PHYTOREMEDIATION OF POTENTIALLY TOXIC METALS CONTAMINATED AGRICULTURAL SOIL USING PUTATIVE *Brassica napus* AND *Raphanus raphanistrum* IN UASIN GISHU COUNTY, KENYA

BY

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DECLARATION

Declaration by the Student

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DEDICATION

To my dear parents, whose love for education guaranteed that I achieved and fulfilled the dream they had longed for.

ABSTRACT

Potentially toxic metals (PTMs), Arsenic (As), Cadmium (Cd), Chromium (Cr), Mercury (Hg), and Lead (Pb) naturally occur in soil within the environment, but human activities such as largescale farming have increased PTMs concentrations in agricultural soils resulting from the use of agrochemicals, sludge, and wastewater irrigation. This leads to PTM contamination of agrarian soil, making such soil a probable source of ecological and health risks. Hence, this study aimed to assess the concentrations and possibility of phytoremediation of PTMs in agricultural soils from selected farms in Kaprobu, Kosyin, Moiben, Naiberi, and Ziwa in Uasin Gishu County, Kenya. *Brassica napus* (canola) and *Raphanus raphanistrum* (wild radish) were used in this study. The selected PTM concentrations within the study areas, physicochemical parameters, and *in vitro* bioaccessibility in agricultural soils were determined using standard methods. In addition, wild Brassicaceae, *Brassica napus,* and *Raphanus raphanistrum* were identified and chemically treated for the possibility of phytoremediation of the polluted soils. Field surveys and completely randomized experimental designs were adopted to collect soil and seed samples. Standard lab procedures were applied to determine PTM concentrations in soil samples, physicochemical parameters of soil, *in vitro* bioaccessibility, seed germination rate, colchicine modification of seeds, and phytoremediation of PTMs in soils. Descriptive statistics, regression analysis, and analysis of variance (ANOVA) were used in analyzing the data, and the results are presented in tables and graphs. The mean concentrations of PTMs in agricultural soils ranged from 2.90 to 6.40 mg/Kg As, 0.06 to 0.13 mg/Kg Cd, 14.31 to 48.19 mg/Kg Cr, and 16.46 to 35.89 mg/Kg Pb, while Hg was not detected (ND). Chromium and Lead had relatively high concentrations across the study areas as Moiben recorded the highest of the two, 48.19 mg/Kg Cr and 35.89 mg/Kg Pb. Physicochemical parameters, pH, organic matter (%OC), Al^{+3} (Cmol+kg-1), and H^{+} (Cmol+kg-1) in agricultural soils from Moiben were low. *In vitro* bioaccessibility measured was low, 0.77% Cr and 11.88% Pb. *Raphanus raphanistrum* (RR) and *Brassica napus (BN)* were selected among locally identified Brassicaceae species and their germination rates were tested using germination agents. Gibberellic acid (GA3) gave an efficiency of 80% and 90% for RR and BN, respectively. The seeds were further treated with different concentrations of colchicine to heighten growth and morphological development in possible enhanced phytoremediation of PTMs to agricultural soil. The PTMs assessment results showed that Cr and As were above the USEPA agricultural soil regulatory standards. The assessed ecological risk indices ranged from low to extremely high Geo-Accumulation Factors for all PTMs, and low to moderate Ecological Risk Index. Health risks assessed via work-related exposures to agricultural soils posed no significant carcinogenic and non-carcinogenic risks. A negative correlation was recorded between the physicochemical parameters, soil pH, organic matter, and *in vitro* bioaccessibility of Pb and Cr. The putative mutant plants, *B.napus* and *R.raphanistrum* treated with a 0.50% dose of colchicine had hyperaccumulation potential at M_1 and M_2 generations for Cr and Pb decontamination. Both plants bioaccumulated high amounts of metals, Cr and Pb that could pose environmental and health risks. This study finding contributes greatly to enhanced phytoremediation techniques in environmental restoration that can be cascaded on different PTMs contaminated fields.

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LIST OF ABBREVIATIONS AND ACRONYMS

Abbreviation	Definition
ADI	Average Daily Intake
AAS	Atomic Absorption Spectroscopy
BARGE	BioAccessibility Research Group Europe
BCR	Community Bureau of Reference
BioAC	Bioaccessibility
$\mathbf{B_n}$	Background value of PTMs in Preindustrial soil
BN	*Brassica napus*
CEC	Cation Exchange Capacity
$\mathbf{C_{deg}}$	Degree of contamination of PTMs in soil
$\mathbf{C_n}$	The concentration of PTMs in Soil
CR	Carcinogenic Risk
CSF	Cancer Slow Factor
CTR	Target Carcinogenic Risk
Eqn	Equation
GI	Germination Index
HI	Hazard Index
HQ	Hazard Quotient
Igeo	Geo-accumulation factor
ISO	International Organization for Standard
IWD	Inverse Distance Weighted
ICP-MS	Inductively Coupled Plasma Mass Spectrometer
MAD	Microwave-Assisted Digester/Digestion
MGT	Mean Germination Time

MTF	Metal Transfer Factor
PBET	Physiologically Based Extraction Test
PTMs	Potentially Toxic Metals
RBALP	Relative Bioavailability Leaching Procedure
RI	Ecological risk index
RIVM	in vitro Digestion Model
rpm	Run per minute
RR	*Raphanus raphanistrum*
SBRC	Solubility Bioaccessibility Research Consortium assay
SDGs	Sustainable Development Goals
TOC	Total Organic Carbon
TsD	Days of the first count
UBM	United Barge Method United
USEPA	The United States Environmental Protection Agency
WHO	World Health Organization

