study, indicating that the difference in phytoremediation performance between the species used in both studies might be less significant. Martins *et al*. (2014) reported TPH reduction of 10% by *Helianthus annuus* when compared with the control after 40 days in multi-contaminated soils. Comparing the results from the current study to the later, *Helianthus annuus* reduction of TPH was significantly higher in the current study and this could be attributed to experiment duration as the experiment duration of the current study was significantly longer than Martins *et al*. (2014). Although it is unlikely that the TPH reduction in the referenced study would match or exceed that of the current study, it can be argued that extending the experiment duration might be efficacious in decreasing the margin of disparity in the results of both studies.

The current study showed disparities in TPH reductions between both species and at various contaminant levels with TPH reductions for both species reducing significantly at the higher contaminant level (1.6% SEO + 10% mine-spoils). This could be attributed partly to disparities in plant biomass production which affects the rate of phytoremediation. This was observed as *Helianthus annuus* generally had a higher dry biomass than *Brassica juncea* which might explain why the former had higher TPH reductions compared to the former. Both plant species also showed significant reduction in dry biomass at 1.6% SEO + 10% mine-spoils when compared to 0.8% SEO + 10% mine-spoils which might explain why TPH reduction was significantly less for both species at 1.6% SEO + 10% mine-spoils. Effects of various plant species on petroleum degrading bacteria could also be responsible for the differences in TPH reduction by both species. Similar observations were reported by Xie *et al*. (2017) who reported higher petroleum microbiota in bristle grass soils when compared to alfalfa treated soils. They also reported that TPH reduction was 6.5% - 18.9% higher in bristle grass treatments when compared to alfalfa treatments, which further suggests that a relationship might exist between the ability of a specie to influence petroleum degrading microbial populations and the TPH reduction in soils. Their study also reported a decline in TPH reduction with increase in contamination, with TPH reductions being lower with higher biomass loss. This aligns the finding of the current study which suggests that plant biomass production, ability to increase petroleum degrading microbiota, and contamination levels all play an important role in the reduction of TPH in contaminated soils.

5.4.1 Effects of Nutrient Supplementation on TPH Reductions in SEO and Mine-Spoils Co-Contaminated Soils

The effects of nutrient supplementation on the TPH reduction in *Helianthus annuus* and *Brassica juncea* soils could be related to its effects on dry biomass production. This was only the case for *Helianthus annuus,* for instance, struvite supplementation only yielded positive results (up to 34.7%) in terms of reduction in TPH at 1.6% SEO + 10% mine-spoils when compared to the unamended treatments (**Figure *4.28***). This corresponds with the effects of struvite on the dry biomass of *Helianthus annuus* with dry biomass being 68% higher than unamended treatments at 1.6% SEO + 10% mine-spoils. Another instance is that struvite amended treatments were 34.8% and 18.1% higher than NPK treatments which yielded lower biomass at 0.8% SEO + 10% mine-spoils and 1.6% SEO + 10% mine-spoils respectively. Similar findings were reported by Nero (2021) whose findings suggest a relationship between increase in plant growth and TPH reduction. Their study showed that compost and fertilizer supplementation enhanced plant growth when compared to unamended treatments for *Jatropha curcas*. They reported that TPH reductions were also significantly higher in supplemented treatments in the order that they increased plant growth (compost >fertilizer>) when compared to the unamended treatments which aligns with the findings of the current study. However, when comparing the performance of struvite fertilizer used in my study to the NPK fertilizer used in their study in enhancing TPH reduction, struvite was more efficacious as it yielded a 34.7% improvement in TPH reduction with *Helianthus annuus* while NPK fertilizer yielded a 27.9% improvement in TPH reduction with *Jatropha curcas*in their study.

The present study showed contrasting results for *Brassica juncea*. This is because although struvite and NPK supplementation significantly increased dry biomass, TPH reductions were significantly higher in the unamended treatments when compared with treatments amended with struvite and NPK (**Figure *4.28*** and **Figure *4.30***). This could mean that although both amendments were beneficial for the growth and biomass production in *Brassica juncea*, they might have had a negative effect on the activities of petroleum degrading bacteria and these differences in microbial activities between amended and unamended treatments could account for why TPH reductions were less in amended treatments (Xie *et al*. 2017).

Comparing the performances of *Helianthus annuus* and *Brassica juncea* in the reduction of TPH in SEO and mining soil co-contaminated soils, *Helianthus annuus* proved to be a better specie making it a better choice for the phytoremediation of TPH. In terms of nutrient amendments used, struvite performed better than NPK for both plant species in the reduction of TPH, although none of the amendments were successful for *Brassica juncea* when compared with unamended treatments.

5.5 Total PAH Reductions in *Helianthus annuus* and *Brassica juncea* Soils Co-Contaminated with SEO and Mine-Spoils

Reduction of total PAHs in *Helianthus annuus* and *Brassica juncea* planted soils was only observed at 1.6% SEO + 10% mine-spoils with total PAH reductions of 43.6% and 40.4%

recorded for *Helianthus annuus* and *Brassica juncea* respectively when compared with the unplanted treatments (**Figure *4.32***). On the other hand, total PAH reduction at 0.8% SEO + 10% mine-spoils was higher in unplanted treatments than in planted treatments particularly when compared with *Brassica juncea* which had total PAH reduction that was 13% less than the unplanted treatments. This could partly be because the concentration of PAHs in the SEO was quite low especially at the 0.8% SEO + 10% mine-spoils where total PAHs were 3.6 mg/kg. The presence of high doses of Pb combined with lower microbial activities due to lower SEO concentration at that contamination level could have played a part in reducing the percentage of PAHs removed in the planted treatments. This is supported by Kluk and Steliga (2019) who reported a 2.1%, 2.1%, 2.7% and 5.9% reduction in the removal of naphthalene, phenanthrene, fluoranthene and chrysene respectively for *Helianthus annuus* treatments in soils co-contaminated with petroleum hydrocarbons and heavy metals when compared with treatments containing only petroleum hydrocarbons after a 6-month experimental period.

When comparing the performances of the two species used in my study, *Helianthus annuus* generally performed better especially at the 0.8% SEO + 10% mine-spoils where the former had total PAH reductions that was 11.3% higher than the later. This suggests that *Helianthus annuus* has potential as a phytoremediation specie for PAH contaminated soils. Similar observation was made by Zand and Hoveidi (2016) who reported a *Helianthus annuus* induced 49.42% reduction in petroleum hydrocarbons in soils polluted with 5000 mg/kg gasoline after 60 days. Kluk and Steliga (2019) also showed promising results for *Helianthus annuus* with a 23.9%, 21.2%, 21.6 and 13.3% reduction of naphthalene, phenanthrene, fluoranthene and chrysene respectively after a 6-month period when compared to the unplanted shows that it was better suited for the removal of PAHs. The results from these studies in conjunction with my study further demonstrate that *Helianthus annuus* is a viable option for the phytoremediation of soils contaminated with petroleum hydrocarbons, and the impacts of hydrocarbon co-contamination with heavy metals necessitates further experimentation with the use of amendments to attenuate the concomitant performance reductions with a view to enhance the versatility in its application.