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CHAPTER ONE

INTRODUCTION

1.1 Background

Potentially toxic metals/metalloids (PTMs) refer to a group of elements particularly found in the environment according to Pourret *et al.* (2019), that have a high potential to cause toxicity to humans and other organisms in trace amounts. Some commonly found PTMs in the environment are Arsenic (As), Cadmium (Cd), Chromium (Cr), Mercury (Hg), and Lead (Pb). PTMs are naturally occurring elements in the soil in disproportionate amounts. However, anthropogenic activities resulting from an accelerated rate of population growth and demand for food production have been linked to increased extensive farming as reported by Nazli *et al.* (2020). Increased agricultural activities and nutrient-leached soils have resulted in increased use of chemical amendments, recycled sewerage sludge, and surface runoff irrigation to replenish macro and micronutrients have led to an increase in the amounts of PTMs in agrarian soils. Further, atmospheric deposition from farm machinery exhaust emissions in mechanized farming has led to an increased distribution of PTMs in agricultural soil (Qin *et al.*, 2021). PTMs-contaminated soils are likely sources of ecological and health risks in the environment (Munishi *et al.*, 2021). Increased PTMs concentrations in agricultural soils subsequently lead to bioaccumulation of the metals into plants and crops and may result in phytotoxicity as described by Nazli *et al.*, (2020). As in the case of agrochemicals (fertilizers and pesticides) use to improve farm soil consequently leads to phytoextraction of PTMs in plants, hence their ultimate bioaccumulation, bioconcentration, and bioavailability in the food chain (Saha *et al.*, 2017). This may result in deleterious effects on human health, thus latest technology and electrochemical apt sensors are used with high sensitivity, specificity, and accuracy to determine Hg, Pb, Cd, and As in food and other consumer products. This is a promising technology that rapidly determines PTM concentrations online with high confident levels according to Wang *et al.* (2020).

Various states of potentially toxic metals exist within soils. The existing states of PTMs, the physicochemical properties, and the geochemistry of the surrounding environments have a substantial effect on the concentration levels in soils and the process by which PTMs are transferred into crops (Adamo *et al.*, 2018). This biogeochemistry also exacerbates the probable ecological and health risks associated with PTMs. Several studies have reported that human exposure to PTMs including As, Cd, Cr, Hg, and Pb may cause mutagenic, carcinogenic, and genotoxic effects as reported by Mishra *et al.* (2019). Moreover, humans, both adults, and children are exposed to PTMs in agro-ecological zones through multiple routes of exposure, but most frequently through the dietary intake of contaminated foods, incidental absorption of polluted soil, bodily absorption of polluted soil, and inhalation of polluted dust. Depending on the duration and measurable doses of chemical species (As, Cd, Cr, Hg, or Pb) by an individual or organism, these metals may cause organ toxicity and public health issues in a population (Tchounwou *et al.*, 2012). Albeit, among these multiple exposure routes aforementioned, dietary intake of vegetables, fruits, cereals, and other foodstuffs are the most common routes of PTMs into humans (Agrelli, Duri, *et al.*, 2020; Nawab *et al.*, 2018). PTMs generally are not biodegradable inorganic chemical species that remain in the environment for a longer time period during which time they mutate and potentially induce environmental and health issues (Khan and Sajad, 2013). Research reports from different parts of the World including Asia and Africa have shown that contamination of food as a result of PTMs is critical in human health exposure assessment (Liu *et al.*, 2013). In Kenya, a study on PTMs showed that there are high levels of trace elements-As, Cd, Cr, Cu, Pb, Hg, Ni, and Zn in agricultural soils above the World Health Organization (WHO) allowable limits as reported by Mungai *et al.* (2016). Also, a study of PTMs pollutions in reclaimed farmlands linked the increased levels of PTMs in soils to inorganic fertilizers amendments and atmospheric deposition from fossil fuel combustions as reported by Gu *et al.* (2014).

To minimize the likely associated impacts of PTMs in agriculture soils and consequently in the food chain, several remediation techniques including physical soil replacement, chemical leaching, microbial digestion, and industrial methods are available (Gang *et al.*, 2010). Unfortunately, a number of these methods are costly, labor-intensive, and time-consuming. Many of the challenges are largely managed by bioremediation, particularly the use of plant phytoremediation (Sidhu, 2016). Phytoremediation is an eco-friendly bioremediation technology used to decontaminate polluted soils using plants (Wuana *et al.*, 2011). This environmental abatement technology is less expensive and sustainable. It involves the use of known plant species, for example, Brassicaceae that are grown onsite for hyperaccumulation (extract and store) of potentially toxic metals, absorb volatile compounds and stabilize PTMs in soils (Dowling & Doty, 2009). The phytoremediation mechanism involves extraction, filtration, and stabilization of potentially toxic metals from contaminated soils using macrophytes (Mani & Kumar, 2014; Nwoko, 2010). It can be achieved directly by planting the identified plants or indirectly by enhancing the identified plant's PTM uptake capacity. Enhanced phytoremediation involves the use of physical and chemical agents to induce hyperaccumulation in selected plants through chromosomal or genetic modification to increase PTMs absorption by plants or the use of chemical reagents to rise the bioavailability of PTMs in soil (Rahman *et al.*, 2016). It can also include the use of induced seed mutation that comprises of pretreatment of selected seeds by physical, chemical, or radial means to yield anticipated variants (Oladosu *et al.,* 2016). An induced seed mutation technique is widely used in plant breeding to cultivate plant varieties that can adapt to different environmental stress conditions through gene modification or dormancy breaking (Ahloowalia *et al.*, 2004). Physically induced mutation encompasses irradiation of seeds using gamma rays or neutrons, whereas chemical mutation involves the use of chemical compounds such as Ethyl methyl sulfonate (EMS) and N-Nitroso-N-Methyl urea (NMU) and colchicine to initiate variants in a plant species. The latter has proved effective and yielded comparative results with physical irradiation in mutagenicity (Kharkwal, 1998; Wani, 2017). Many plants and enhancement techniques have been tested in phytoremediation studies. Some techniques treat seeds, while others use roots and stems. Plants typically called hyperaccumulating agents have drawn more research attention due to their capacity to remove and store excess PTMs in their biomass (Vamerali *et al.*, 2010). The family Brassicaceae is dominant among groups of plants classified as hyperaccumulators. Several species of Brassicaceae are known to efficiently phytoextract potentially toxic metals from soils. Hence, the transformation of these Brassicaceae plants through chemical enhancement will provide a promising future for the biological remediation of PTMs in an environmentally friendly manner (Agnihotri *et al.*, 2019). The chemical reagent, colchicine is widely used in to improve growth characteristics, increase biomass, and support environmental stress resistance. In the process, colchicine interferes with mitosis and causes the doubling of chromosomes in plant cells. This process is influenced by numerous factors, mainly the concentration of colchicine dose, exposure duration, and explant materials (Eng *et al.*, 2019).