5.5.1 Effects of Nutrient Amendments on the Reduction of Total PAHs in SEO and Mine-Spoils Co-Contaminated Soils

The results in sections **4.10** and **4.10.1** showed no significant differences between total PAH reduction in struvite amended and unamended treatments for both plant species except for *Helianthus annuus* at 1.6% SEO + 10% mine-spoils where total PAHs was 11% higher in the struvite amended treatments when compared to unamended treatments. However, NPK supplementation negatively impacted total PAHs reduction in *Helianthus annuus* treatments. This was evidenced with the 19% and 23.8% reduction in total PAHs dissipation at 0.8% SEO + 10% mine-spoils and 1.6% SEO + 10% mine-spoils respectively in *Helianthus annuus* treated soils. This corresponds with Olson *et al*. (2008) who reported significant reduction in PAH dissipation in NPK fertilizer amended treatments when compared to unamended and even unplanted treatments. They suspected this to be a result of competition for organic nutrients between plants, PAH degrading bacteria and non-PAH degrading microbial communities. This was because they observed a decline in PAH degrading bacterial population in planted soils with NPK supplementation when compared to the unplanted counterparts that also had NPK supplementation. However, this was not the case for *Brassica juncea* in the present study with total PAHs being generally higher in NPK treatments at both contamination levels when compared to struvite amended treatments although these differences were not statistically significant (**Figure *4.35***). When NPK was compared with struvite amendment, total PAH reduction was significantly higher in struvite amended *Helianthus annuus* planted treatments by 16.5% and 34.8% at 0.8% SEO + 10% mine-spoils and 1.6% SEO + 10% mine-spoils respectively (**Figure *4.34***). Although the differences between NPK and struvite amended treatments were not statistically significant for *Brassica juncea*, NPK amended treatments were 5.1% higher at 1.6% SEO + 10% mine-spoils when compared with unamended treatments. Plant root induced increased microbial activity and contact time between PAHs and microbes are instrumental for the phytoremediation of PAHs (Smith *et al*. 2011). This could mean that disparities in plant – amendment interaction with concomitant effects on PAH bioavailability and microbial breakdown could account for the selectivity of both plant species for different amendments in terms of successful total PAH reduction. This was particularly true in the present study as both plant species exhibited varying affinities for nutrient amendments with struvite being most effective for *Helianthus annuus* and NPK for *Brassica juncea*.

Worthy of note is that both amendments only proved to be significantly successful at 1.6% SEO + 10% mine-spoils which could be attributed to microbial responses to SEO concentrations as petroleum degrading bacteria are known to increase in soils with high concentrations of petroleum hydrocarbons. Similar trends were observed by Gao *et al*. (2022) who reported a 3.91%–57.01% increase in *Proteobacteria* phylum abundance in soils with heavy petroleum contamination. Haim and Al-Ani (2019) also reported similar findings where concentrations of kerosine, diesel and waste engine oil significantly increased hydrocarbon utilizing bacterial populations in soils.

Overall, both soil amendments used in the current study showed benign potential for enhancing total PAH reductions when compared to the reduction of Pb in soils co-

contaminated with SEO and mine-spoils. This is consistent with literature that has shown relatively poor to mediocre results using inorganic fertilizers to enhance the phytoremediation of petroleum hydrocarbons (Nwaichi et al. 2015, Olson *et al.* 2008). However, combining the fertilizers used in the present study with organic nutrient amendment types like poultry droppings and cassava peels might be more optimal for the simultaneous phytoremediation of heavy metals and petroleum hydrocarbons in co-contaminated soils (Jidere, Akamigbo, and Ugwuanyi 2012).

5.6 Phytoremediation of Pb by *Helianthus annuus* and *Brassica juncea* in Soils Co-Contaminated with SEO and Mine-Spoils

The significantly higher Pb reduction in planted treatments (32.5% and 20.3% for *Helianthus annuus* and *brassica juncea* respectively) when compared to unplanted treatments is indicative of the fact that both species had positive impacts on Pb dissipation in SEO and mine-spoils co-contaminated soils (**Figure** ***4.36***). Comparable results can be seen in a study by Shehata, Badawy, and Aboulsoud (2019) who reported a 31.1%, 37.37%, 11.26% and 24.52% dissipation of Co, Cr, Cd and Mn respectively in *Hibiscus cannabinus L*. planted soils. *Helianthus annuus* had the highest percentage reduction of Pb in soil when compared to *Brassica juncea* and unplanted treatments and these could be attributed to biomass production as the former had significantly higher dry biomass compared to the latter. Biomass production has been associated with greater heavy metal uptake in contaminated soils and this was demonstrated in the present study with Pb reductions being 12.2% and 17.7% higher in *Helianthus annuus* soils when compared to *Brassica juncea* soils at 0.8% SEO + 10% mine-spoils and 1.6% SEO + 10% mine-spoils respectively (**Figure** ***4.15***). This was most likely the case as the reduction of Pb was observed to reduce at the higher contaminant doses for both species. This could be attributed to the lower biomass production by both plant species at the higher contaminant dose (1.6% SEO + 10% mine-spoils). This was supported by Lothe, Hansda, and Kumar (2016) who reported the highest Cu removal efficiency of 42% for *Brassica nigra* at the lowest contamination level where it's biomass production was higher.

The present study further showed an indication of a relationship between biomass production and uptake of heavy metals, and this was evidenced by *Helianthus annuus* having an 82% higher total Pb uptake when compared to total Pb uptake in *Brassica juncea.* The differences in Pb uptake and reduction in co-contaminated soils could be due to their varying tolerances for contaminant stress and *Helianthus annuus* exhibited far superior tolerance for contaminant stress when compared to *Brassica juncea*.

Overall, the results of the comparison between *Helianthus annuus* and *Brassica juncea* in terms of uptake of Pb as well as reduction of Pb concentration in contaminated soils, and this is consistent with all the above sections. This is an indication that the former has more potential for use in the phytoremediation of Pb in hydrocarbon and heavy metal co-contaminated soils when compared to the later.