Brassicaceae plants have been modified using colchicine and provided likely characteristic traits in the family. Colchicine, $C_{22}H_{25}NO_6$ (Figure 1.1) is a mitotic poison and also use as a medication to treat gout (Nasr *et al.*, 2020).

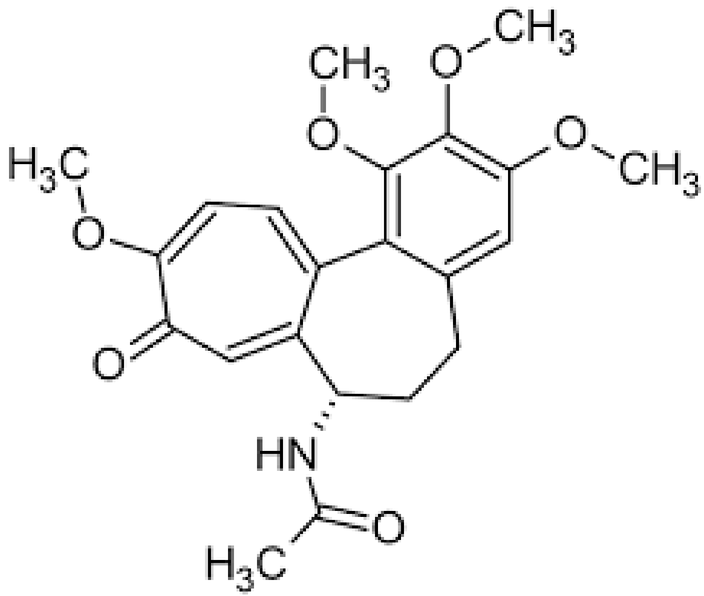

Figure 1.1: Colchicine structural formula

Several studies have reported that the colchicine enhancement technique improved plants' growth, and physical and biological resistance among the many advantages conferred by plants (Chen *et al.*, 2022; Mwathi *et al.*, 2020). *B. napus* and *R. raphanistrum* are members of this big family of Brassicaceae that are wildly distributed in the environment. The latter is an aggressive species as a result of its prolific seed production, is resistant to numerous herbicides, and reduces loss in crop yield (Kebaso *et al.*, 2020). Though some studies have shown it contains beneficial compounds enhancement of *Raphanus raphanistrum* to decontaminate PTMs in polluted soil will provide an additional value, phytoremediation to what is called Harvest weed seed control (HWSC) (Sun *et al.*, 2021).

1.2 Statement of the Problem

PTMs Cr and Pb are essentially useful industrial elements found in the environment that support life. Notwithstanding, many PTMs are toxic that bioaccumulate in living organisms via physical interaction (UNEP/GPA, 2006). Common sources of PTMs in the environment are agricultural fields, mine tailing, pesticide application on farms, fertilizers production, and applications in farming. In Kenya, agriculture contributes about 33% to the GDP, it provides job opportunities for more than 40% percent of the entire population, and 70% of the rural population (USAID, 2022). Uasin Gishu County is among the central farming areas referred to as the breadbasket in the Rift Valley region of Kenya. Increased farming activities, especially large-scale farming uses more agrochemicals to maximize yields. Inorganic metals including Cd, Pb, Cr, and As are among the active constituent ingredients of synthetic fertilizers and pesticides used in agriculture. These metals cause ecological and health risks to the environment through soil and water pollution, used to grow food. Contaminated food consumption leads to numerous human health threats and risks to the viability of the ecosystem (Sarwar *et al.*, 2017). The continuous application of agrochemicals in farming including recycled sludge results in PTMs buildup in agricultural soils that subsequently bioaccumulate into crops. Different PTMs are known to cause several health effects in humans such as teratogenicity, cancer, and retarded brain development, especially in children. As a result, remediation of PTMs contaminated soils is an essential pillar of sustainable ecological and environmental management practice.