5.6.1 Effects of Nutrient Supplementation on the Phytoremediation of Pb by *Helianthus annuus* and *Brassica juncea* in Soils Co-Contaminated with SEO and Mine-Spoils

Various forms of nutrient supplementation have been employed in phytoremediation studies to combat the reduction in nutrient bioavailability and uptake by plants in soils contaminated with petroleum hydrocarbons and heavy metals (Wei *et al*. 2010; Bryson and Barker 2007;

González-Alejandre *et al*. 2018). The present study experimented with NPK and Struvite fertilizers (**sections 4.11.1** and **4.11.2**), and struvite fertilizers either had no significant effect on or significantly inhibited the reduction in Pb concentrations in *Helianthus annuus* and *Brassica juncea* planted soils except for *Helianthus annuus* at 0.8% SEO + 10% mine-spoils where Pb reduction was 8.1% higher in struvite amended treatments when compared with the unamended treatments (**Figure *4.37*** and **Figure *4.38***). NPK however, had significantly lower total Pb reduction in *Helianthus annuus* planted soils while the opposite was observed in *Brassica juncea* planted pots (**Figure *4.39*** and **Figure *4.40***). This could be due to the potential effects of plant-amendment interaction on metal bioavailability and solubility which influence root uptake of metal, and ultimately lead to metal loses from soils via plant uptake (Rieuwerts *et al*. 2015). Regardless of the disparities in effects of the nutrient amendments studied, struvite and *Helianthus annuus* combination recorded the highest total Pb reduction (49.5% Pb reduction).

For total Pb uptake, *Helianthus annuus* and *Brassica juncea* showed varied responses to struvite and NPK fertilizers. For instance, struvite yielded better results for *Helianthus annuus* when compared to NPK treatments and on the other hand, NPK yielded better results for *Brassicca juncea* treatments when compared to struvite treatments (see **Figure *4.44*** and **Figure *4.45***). This could be because the plant-amendment interaction might have affected the soil pH, organic matter content and cation exchange capacity, all of which are important factors affecting the uptake of heavy metals in soils as pointed out by Jung (2008). Similarly, Cataldo and Wildung (1978) argued that metabolic processes related to nutrient absorption by plant roots regulate the selectivity and uptake rates of specific non-nutrient ions. This could mean that NPK and struvite fertilizers might have affected the affinity of *Helianthus annuus* and *Brassica juncea* for Pb uptake and that might account for the disparities in results achieved by both nutrient amendments. Overall, struvite fertilizer in tandem with *Helianthus annuus* yielded the highest enhancement of Pb uptake reaching 82.8% increase at 1.6% SEO + 10% mine-spoils when compared with the unamended counterpart. In comparison to the results obtained for NPK in a study by However, *Helianthus annuus* and struvite fertilizer combination proved to be the most efficacious in the total uptake of Pb when compared with all other treatments, thereby, making this a promising combination for the phytoremediation of Pb in hydrocarbon and heavy metal contaminated soils.

5.6.2 Fate of Contaminated Plant Biomass Post-Phytoremediation

The uptake of contaminants by plants, albeit beneficial for the phytoremediation process, raises questions and concerns surrounding secondary contamination if contaminated plant biomass is not properly handled/disposed of at the end of a Phyto-management cycle. This has prompted research into safe disposal and re-use possibilities of contaminated biomass post-phytoremediation. Zhong et al. (2015) pointed out that aside environmental considerations related to the safe disposal of contaminated biomass from phytoremediation, economic viability plays an important role in the choice of disposal method. In view of this sentiment, a review on disposal and utilization of phytoremediation species containing heavy metals by Liu and Tran (2021) showed that heat treatment (particularly incineration) could be a viable disposal method. This is because it not only significantly reduces the volume of biomass generated with concomitant benefits for transportation, but also has other potential benefits from the incineration process like power generation from excess heat production. Concerns surrounding volatilization and evaporation of copious amounts of heavy metals into the atmosphere from incinerating contaminated biomass have been raised but a study by Wu et al. (2013) revealed that kaolin and activated carbon significantly reduced the concentrations of Cd and PAHs in the flue gas during the incineration of contaminated *Sedum* *plumbizincicola* biomass in a laboratory-scale entrained flow tube furnace. Although more trials might be required to test the efficacy of kaolin and activated carbon in reducing contaminant volatilization into the atmosphere, the results from the referenced study seemed promising in combating the environmental risks related to incineration, thereby making incineration an even more attractive choice for disposal.

Aside disposal, the biomass produced from phytoremediation projects also present opportunities for more sustainable practices from a life cycle assessment standpoint. This is particularly relevant as the biomass generated could serve as valuable feedstock for the green-energy industry in terms of production of biodiesels, bioethanol, biofuels, and power generation from heat generated through incineration (Grifoni *et al*. 2021). This opens multiple opportunities like reducing demand on arable land used in the cultivation of energy crops for feedstock supply to renewable energy industries, giving economic value to biomass from phytoremediation projects and converting contaminated land which is often viewed as waste lands to valuable sites for renewable energy biomass feedstock production.

Furthermore, they provide fibers suitable for textiles and other fiber-based industrial applications, presenting a renewable resource for the textile industry. Moreover, the oil extracted from its seeds finds utilization in various industrial products such as cosmetics, lubricants, and soaps, underscoring its importance in manufacturing sectors. *Brassica juncea* and *Helianthus annuus* have demonstrated potential as biopesticides, offering an eco-friendly alternative to synthetic chemical pesticides in agriculture, contributing to sustainable farming practices (Popova, Dubie, and Morra 2017, Nchimbi 2020, Acheuk et al. 2022, Mirpoor, Giosafatto, and Porta 2021). They can also be used as green manure improves soil structure, boosts water retention, and inhibits weed growth, leading to enhanced soil health and fertility. This eco-friendly approach also reduces reliance on synthetic fertilizers, promoting sustainable agricultural practices and contributing to long-term environmental sustainability.

Chapter 6: CONCLUSION

The investigation of the phytoremediation potential of *Helianthus annuus* and *Brassica juncea* in hydrocarbon and hydrocarbon – heavy metal co-contamination scenarios, nutrient supplementation, and mixed cropping agronomic practices, presented a rigorous assessment of the phytoremediation potential of both species. Plant growth and development under contaminant stress are important indicators of the suitability of species for phytoremediation. Although both species were significantly impacted under contaminant stress in terms of germination, height, laminar leaf area, number of leaves and dry biomass production, *Helianthus annuus* was significantly more tolerant than *Brassica juncea* under contaminant stress. In terms of phytoremediation of TPH, total PAHs and Pb, both species significantly reduced contaminant concentrations in soils and were able to uptake Pb in contaminated soils. However, *Helianthus annuus* performed significantly better than *Brassica juncea* in the reduction of TPH, total PAHs and Pb and in the total uptake of Pb, making it a more suitable phytoremediation species.