Hence, to curb the probable ecological and health risks that result from induced anthropogenic high concentrations of PTMs in the environment, the phytoremediation technique is essentially promising. Phytoremediation is an inexpensive, easily achievable green technology that can certainly be workable almost in every setting. However, plants' morphometries including low biomass and limited growth rate reduce extensive utilization of natural phytoremediation. Therefore, this study focuses on the possibility of enhanced phytoremediation, which involves chemical treatment of *B. napus* and *R. raphanistrum* to increase PTMs, Cr, and Pb phytoextraction from contaminated soils and subsequently reduce their likely associated adverse effects.

1.3 Objectives

1.3.1 General Objective

This study aims to assess PTMs: As, Cd, Cr, Hg, and Pb concentrations and the possibility of enhanced phytoremediation using *R. raphanistrum* (wild radish) and *B. napus* (canola) plants to reduce the concentration of Cr and Pb in agricultural soils in Moiben Uasin Gishu County.

1.3.2 Specific Objectives

1. To assess concentrations of PTMs As, Cd, Cr, Hg, and Pb in agricultural soils from selected farms in Uasin Gishu County;
2. To determine the levels of physicochemical parameters: pH, organic carbon, $Al^{+3,}$ and H^{+} in soil from the study areas;
3. To determine *in vitro* bioaccessibility (IVB) of PTMs and evaluate their ecological and health risks in agricultural soils in the study area;
4. To identify and determine the germination rate of *R. raphanistrum* and *B. napus* seeds from the study areas;
5. To assess the possibility of phytoremediation of PTMs contaminated soil using modified *B. napus* and *R. raphanistrum*.

1.4 Research Questions

1. What are the concentration levels of As, Cd, Cr, Hg, and Pb in agricultural soils in the selected study areas?
2. What are the levels of physicochemical parameters of the soils in the study area?
3. What are the *in vitro* bioaccessibility levels, ecological risk, and health risk levels of Cr and Pb in contaminated soil?
4. What are the germination rates of wild *B. napus* and *R. raphanistrum* seeds?
5. How effective are *B. napus* and *R. raphanistrum* in the phytoremediation of Cr and Pb in soil?

1.5 Justification of the Study

Uasin Gishu County is one of the areas called the national breadbasket of Kenya. This area relies on agriculture for socioeconomic and sustainable livelihood practices. It is a major supplier of foods including maize, wheat, and animal feeds to the local and national markets. High production and supply of agricultural produce heavily depend on amplified agrochemicals inputs that source and increase the flow of PTM concentrations along the food chain. High levels of PTMs in the food supply are the recipe for potential health risks to the consuming populace. Human exposure to PTMs causes a health burden to society. In many developing countries, most diseases and death are caused by soil, water, and air pollution (Murray *et al.*, 2012). Environmental pollution is the primary cause of more than a quarter of global death (Xu *et al.*, 2018). In this light, this study involves the use of wild Brassicaceae plants– *B. napus* and *R. raphanistrum* to bioremediate selected potentially toxic metals in agricultural contaminated soils. The study adopts enhanced phytoremediation techniques to induce the plants' hyperaccumulation capacity in PTMs decontamination of polluted agricultural soils. This will improve agricultural soil health and reduce ecological and health risks in the study areas. This is in line with the framed environmentally sensitive development program, the United Nations Sustainable Development Goals (SDGs) that many countries follow to achieve (Farmer, 2017). The SDGs sets goals and targets, including goals 1, 6, and 11 to 15, aiming at the soil, water, and air pollution controls through research, innovation, and technology transfer for health generation (WHO, 2016).

1.6 Scope of the study

This research work centers on the assessment and enhanced phytoremediation of potentially toxic metals in agricultural soils using chemically modified *Brassica napus* and *Raphanus raphanistrum* to reduce concentrations of the PTMs in soils and lower the probable associated ecological and health risks impacts. This study also put direct emphasis on enhanced phytoremediation taking into account the determination of soil PTM concentrations, measurement of physicochemical characteristics of the soil and their influence on *in vitro* bioaccessibility, wild Brassicaceae plant identification, germination, and modification for application in PTMs phytoremediation. Furthermore, the study determined the ecological and health risks of PTMs, as well as probable cancer and non-cancer risks of exposed individuals through oral incidental absorption and bodily absorption. In addition, the research investigates the processes of chemical colchicine dosing on Brassicaceae plants and their potential efficiency to uptake PTMs Cr and PB from contaminated soil. It also recommends appropriately enhanced phytoremediation of PTMs polluted agricultural soils in the study areas.

CHAPTER TWO

LITERATURE REVIEW

2.1 Background Information

The agricultural food production system feeds over seven million people globally, despite this fact, it is a major contributor to environmental deterioration (Clark *et al.*, 2017). Agriculture has been identified as one of the human activities that contribute to high levels PTMs in soils, according to several studies that have been conducted in different countries and regions across the globe. (Huang *et al.*, 2019; Nouri *et al.*, 2008; Shifaw, 2018). The buildup of non-biodegradable potentially toxic metals in food chains causes ecological and health hazards, as well as a reduction in the ecosystem's capacity to support human life, plant life, and animal life. These risks are caused by an increase in PTMs in soils (Masindi *et al.*, 2018). The manufacturing of industrialized food also adds to the contamination of the environment and the acceleration of climate change. The contribution of Africa's agricultural sector to global greenhouse gas emissions is significantly rising, with East Africa taking the top rank on the ladders in the continent (Tongwane *et al.*, 2018). The effects of climate change are already being felt, particularly in the agricultural sector of Kenya, where a large number of farmers who rely on the rain-fed agriculture system are dealing with the unpredictable rainfall pattern and rising temperature caused by global warming (Kogo *et al.*, 2021). PTMs in the environment are typically caused by pollutants from vehicles, agricultural inputs, and wastewater from industrial sources that have been absorbed into the soil aggregates (Proshad *et al.*, 2021). Agricultural amendments, most commonly fertilizers are responsible for high levels of PTMs, most notably cadmium, in agricultural soils.

Micro-dosing helps to keep PTM pollution in agricultural soils to a minimum, especially when combined with sustainable farming practices (Aune *et al.*, 2015). In addition, conservation agricultural methods such as organic farming and bioremediation techniques including phytoremediation, mycoremediation, and improved micro-bacterial remediation are promising possible solutions to the problem of PTM contamination in agricultural soils (Ali *et al.*, 2015). It is possible to bind PTMs and render them inaccessible to plants and other organisms through a process known as the immobilization of potentially toxic metals using appropriate chemical reagents. It is vital to increase crop production while simultaneously reducing the quantity of PTMs that are found in agrarian soils. This can be accomplished by making use of one or more of the numerous inorganic and organic amendments which are already available on the market (Sharma *et al.*, 2018).

2.2 Sources of Potentially Toxic Metals in the Environment

Environmental pollution is a worldwide issue that affects the ecosystem in full. Pollutants revolve from industrial wastes and are transferred to the environment including agricultural fields in crops, livestock, and consequently to man over the food chain. Discussions on the health and ecological impacts of potentially toxic metals and environmental pollution have been highlighted in many scientific symposiums and conferences in the recent past as reported by Okereafor *et al.* (2020). As reported by Fei *et al.* (2020), agricultural activities and industrial actions are among the key drivers of PTMs: Cd, As, Hg, and Pb pollution in soils in China. In Kenya, several studies have reported that potentially toxic elements pollution in water, soils, and sediments comes from diverse natural and anthropogenic sources including agriculture, industries, mining, and smelting as reported by Githaiga *et al.*

(2021); Mungai *et al.* (2019). Agricultural produce quality can be negatively impacted when there is an excessive amount of pollution in agricultural soils caused by potentially toxic metals. The continued use of metal-containing inorganic fertilizers and pesticides causes high amounts of pollutant compounds in agricultural soil, which can then be passed into the food produced therein (Kelepertzis, 2014). Also, waste disposal sites, municipal effluents, and industrial byproducts are often overloaded with high amounts PTMs that are dispersed to the environment through irrigation and flood (Opaluwa *et al.*, 2012). Increased concentrations of PTMs including Cd, Cr, Pb, Hg, and As within an environment are commonly found in soil, air, and water samples including sediment. These elements are potential a threat to the human health and plants that are exposed to them. Children who are exposed to potentially toxic metals are more likely to acquire neurotoxic deficits and morphological deformities in their developing body parts (Rahman *et al.*, 2019). Additionally, potentially toxic metal pollution has a major negative effect on the microbial ecology of the soil. Increased levels of harmful metal concentrations affect the number, variety, and bioactivity of soil microbes (Xie *et al.*, 2016).

The existence of PTMs in agricultural and urban soils has given rise to environmental concerns about the sensitive matter in many different areas (Zhang, Zhu, *et al.*, 2018). Waterbody and sediments are impacted by land use activities in human settlements such as agriculture, road transportation, and industrial plants, these activities cause silting of surface runoff, which increases the concentration of PTMs in these environmental media. (Zeng *et al.*, 2020). Other human activities, such as the requirement of extracting jewelry and valuable metals in mines close to agricultural land, are key contributors to the widespread distribution of PTMs in contaminated terrestrial and aquatic environments. These elements are not biodegradable, so they build up in the soil and contaminate food. These locations present a health threat to human, particularly the children population's environmental health (Ali, Khan, *et al.*, 2013). Increasing levels of toxic metals in soil, plants, and the atmosphere come from industries, combustion of fossil fuels in transportation, chemical wastes, inorganic fertilizers, and pesticide use in agriculture (Falahi-Ardakani, 1984).

The extraction of natural resources generates high concentrations of potentially toxic metals in the environment. High potential ecological risks assessment of metals in soils, crops, and human hair in China's Xiaoqinling gold mining region showed high exposure to potentially toxic metals-Hg, Cd, Pb, Cu, Cr, As, and Zn, respectively (Wu *et al.*, 2010). Similarly, research on toxic elements in Africa showed a steady accumulation of toxic metals in vegetables, fish, water, soil, and animal feed above acceptable limits, especially Cd and Pb (Yabe *et al.*, 2010). Potentially toxic metals pollution evaluation in the environment is made possible using several ecological and risk assessments including single and multiple elements indices. Different methods are used to evaluate different environmental matrices-water, air, sediment, and soil. Widely used indices include the contamination factor (Cf), Geo-accumulation Factor/index (Igeo), enrichment factor (CEF), risk index (RI), and contamination security index (CSI), which together they give provide detailed insight into potentially toxic metals contamination within the environment (Kowalska *et al.*, 2018). The contamination factor (Cf) determines the contamination of PTMs by measuring the ratio of the concentration in sediment and/or soil to the background levels of sediment and soil. The geo-accumulation index measures the anthropogenic pollution of the soils and compares the contamination of individual potentially toxic metals in the selected study areas in different concentration ranges. It is used to check PTM pollution in soils and sediments (Hassaan *et al.*, 2016). The ecological risk index is also used to assess PTM contamination in sediment and/or soil. It was devised by Hakanson (Hakanson, 1980).