Mixed cropping is an agronomic practice that has been used to improve crop yield, thus, possesses potential benefits for enhancing crop growth under contaminant stress. The present studied showed compatibility issues between *Helianthus annuus* and *Brassica juncea* in unpolluted and SEO polluted treatments which manifested in growth inhibitions in unpolluted treatments and no notable differences in SEO contaminated soils when compared with single crop treatments. This proves that combining both said species would not be a suitable phytoremediation enhancement in hydrocarbon contaminated soils. Nutrient supplementation with NPK and Struvite fertilizers proved beneficial on improving the growth, total Pb uptake and dissipation of Pb, TPH and total PAHs in co-contaminated soils. However, struvite fertilizer was most promising in improving contaminant dissipation, Pb uptake and growth under contaminant stress when combined with *Helianthus annuus*.

Overall, *Helianthus annuus* has more potential as a phytoremediation species for low to medium SEO contaminated soils in comparison to *Brassica juncea*. However, the length of time required for complete soil treatment could present a bottleneck in the commercialization of this technology. Other factors that could affect the economic viability of using these species is the fact that they are both important economic crops, which creates the dilemma as to what would constitute more responsible use of the species especially from a food security standpoint. However, a different perspective could be drawn from the potential uses they could be put to after phytoremediation projects. This presents an opportunity for material injection into the circular economy as they have applications in non-edible contexts like the production of biodiesel, bioplastics, biopesticides, cosmetics, provision of green manure, contributing to carbon offset and capture during large scale phytoremediation projects, carbon savings from the utilization of less carbon intensive remediation alternatives, and the remediation of land which could be utilized for agricultural purposes with upsides for food security. At the end of the day, taking a more balanced and view with careful consideration of all the positive downstream cascade of opportunities highlighted above helps to better put things into perspective.

Finally, the overarching goal of this research is to serve as a pivotal step towards a promising future, where the principles of the circular economy guide our decisions and actions, fostering a harmonious coexistence with our planet. With a collective dedication to sustainable practices, we can forge a greener, more equitable world that cherishes both the well-being of humanity and the preservation of our precious environment.

6.1 LIMITATIONS

Despite the success the present study had in terms of fulfilling its aim and objectives, there were limitations to this study which would ultimately create opportunities for further studies. One key limitation was significant loss of time from limited laboratory access because of the Covid-19 pandemic and other lab delays from equipment setups and instrument training. These delays limited the amount of laboratory studies (such as microbial studies, plant analysis for TPH and PAHs and extensive characterization of the SEO used) which could have potentially been carried out. The loss of time also impacted the amount of greenhouse experiments that could be carried out especially taking into consideration the amount of time required for growing of crops. This meant that further phytoremediation trials could not be carried out to determine the total amount of time required for both species to completely clean up the contaminated soils with and without soil amendments.

The study was limited to greenhouse simulations of the phytoremediation of contaminated soils, and without field trials, it is limited to being an academic study. Measurement of Pb uptake by both species as total uptake without examining Pb concentrations in the different parts of the plants was a created a lack of understanding of the mechanisms of remediation under different treatment conditions, hence, presenting a limitation in this study. Lastly, the study was limited to a single heavy metal (Pb) because of the absence of more heavy metals in the mine spoils used for the co-contaminated soil substrate.

6.2 Recommendations for Further Studies

The findings and limitations of this study present opportunities for further studies in the following areas

- Investigating the potential root and shoot uptake of TPH and PAHs in both SEO contaminated soils and SEO and mining soil co-contaminated soils.
- Extensive microbial studies to identify microbial communities and investigate the impact of individual species, mixed cropping, and nutrient amendments on the activities of petroleum degrading microorganisms in SEO contaminated soils.
- Investigate combination of NPK and Struvite fertilizers at various ratios to determine if any combination ratio could be beneficial in reducing contaminant induced stress and in enhancing contaminant dissipation and uptake.
- Experimenting more doses of struvite amendment to determine the optimum dose for the best results.

- Repeated phytoremediation cycles to determine the time required for the total remediation of hydrocarbon and heavy metals contaminated soils by *Helianthus annuus* and *Brassica juncea*.
- Conducting field scale studies would be recommended as this would provide more information on real life/practical applications of the findings of this study.
- Sourcing mine spoils containing multiple heavy metals and varying the concentration of mine spoils in co-contamination with SEO is recommended to facilitate the study of the phytoremediation of various concentrations of multiple heavy metals in this type of co-contamination.

Chapter 7: References

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Chapter 8: Appendices

8.1 Appendix A

Two-Factor ANOVA Comparing the Differences in Percentage Germination of *Helianthus annuus* and *Brassica juncea* in Soils with Various SEO Concentrations

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	138.2976	1	138.2976	1535.31	2.8692E-23	4.26
Columns	47125.6882	5	9425.13764	104633.33	3.1483E-51	2.62
Interaction	172.8564	5	34.57128	383.79	4.4975E-22	2.62
Within	2.16186667	24	0.09007778			
Total	47439.0041	35				

8.2 Appendix B

Two-Factor ANOVA Comparing the Differences in the Height of *Helianthus annuus* and *Brassica juncea* in SEO Polluted and Unpolluted Soils

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	198.046875	1	198.046875	228.900795	3.605E-07	5.31765507
Columns	275.041875	1	275.041875	317.890922	1.0029E-07	5.31765507
Interaction	0.541875	1	0.541875	0.62629425	0.45154134	5.31765507
Within	6.92166667	8	0.86520833			
Total	480.552292	11				

Two-Factor ANOVA Comparing the Differences in the Laminar Leaf Area of *Helianthus annuus* and *Brassica juncea* in SEO Polluted and Unpolluted Soils

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	151.514133	1	151.514133	2822.8064	1.7461E-11	5.31765507
Columns	717.9627	1	717.9627	13376.1099	3.4911E-14	5.31765507
Interaction	93.2976333	1	93.2976333	1738.19531	1.2068E-10	5.31765507
Within	0.4294	8	0.053675			
Total	963.203867	11				

Two-Factor ANOVA Comparing the Differences in the Number of Leaves of *Helianthus annuus* and *Brassica juncea* in SEO Polluted and Unpolluted Soils

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	18.75	1	18.75	75	2.4568E-05	5.31765507
Columns	52.0833333	1	52.0833333	208.333333	5.191E-07	5.31765507
Interaction	4.08333333	1	4.08333333	16.3333333	0.00372822	5.31765507
Within	2	8	0.25			
Total	76.9166667	11				

8.3 Appendix C

Two-Factor ANOVA Comparing the Differences in the Height of *Helianthus annuus* in Mixed Cropping and Unmixed Treatments in SEO Polluted and Unpolluted Treatments

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	80.341875	1	80.341875	31.7165	0.0005	5.3177
Columns	96.0502083	1	96.0502083	37.9177	0.0003	5.3177
Interaction	36.5752083	1	36.5752083	14.4388	0.0052	5.3177
Within	20.265	8	2.533125			
Total	233.232292	11				

Two-Factor ANOVA Comparing the Differences in the Laminar Leaf Area of *Helianthus annuus* in Mixed Cropping and Unmixed Treatments in SEO Polluted and Unpolluted Treatments

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	115.816533	1	115.816533	154.17	1.6523E-06	5.32
Columns	681.616133	1	681.616133	907.34	1.6011E-09	5.32
Interaction	107.042133	1	107.042133	142.49	2.2317E-06	5.32
Within	6.0098	8	0.751225			
Total	910.4846	11				

Two-Factor ANOVA Comparing the Differences in the Number of Leaves of *Helianthus annuus* in Mixed Cropping and Unmixed Treatments in SEO Polluted and Unpolluted Treatments

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	0.08333333	1	0.08333333	0.5	0.49957589	5.32
Columns	18.75	1	18.75	112.5	5.4594E-06	5.32
Interaction	0.75	1	0.75	4.5	0.066688	5.32
Within	1.33333333	8	0.16666667			
Total	20.9166667	11				

Two-Factor ANOVA Comparing the Differences in the Height of *Brassica juncea* in Mixed Cropping and Unmixed Treatments in SEO Polluted and Unpolluted Treatments

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	41.4408333	1	41.4408333	23.6186179	0.0012560	5.3176551
Columns	546.75	1	546.75	311.6124436	0.0000001	5.3176551
Interaction	36.75	1	36.75	20.9451437	0.0018101	5.3176551
Within	14.0366667	8	1.75458333			
Total	638.9775	11				

Two-Factor ANOVA Comparing the Differences in the Laminar Leaf Area of *Brassica juncea* in Mixed Cropping and Unmixed Treatments in SEO Polluted and Unpolluted Treatments

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	20.0725333	1	20.0725333	449.30	2.5791E-08	5.32
Columns	164.7243	1	164.7243	3687.17	6.0125E-12	5.32
Interaction	18.5008333	1	18.5008333	414.12	3.5546E-08	5.32
Within	0.3574	8	0.044675			
Total	203.655067	11				

Two-Factor ANOVA Comparing the Differences in the Number of Leaves of *Brassica juncea* in Mixed Cropping and Unmixed Treatments in SEO Polluted and Unpolluted Treatments

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	0.33333333	1	0.33333333	1	0.34659351	5.32
Columns	96.3333333	1	96.3333333	289	1.4552E-07	5.32
Interaction	0.33333333	1	0.33333333	1	0.34659351	5.32
Within	2.66666667	8	0.33333333			
Total	99.6666667	11				

8.4 Appendix D

Two-Factor ANOVA on the Effects of SEO and Mine-Spoils Co-Contamination on the Height of *Helianthus annuus* and *Brassica juncea*

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	106.58	1	106.58	65.0318644	3.464E-06	4.74722535
Columns	2623.18778	2	1311.59389	800.294576	1.698E-13	3.88529383
Interaction	195.43	2	97.715	59.6227119	5.8423E-07	3.88529383
Within	19.6666667	12	1.63888889			
Total	2944.86444	17				

Two-Factor ANOVA on the Effects of SEO and Mine-Spoils Co-Contamination on the Number of Leaves of *Helianthus annuus* and *Brassica juncea*

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	3.55555556	1	3.55555556	21.3333333	0.00059141	4.74722535
Columns	70.1111111	2	35.0555556	210.333333	4.5516E-10	3.88529383
Interaction	2.77777778	2	1.38888889	8.33333333	0.00538052	3.88529383
Within	2	12	0.16666667			
Total	78.4444444	17				

Two-Factor ANOVA on the Effects of SEO and Mine-Spoils Co-Contamination on the Laminar Leaf Area of *Helianthus annuus* and *Brassica juncea*

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	14.2222222	1	14.2222222	8.36327997	0.01352746	4.74722535
Columns	761.547778	2	380.773889	223.911467	3.159E-10	3.88529383
Interaction	75.6144444	2	37.8072222	22.232277	9.2137E-05	3.88529383
Within	20.4066667	12	1.70055556			
Total	871.791111	17				

8.5 Appendix E

Two-Factor ANOVA Comparing the Percentage Reduction in the Height of *Helianthus annuus* and *Brassica juncea* in Soils Co-Contaminated with SEO and Mine-Spoils

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	2647.77338	1	2647.77338	425.34	3.1999E-08	5.32
Columns	2798.70454	1	2798.70454	449.59	2.5727E-08	5.32
Interaction	195.793485	1	195.793485	31.45	0.00050536	5.32
Within	49.8004776	8	6.2250597			
Total	5692.07188	11				

Two-Factor ANOVA Comparing the Percentage Reduction in the Number of Leaves of *Helianthus annuus and Brassica juncea* in Soils Co-Contaminated with SEO and Mine-Spoils

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	504.403333	1	504.403333	20.03	0.00206835	5.32
Columns	1482.96333	1	1482.96333	58.89	5.8854E-05	5.32
Interaction	448.963333	1	448.963333	17.83	0.0029067	5.32
Within	201.466667	8	25.1833333			
Total	2637.79667	11				

Two-Factor ANOVA Comparing the Differences Percentage Reduction in the Laminar Leaf Area of *Helianthus annuus and Brassica juncea* in Soils Co-Contaminated with SEO and Mine-Spoils

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	651.213333	1	651.213333	22.20	0.00151774	5.32
Columns	1260.75	1	1260.75	42.98	0.0001773	5.32
Interaction	250.253333	1	250.253333	8.53	0.01926129	5.32
Within	234.64	8	29.33			
Total	2396.85667	11				

Two-Factor ANOVA Comparing the Dry Biomass of *Helianthus annuus* and *Brassica juncea* in SEO and Mine-Spoils Co-Contaminated Soils

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	208.4201389	1	208.4201389	306.31	6.5828E-10	4.75
Columns	447.2308333	2	223.6154167	328.64	3.322E-11	3.89
Interaction	76.46527778	2	38.23263889	56.19	8.0646E-07	3.89
Within	8.165	12	0.680416667			
Total	740.28125	17				

8.6 Appendix F

Two-Factor ANOVA Comparing the Differences in Height of *Helianthus annuus* in Struvite Amended and Unamended Soils with SEO and Mine-Spoils Co-Contamination

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	5.20083333	1	5.20083333	3.54	0.09676367	5.32
Columns	91.3008333	1	91.3008333	62.11	4.8637E-05	5.32
Interaction	175.5675	1	175.5675	119.43	4.358E-06	5.32
Within	11.76	8	1.47			
Total	283.829167	11				

Two-Factor ANOVA Comparing the Differences in Laminar Leaf Area of *Helianthus annuus* in Struvite Amended and Unamended Soils with SEO and Mine-Spoils Co-Contamination

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	1.76333333	1	1.76333333	14.90	0.00480733	5.32
Columns	11.6033333	1	11.6033333	98.06	9.133E-06	5.32
Interaction	6.16333333	1	6.16333333	52.08	9.0933E-05	5.32
Within	0.94666667	8	0.11833333			
Total	20.4766667	11				