Therefore, in-depth monitoring is required to reduce the excessive buildup of potentially toxic metals in the food chain through agricultural and industrial activities. Small-scale artisanal gold mining uses mercury to extract gold; this accounts for about 10% of the universal anthropogenic emission and environmental pollution resulting from Hg (Lacerda, 1997; Veiga *et al.*, 2006). Another focal source of potentially toxic metal contamination in developing countries is electronic waste. A study conducted at Lagos Alaba International e-waste Market showed that potentially toxic metal concentrations in soil and water around the site exceeded the accepted World Health Organization (WHO) levels (Olafisoye *et al.*, 2013). Likewise, in Guiyu, the most prominent e-waste dismantling and processing center in China, environmental and human health assessments from dust sampled in the nearby environs showed that surrounding communities were adversely impacted, putting residents' health at high risk (Leung *et al.*, 2008). In e-wastes from modern technology, equipment such as Liquid Crystal Displays (LCD) and Plasma televisions could present lesser potentially toxic metals toxicity to humans compared to the previous Cathode-Ray tubes (CRTs) televisions. Cathode-ray televisions are heavily built with high Hg content that makes hazardous waste during disposal (Lim *et al.*, 2010). In addition, in many developing countries landfills and open dumpsites receive unsorted wastes from construction work, manufacturing industries, municipalities, and households that contained PTMs and eventually end up in the soils at these sites. Through leaching and siltation, these metals spread into surrounding soils, groundwater, and nearby river that are used for gardening, irrigation, and other domestic activities (Gworek *et al.*, 2016).

2.3 *In vitro* bioaccessibility

The measurement of *in vitro* bioaccessibility of potentially toxic metals such as Arsenic, Cadmium, Chromium, and Lead in soils is a critical pollution assessment venture. As reported in many previous studies, i*n vitro bioaccessibility* of PTMs greatly depends on the soil's physicochemical properties (Du *et al.*, 2020; Zhu *et al.*, 2016). The physicochemical parameters of soils and sediment such as soil texture, particle size, pH, electric conductivity, cation exchange capacity, and organic matter are crucial to the bioaccessibility and bioavailability of potentially toxic metals in soil (Motsara, 2008; Yao *et al.*, 2013). The bioavailability of toxic metals in soil is the amount of As, Cr, Cd, and Pb available for animals, plants, or organisms uptake that can physiologically enhance bioaccumulation or cause supplementary effects in plants from the sum of available As, Cd, Cr, and Pb present in the soil (Kim *et al.*, 2015). Similarly, it measures the amount of PTMs that are available for absorption into the systemic organic from the bloodstream in the animal. There are several methods of bioavailability analysis in soil samples including the Tessier Community Bureau of Reference (BCR) (Zimmerman *et al.*, 2010). And *in vitro* methods, including relative bioaccessibility leaching procedure (RBALP), Bioaccessibility Research Group of Europe (BARGE), Unified BRGE Method (UBM), Solubility/Bioaccessibility Research Center (SBRC), Physiologically Based Extraction Test (PBET), the United States Environmental Protection Agency method 1340 (USEPA) and the National Institute for Public Health and Environment in the Netherlands (RIVM). Depending on the technique chosen, PTMs pollution level in soil, and extraction techniques, the results of these methods may vary to some extent (Mungai *et al.*,, 2016). *In vitro* bioaccessibility study relies heavily on the chemical speciation of potentially toxic metals and other trace elements. Additionally, the sequential extraction technique, which was formerly known as the Community Bureau of reference (BCR) for soils and sediments, provides a reasonable basis, and its results are extremely comparable to those obtained by other procedures (Hlavay *et al.*, 2004). The various methods of *in vitro* bioaccessibility and bioavailability of potentially toxic metals in soil and sediment are used to assess health and ecological risks and exposure in the environment. *In vitro* bioaccessibility particularly provides insightful information on the oral bioavailability of PTMs (Griggs *et al.*, 2021).

The bioavailability of PTMs measures the quota of metals or metalloids that are characteristically absorbable by the cellular membrane of an organism. At the same time, bioaccessibility is the fraction of a metal or metalloid that is readily available to the cellular membrane of an organism when exposed to PTMs (Ng *et al.*, 2015; Semple *et al.*, 2004). The bioavailability of potentially toxic metals is certainly tested through oral ingestion and gastrointestinal pathways (*In vivo*) in lower-class animals such as rats, rabbits, and worms. However, due to stringent ethical and legal issues associated with experimental animals worldwide, *in vitro* bioaccessibility methods have been developed and validated which give comparable results to *in vivo* bioavailability studies (Xia *et al.*, 2016). The bioavailability of PTMs in the soil is further defined as the number of potentially toxic metals available for uptake that can physiologically enhance bioaccumulation or cause supplementary effects in plants from the sum of selected PTMS present in the soil (Kim *et al.*,, 2015). *In vitro,* the bioaccessibility of PTMs is a promising environmental pollution assessment tool. It estimates the amount in percent called bioavailable fractions of PTMs that are absorbed into the gastric and systemic circulation when PTMs contaminated foods are ingested (Griggs *et al.*,, 2021). The many *in vitro* treatments all have the same overarching objective, which is to evaluate the effects of PTMs and metalloids on health and ecological risk in studies (Chen, Singh, *et al.*, 2020). Oral bioavailability testing, even though it provides a more accurate representation of PTM toxicity *in vivo* studies, it is not commonly done in many developing countries because of the difficulties associated with it. These difficulties include issues of technology, ethics, cost, and time-intensity. Alternately, *in vitro* digestion methods are frequently utilized in research settings. These methods imitate the physiological extraction process in animals (oral bioavailability). (Darko *et al.*, 2017). In this research, we selected to use the USEPA method 1340, which is quite comparable to the SBRC procedure and is one of the most common in vitro bioaccessibility procedures using single extraction techniques. (Gu *et al.*, 2018; USEPA, 2012).