Two-Factor ANOVA Comparing the Differences in Number of Leaves of *Helianthus annuus* in Struvite Amended and Unamended Soils with SEO and Mine-Spoils Co-Contamination

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	0.75	1	0.75	1	0.34659351	5.32
Columns	2.08333333	1	2.08333333	2.78	0.13414064	5.32
Interaction	2.08333333	1	2.08333333	2.78	0.13414064	5.32
Within	6	8	0.75			
Total	10.9166667	11				

Two-Factor ANOVA Comparing the Differences in Height of *Brassica juncea* in Struvite Amended and Unamended Soils with SEO and Mine-Spoils Co-Contamination

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	156.240833	1	156.240833	149.39	1.8634E-06	5.32
Columns	505.700833	1	505.700833	483.54	1.9312E-08	5.32
Interaction	58.5208333	1	58.5208333	55.96	7.0577E-05	5.32
Within	8.36666667	8	1.04583333			
Total	728.829167	11				

Two-Factor ANOVA Comparing the Differences in Laminar Leaf Area of *Brassica juncea* in Struvite Amended and Unamended Soils with SEO and Mine-Spoils Co-Contamination

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	6.75	1	6.75	82.65	1.7204E-05	5.32
Columns	37.4533333	1	37.4533333	458.61	2.379E-08	5.32
Interaction	5.33333333	1	5.33333333	65.31	4.0603E-05	5.32
Within	0.65333333	8	0.08166667			
Total	50.19	11				

Two-Factor ANOVA Comparing the Differences in Number of Leaves of *Brassica juncea* in Struvite Amended and Unamended Soils with SEO and Mine-Spoils Co-Contamination

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	0.08333333	1	0.08333333	0.5	0.49957589	5.32
Columns	36.75	1	36.75	220.5	4.1672E-07	5.32
Interaction	0.08333333	1	0.08333333	0.5	0.49957589	5.32
Within	1.33333333	8	0.16666667			
Total	38.25	11				

8.7 Appendix G

Two-Factor ANOVA Comparing the Differences in the Dry Biomass of *Helianthus annuus* in Struvite Amended and Unamended Soils Co-Contaminated with SEO and Mine-Spoils

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	15.7552083	1	15.7552083	21.06	0.00178072	5.32
Columns	36.5752083	1	36.5752083	48.89	0.00011353	5.32
Interaction	25.0852083	1	25.0852083	33.53	0.00040954	5.32
Within	5.985	8	0.748125			
Total	83.400625	11				

Two-Factor ANOVA Comparing the Differences in the Dry Biomass of *Brassica juncea* in Struvite Amended and Unamended Soils Co-Contaminated with SEO and Mine-Spoils

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	4.32	1	4.32	27.43	0.00078593	5.32
Columns	24.0833333	1	24.0833333	152.91	1.7049E-06	5.32
Interaction	3.20333333	1	3.20333333	20.34	0.00197624	5.32
Within	1.26	8	0.1575			
Total	32.8666667	11				

Two-Factor ANOVA Comparing the Differences in the Height of *Helianthus annuus* in Struvite and NPK amended Soils Co-Contaminated with SEO and Mine-Spoils

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	0.10083333	1	0.10083333	0.09	0.77	5.32
Columns	0.10083333	1	0.10083333	0.09	0.77	5.32
Interaction	11.4075	1	11.4075	10.54	0.01	5.32
Within	8.66	8	1.0825			
Total	20.2691667	11				

Two-Factor ANOVA Comparing the Differences in the Laminar Leaf Area of *Helianthus annuus* in Struvite and NPK amended Soils Co-Contaminated with SEO and Mine-Spoils

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	29.7675	1	29.7675	425.25	3.2026E-08	5.32
Columns	46.0208333	1	46.0208333	657.44	5.7396E-09	5.32
Interaction	34.3408333	1	34.3408333	490.58	1.8242E-08	5.32
Within	0.56	8	0.07			
Total	110.689167	11				

Two-Factor ANOVA Comparing the Differences in the Number of Leaves of *Helianthus annuus* in Struvite and NPK amended Soils Co-Contaminated with SEO and Mine-Spoils

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	0.33333333	1	0.33333333	0.25	0.63	5.32
Columns	0.33333333	1	0.33333333	0.25	0.63	5.32
Interaction	0.33333333	1	0.33333333	0.25	0.63	5.32
Within	10.6666667	8	1.33333333			
Total	11.6666667	11				

Two-Factor ANOVA Comparing the Differences in the Height of *Brassica juncea* in Struvite and NPK amended Soils Co-Contaminated with SEO and Mine-Spoils

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	149.1075	1	149.1075	181.29	8.8761E-07	5.32
Columns	254.840833	1	254.840833	309.84	1.1087E-07	5.32
Interaction	200.900833	1	200.900833	244.26	2.8018E-07	5.32
Within	6.58	8	0.8225			
Total	611.429167	11				

Two-Factor ANOVA Comparing the Differences in the Laminar Leaf Area of *Brassica juncea* in Struvite and NPK amended Soils Co-Contaminated with SEO and Mine-Spoils

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	136.6875	1	136.6875	559.81	1.0836E-08	5.32
Columns	2.52083333	1	2.52083333	10.32	0.01236597	5.32
Interaction	100.340833	1	100.340833	410.95	3.6636E-08	5.32
Within	1.95333333	8	0.24416667			
Total	241.5025	11				

Two-Factor ANOVA Comparing the Differences in the Number of Leaves of *Brassica juncea* in Struvite and NPK Amended Soils Co-Contaminated with SEO and Mine-Spoils

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	12	1	12	18	0.0028	5.3177
Columns	27	1	27	40.5	0.0002	5.3177
Interaction	1.33333333	1	1.33333333	2	0.1950	5.3177
Within	5.33333333	8	0.66666667			
Total	45.6666667	11				

Two-Factor ANOVA Comparing the Dry Biomass of *Helianthus annuus* in Struvite and NPK Amended Soils Co-Contaminated with SEO and Mine-Spoils

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	6.60083333	1	6.60083333	38.08	0.00026776	5.32
Columns	3.10083333	1	3.10083333	17.89	0.00287797	5.32
Interaction	0.52083333	1	0.52083333	3.00	0.12124719	5.32
Within	1.38666667	8	0.17333333			
Total	11.6091667	11				

Two-Factor ANOVA Comparing the Dry Biomass of *Brassica juncea* in Struvite and NPK Amended Soils Co-Contaminated with SEO and Mine-Spoils

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	5.88	1	5.88	51.13	9.7039E-05	5.32
Columns	6.163333333	1	6.16333333	53.59	8.2219E-05	5.32
Interaction	17.76333333	1	17.7633333	154.46	1.6403E-06	5.32
Within	0.92	8	0.115			
Total	30.72666667	11				

8.8 Appendix H

Two-Factor ANOVA Comparing the Differences in Percentage TPH Reduction Between *Helianthus annuus* and Unplanted Treatments in Soils Co-Contaminated with SEO Concentrations and Mine-

Spoils

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	8448.213333	1	8448.21333	4423	2.9071E-12	5.32
Columns	1008.333333	1	1008.33333	528	1.3659E-08	5.32
Interaction	533.3333333	1	533.333333	279	1.6641E-07	5.32
Within	15.28	8	1.91			
Total	10005.16	11				

Two-Factor ANOVA Comparing the Differences in Percentage TPH Reduction Between *Brassica juncea* and Unplanted Treatments in Soils Co-Contaminated with SEO Concentrations and Mine-Spoils

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	6960.083333	1	6960.08333	565.1712	1.0436E-08	5.31765507
Columns	420.0833333	1	420.083333	34.1115171	0.00038692	5.31765507
Interaction	140.0833333	1	140.083333	11.3750169	0.00974669	5.31765507
Within	98.52	8	12.315			
Total	7618.77	11				

Two-Factor ANOVA Comparing the Differences in Percentage TPH Reduction Between *Brassica juncea* and *Helianthus annuus* in Soils Co-Contaminated with SEO Concentrations and Mine-Spoils

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	72.03	1	72.03	5.08	0.05	5.3
Columns	1900.083333	1	1900.08333	133.98	2.8205E-06	5.3
Interaction	126.75	1	126.75	8.94	0.02	5.3
Within	113.4533333	8	14.1816667			
Total	2212.316667	11				

8.9 Appendix I

Two-Factor ANOVA Comparing the Differences in Percentage TPH Reduction Between Struvite Amended *Helianthus annuus* soils and Unamended *Helianthus annuus* soils co-contaminated with SEO and Mine-Spoils

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	880.6533333	1	880.653333	262.7	2.1113E-07	5.3
Columns	599.2533333	1	599.253333	178.7	9.3707E-07	5.3
Interaction	922.2533333	1	922.253333	275.1	1.7639E-07	5.3
Within	26.82	8	3.3525			
Total	2428.98	11				

Two-Factor ANOVA Comparing the Differences in Percentage TPH Reduction Between Struvite Amended *Brassica juncea* soils and Unamended *Brassica* junce soils co-contaminated with SEO and Mine-Spoils

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	132.6675	1	132.6675	9.4	0.01533198	5.3
Columns	646.8008333	1	646.800833	46.0	0.00014065	5.3
Interaction	47.60083333	1	47.6008333	3.4	0.10315092	5.3
Within	112.56	8	14.07			
Total	939.6291667	11				

Two-Factor ANOVA Comparing the Differences in Percentage Reduction of TPH in *Helianthus annuus* with Struvite and NPK Amendment in Soils Co-Contaminated with SEO and Mine-Spoils

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	2088.240833	1	2088.24083	639.42	6.4066E-09	5.32
Columns	414.1875	1	414.1875	126.82	3.4731E-06	5.32
Interaction	209.1675	1	209.1675	64.05	4.3553E-05	5.32
Within	26.12666667	8	3.26583333			
Total	2737.7225	11				

Two-Factor ANOVA Comparing the Differences in Percentage Reduction of TPH in *Brassica juncea* with Struvite and NPK Amendment in Soils Co-Contaminated with SEO and Mine-Spoils

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	105.020833	1	105.020833	31.49	0.00050337	5.32
Columns	769.600833	1	769.600833	230.76	3.4933E-07	5.32
Interaction	84.8008333	1	84.8008333	25.43	0.00099843	5.32
Within	26.68	8	3.335			
Total	986.1025	11				

8.10 Appendix J

Two-Factor ANOVA Comparing the Differences in Percentage Total PAH Reduction Between *Helianthus annuus* and Unplanted Treatments in Soils Co-Contaminated with SEO Concentrations and Mine-Spoils

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	1324.2603	1	1324.2603	486.27	1.8888E-08	5.32
Columns	2478.537633	1	2478.53763	910.12	1.5817E-09	5.32
Interaction	1539.973633	1	1539.97363	565.48	1.0413E-08	5.32
Within	21.7864	8	2.7233			
Total	5364.557967	11				

Two-Factor ANOVA Comparing the Differences in Percentage Total PAH Reduction Between *Brassica juncea* and Unplanted Treatments in Soils Co-Contaminated with SEO Concentrations and Mine-Spoils

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	553.5208333	1	553.520833	80.50	1.8956E-05	5.32
Columns	1803.200833	1	1803.20083	262.25	2.125E-07	5.32
Interaction	2168.140833	1	2168.14083	315.33	1.0351E-07	5.32
Within	55.00666667	8	6.87583333			
Total	4579.869167	11				

Two-Factor ANOVA Comparing the Differences in Percentage Total PAH Reduction Between *Brassica juncea* and *Helianthus annuus* in Soils Co-Contaminated with SEO Concentrations and Mine-Spoils

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	165.0208333	1	165.020833	22.606	0.001	5.318
Columns	10.2675	1	10.2675	1.407	0.270	5.318
Interaction	53.34083333	1	53.3408333	7.307	0.027	5.318
Within	58.4	8	7.3			
Total	287.0291667	11				

8.11 Appendix K

Two-Factor ANOVA Comparing the Differences in Percentage Total PAH Reduction Between Struvite Amended *Helianthus annuus* soils and Unamended *Helianthus annuus* soils co-contaminated with SEO and Mine-Spoils

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	53.50963333	1	53.5096333	17.66	0.00298436	5.32
Columns	1.293633333	1	1.29363333	0.43	0.53176281	5.32
Interaction	136.4176333	1	136.417633	45.04	0.00015101	5.32
Within	24.23306667	8	3.02913333			
Total	215.4539667	11				

Two-Factor ANOVA Comparing the Differences in Percentage Total PAH Reduction Between Struvite Amended *Brassica juncea* soils and Unamended *Brassica juncea* soils co-contaminated with SEO and Mine-Spoils

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	9.013333333	1	9.01333333	1.04	0.33716386	5.32
Columns	29.45333333	1	29.4533333	3.41	0.10217449	5.32
Interaction	3.63	1	3.63	0.42	0.53519751	5.32
Within	69.18	8	8.6475			
Total	111.2766667	11				

Two-Factor ANOVA Comparing the Differences in Percentage Reduction of Total PAH in *Helianthus annuus* with Struvite and NPK Amendment in Soils Co-Contaminated with SEO and Mine-Spoils

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	1976.333333	1	1976.33333	847	2.1037E-09	5.32
Columns	9.013333333	1	9.01333333	3.86	0.08494384	5.32
Interaction	250.2533333	1	250.253333	107.25	6.5322E-06	5.32
Within	18.66666667	8	2.33333333			
Total	2254.266667	11				