2.4 Sources of potentially toxic metals in the environment

2.4.1 Lead

Lead (Pb) is a silvery metal. It gets into the environment from human and natural sources, including different land-use practices, abstraction of mineral resources, recycling, industrial waste, and geological activities. Lead is mainly used in batteries, cable wires, decorative paints, fossil fuels, solders, and metallurgy (Dignam *et al.*, 2019; WHO, 1995). Pb is found in the biota and top soils as a result of atmospheric deposition and sedimentation of leaded fuel combustion from automobiles, industrial, and manufacturing plants (Shigeta *et al.*, 2020). Research on atmospheric deposition of Pb from vehicular emission was texted on roadside vegetable gardening in city areas showed high human health risks through the consumption of mushrooms exceeding WHO-acceptable weekly intake levels (Garcia *et al.*, 1998; Onyari *et al.*, 1991). In a similar study, increased bioavailability of Pb was found in acidic and organic enriched soils was reported in tea from China above WHO allowable levels, posing a probable health risk to the consumers (Jin *et al.*, 2005). Pb is one of the toxic metals that naturally occurs in the earth's crust and causes harm to humans, especially children when exposed to it. Although Pb occurs in the environment, mechanized farming, uncontrolled industrial effluents, and atmospheric deposition from CO_2 emissions are primary anthropogenic sources that increase Pb concentration levels in environment: soil, waterbody, and the atmosphere (Chaney *et al.*, 1996). In addition, exposure to Pb-based décor paints and fuels are weighty sources of Pb in the environment. Pb-based paint dust and soil Pb are common causes of increased Pb blood levels, lead poisoning in children, and lead environmental levels (Mielke *et al.*, 1998). Increased Pb levels in the environment pose health threats to humans and other organisms due to chronic bioaccumulation into bodily tissues and organs. There are concerted efforts to reduce human exposure to Pb through various remediation technologies to minimize future Pb in the global environment (Li *et al.*, 2019).

The manufacturing and processing industries are major Pb emitters in the universe. In the United States of America (USA), a child died reportedly from Pb poisoning upon swollen a low-cost jewelry toy that contained a high Pb level. The high amount of Pb in the toy was embedded during the manufacturing process (Weidenhamer *et al.*, 2007). In a similar case, Pb contamination of pipe-borne water across the USA was reported to have caused retarded growth in children; and hypertension, cancer, or kidney dysfunctions in adults (Renner, 2009). Pb battery recycling and smelting are among the primary anthropogenic sources of Pb environmental poisoning. Research conducted among kindergarten children near a lead battery and smelter recycling factory showed increased Pb levels in the blood of children exposed to contaminated air and soil (Wang *et al.*, 1992). In a related study in Kenya, high concentrations of Lead (Pb) in environmental samples and blood lead levels were reported in children residing in Owino Uhuru community, a settlement near a Pb smelting factory in Mombasa and the factory was subsequently closed (Etiang *et al.*, 2018). A similar case has been reported in Nairobi near an acid battery manufacturing company. The study indicated that Lead levels in the air, wastewater, and plant samples surpassed the WHO, Kenya National Environmental Management Authority (NEMA), and Kenya Bureau of Standards' recommended standards (Otieno *et al.*, 2022).

The use of scalp hairs and nails is one of the several bioassay-sampling methods that have been developed in humans to test for potentially toxic metals. According to published research, the disulfide bonds in hair proteins are key locations for the possible deposition of harmful metals during the formation of hair as well as during its interaction with other foreign particles (Chittleborough, 1980; Martin *et al.*, 2005). Although the results of a study that compared the lead levels in the blood of exposed children to the lead levels in their hair concluded that computing hair Pb concentration is not an effective method for screening children for lead poisoning, however, the study procedure is scientifically conducted in the Pb poisoning test. (Esteban *et al.*, 1999). On the other hand, a different study found a more significant correlation between a doctor's diagnosis of attention deficit hyperactivity disorder (ADHD) and a higher concentration of lead in the scalp hair of youngsters. The researchers concluded that measuring children's chronic exposure to low levels of lead using scalp hair is an appropriate clinical and epidemiological method. (Bermejo-Barrera *et al.*, 1997; Tuthill, 1996).

2.4.2 Cadmium

Cadmium is released into the environment from agrochemicals and later gets into the food chain through crops (Pan *et al.*, 2010; Schroeder *et al.*, 1963). Since the contamination of agricultural soils in Japan with Cd-rich effluent, there has been an increased interest in the study of cadmium poisoning in humans (Asami, 1984; McLaughlin *et al.*, 1999). In a comparable study, Cd concentrations in various parts of crops watered with untreated wastewater proved to be high levels of Cd contamination. The concentrations were higher than the allowable amounts in edible plants (Bakhshayesh *et al.*, 2014). Chronic cadmium toxicosis produced osteoporosis and nephrocalcinosis in foals living near a zinc smelter. The foals' swollen joints were the result of extensive osteochondrosis. (Gunson *et al.*, 1982). In a human-related study, Cd was reported as the cause of Chronic Renal Failure in some parts of Sri Lanka due to the dietary intake of Cd buttressed by increasing fluoride levels in drinking water (Bandara *et al.*, 2008).