Two-Factor ANOVA Comparing the Differences in Percentage Reduction of Total PAH in *Brassica juncea* with Struvite and NPK Amendment in Soils Co-Contaminated with SEO and Mine-Spoils

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	13.33520833	1	13.3352083	3.39	0.10272814	5.32
Columns	61.88020833	1	61.8802083	15.74	0.00413067	5.32
Interaction	0.285208333	1	0.28520833	0.07	0.79445118	5.32
Within	31.44166667	8	3.93020833			
Total	106.9422917	11				

8.12 Appendix L

Two-Factor ANOVA Comparing the Differences in the Percentage Pb Reduction in *Helianthus annuus* and Unplanted Soils Co-Contaminated with SEO and Mine-Spoils

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	2343.6075	1	2343.6075	537.94	1.2683E-08	5.32
Columns	42.1875	1	42.1875	9.68	0.01440574	5.32
Interaction	63.0208333	1	63.0208333	14.47	0.00521183	5.32
Within	34.8533333	8	4.35666667			
Total	2483.66917	11				

Two-Factor ANOVA Comparing the Differences in the Percentage Pb Reduction in *Brassica juncea* and Unplanted Soils Co-Contaminated with SEO and Mine-Spoils

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	516.140833	1	516.140833	358.43	6.2672E-08	5.32
Columns	123.520833	1	123.520833	85.78	1.5004E-05	5.32
Interaction	157.6875	1	157.6875	109.51	6.0419E-06	5.32
Within	11.52	8	1.44			
Total	808.869167	11				

Two-Factor ANOVA Comparing Differences in the Percentage Pb Reduction in *Helianthus annuus* and *Brassica juncea* Soils Co-Contaminated with SEO and Mine-Spoils

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	536.827222	1	536.827222	73.34	1.8593E-06	4.75
Columns	13865.44	2	6932.72	947.16	6.2216E-14	3.89
Interaction	159.004444	2	79.5022222	10.86	0.00202996	3.89
Within	87.8333333	12	7.31944444			
Total	14649.105	17				

8.13 Appendix M

Two-Factor ANOVA Comparing the Differences in the Percentage Reduction of Pb in Struvite Amended and Unamended *Helianthus* annus Soils Co-Contaminated with SEO and Mine-Spoils

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	1.6875	1	1.6875	0.13	0.73021459	5.32
Columns	728.520833	1	728.520833	55.07	7.4691E-05	5.32
Interaction	157.6875	1	157.6875	11.92	0.00866299	5.32
Within	105.833333	8	13.2291667			
Total	993.729167	11				

Two-Factor ANOVA Comparing the Differences in the Percentage Reduction of Pb in Struvite Amended and Unamended *Brassica juncea* Soils Co-Contaminated with SEO and Mine-Spoils

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	0.02083333	1	0.02083333	0.011	0.9173747	5.32
Columns	518.7675	1	518.7675	285.429	1.5276E-07	5.32
Interaction	0.80083333	1	0.80083333	0.441	0.52548561	5.32
Within	14.54	8	1.8175			
Total	534.129167	11				

Two-Factor ANOVA Comparing the Differences in Percentage Pb Reduction in Struvite and NPK Amended *Helianthus annuus* Soils Co-Contaminated with SEO and Mine-Spoils

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	818.400833	1	818.400833	89.77	1.268E-05	5.32
Columns	815.100833	1	815.100833	89.41	1.2872E-05	5.32
Interaction	120.9675	1	120.9675	13.27	0.00656384	5.32
Within	72.9333333	8	9.11666667			
Total	1827.4025	11				

Two-Factor ANOVA Comparing the Differences in Percentage Pb Reduction in Struvite and NPK Amended *Brassica juncea* Soils Co-Contaminated with SEO and Mine-Spoils

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	105.020833	1	105.020833	130.06	3.1573E-06	5.32
Columns	277.440833	1	277.440833	343.58	7.398E-08	5.32
Interaction	27.3008333	1	27.3008333	33.81	0.0003985	5.32
Within	6.46	8	0.8075			
Total	416.2225	11				

8.14 Appendix N

Two-Factor ANOVA Comparing the Differences in Total Pb Uptake of *Helianthus annuus* and *Brassica juncea* in SEO and Mine-Spoils Co-Contaminated Soils

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	8.4672	1	8.4672	72576	4.0353E-17	5.32
Columns	10.3788	1	10.3788	88961.14	1.7876E-17	5.32
Interaction	6.45333333	1	6.45333333	55314.29	1.1958E-16	5.32
Within	0.00093333	8	0.00011667			
Total	25.3002667	11				

Two-Factor ANOVA Comparing the Differences in Total Pb Uptake by *Helianthus annuus* in Struvite Amended and Unamended Soils Co-Contaminated with SEO and Mine-Spoils

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	9.04803333	1	9.04803333	113.02	5.3658E-06	5.32
Columns	22.3041333	1	22.3041333	278.60	1.6789E-07	5.32
Interaction	1.17813333	1	1.17813333	14.72	0.00497439	5.32
Within	0.64046667	8	0.08005833			
Total	33.1707667	11				

Two-Factor ANOVA Comparing the Differences in Total Pb Uptake by *Brassica juncea* in Struvite Amended and Unamended Soils Co-Contaminated with SEO and Mine-Spoils

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	0.00853333	1	0.00853333	17.3559322	0.00313908	5.31765507
Columns	0.3468	1	0.3468	705.355932	4.3446E-09	5.31765507
Interaction	0.00853333	1	0.00853333	17.3559322	0.00313908	5.31765507
Within	0.00393333	8	0.00049167			
Total	0.3678	11				

8.15 Appendix O

Two-Factor ANOVA Comparing the Differences in Total Pb Uptake by *Helianthus annuus* Amended with Struvite and NPK in Soils Co-Contaminated with SEO and Mine-Spoils

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	7.00740833	1	7.00740833	69.49	3.2426E-05	5.32
Columns	4.70000833	1	4.70000833	46.61	0.00013405	5.32
Interaction	2.15900833	1	2.15900833	21.41	0.00169406	5.32
Within	0.80666667	8	0.10083333			
Total	14.6730917	11				

Two-Factor ANOVA Comparing the Differences in Total Pb Uptake by *Brassica juncea* Amended with Struvite and NPK in Soils Co-Contaminated with SEO and Mine-Spoils

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	3.3708	1	3.3708	486.17	1.8903E-08	5.32
Columns	0.08333333	1	0.08333333	12.02	0.00848159	5.32
Interaction	1.2288	1	1.2288	177.23	9.683E-07	5.32
Within	0.05546667	8	0.00693333			
Total	4.7384	11				