However, Cd is a naturally occurring potential toxic metal found in soils, rocks, and marine shales. It is carcinogenic and mostly less abundant compared to other potentially toxic metals in the environment. In many cases, it accumulates gradually into the environment, and agricultural soils from anthropogenic sources smelting, agrochemicals, and sewage slurry, from whence it subsequently gets into the food chain (Thornton, 1992). Other sources of Cd pollution in the environment include waste disposal, volcano emissions, steel, and zinc production (Hutton, 1983). Elevating Cd levels in the environment can also affect groundwater sources for human consumption. Cd is quickly mobilized and can form complexes movable in aquatic environments (Kubier *et al.*, 2019).

An experiment showed adenocarcinomas, mucoepidermoid, epidermoid carcinomas, and carcinomas in inbred rats after exposure to cadmium chloride aerosol. The induced lung cancers were dose-dependent among the experimental rats (Takenaka *et al.*, 1983). Cd is a toxic metal that causes environmental and occupational hazards. It was declared a carcinogen in 1993 by the International Agency for Research on Cancer (IARC) after epidemiological studies showed a causal association with lung cancer in humans resulting from occupational exposure to the metal nickel-cadmium battery industry, including a series of evidence from experimental animals (IARC, 1993; IPCS, 2005-2007; Waalkes, 2000; WHO, 2010). Cd has since been associated with breast, renal, pancreatic, and urinary bladder cancers. The carcinogenicity of Cd and its compounds seems multifactorial (Huff *et al.*, 2007). The allowable monthly Cd intake is 25 µg/kg per body weight, 3µg/l in drinking water, and 5ng/m^3 in the air per year (WHO, 2010).

In recent times, Cd has been associated with prostate cancer. A study showed that dietary Cd exposure between 1998 and 2009 in Sweden proved that Cd exposure possibly has a role in prostate cancer development (Julin *et al.*, 2012). In a similar study, Cd was found as a causative agent in the trans-differentiation of pancreatic cells and increased pancreatic DNA synthesis. It also increased oncogene initiation, making it a probable pancreatic carcinogen in humans (Schwartz *et al.*, 2000). In addition, a study carried out in the state of Louisiana concluded that there is a statistically significant link between exposure to Cd and an increased risk of developing pancreatic cancer. This risk was found to be associated with occupational exposure to polyvinyl chloride (PVC) products and paints, as well as increased consumption of red meat and grains with high levels of Cadmium in urine. (Luckett *et al.*, 2012).

2.4.3 Arsenic

Arsenic (As) is a naturally occurring element found in soil, rock, and groundwater. Long-term exposure to arsenic may lead to cancer and other health problems. Its global profiled index increased above the geogenic level (10 micrograms) due to anthropogenic activities, including agrochemicals, wood preservatives, nonferrous alloys, petrochemicals, mining, etc., and coal combustion (Murcott, 2012). A global eco-geochemistry review showed an increasing trend in As poisoning on a large scale. Several areas in Europe, Australia, New Zealand, and Asia are hotspots due to anthropogenic activities, including mining, waste disposal, use of As-laden pesticides, wood preservatives, and herbicides. In Africa, notably, less research indicated higher As levels in ground and surface water due to mining operations, agricultural wastes, and incineration of municipal wastes (Medunić *et al.*, 2020). The non-renewable resource, that is, fertile farmland needs to be carefully maintained to maintain a level of food production that is both healthy and sustainable (Hou *et al.*, 2020). In addition to its natural occurrence, increased arsenic concentration in agricultural has been reported in several locations globally. The major blame for this can be placed on farmers that utilize fertilizers that are phosphate-based (Jia *et al.*, 2021; Zhou *et al.*, 2018).

Arsenic has been reported in several studies to have carcinogenic effects. It has been related to several cancer types' including lung and bladder. For instance, a recent study report has shown that early life exposure to As is linked to low birth weight, anemia, Kidney problem, pulmonary diseases in children; and also in adults breast and laryngeal cancers including type 2 diabetes (Khan *et al.*, 2020). Arsenic pollution of groundwater, soil, food, and drinks including portable is a universal health problem. It is reported that more than 300 million people globally are affected by As contamination (Quansah *et al.*, 2015). Arsenic, like other PTMs, often gets into the food system from agricultural production in arsenic-contaminated soil and water. The WHO has set allowable standards of Arsenic for many food produce, for example, in polished rice, the allowable standard is 0.2mg/Kg. however, the European Union (EU) and the United States of America (USA) are to set regulatory standards for arsenic in rice products (Biswas *et al.*, 2020).

Increased agricultural field arsenic contamination is a growing concern to arsenic pollution in foods that needs a concerted effort to counter the present and future associated health and ecological risks. It accumulates over time in agricultural soil from As contaminated groundwater irrigation and gradually gets into the food chain through the transfer of organometallic compounds (Shrivastava *et al.*, 2017). Arsenic-contaminated soil and water pose high health hazards to humans and the ecosystem, thus minimizing its impacts, effective remediation techniques such as microbial, electro-kinetic processes, and phytoremediation are leading Arsenic removal techniques from soils and water with minimum drawbacks (Kumar *et al.*, 2020; Singh *et al.*, 2015). In a bioremediation study, organic matter and phosphorus used microbial transformation of extractable arsenic proved efficient and reduced arsenic pollution in soil significantly (Das *et al.*, 2020). As is the case with other types of PTMs, phytoremediation of arsenic in polluted soil has been attempted using a variety of plant species. However, the difficulties associated with asphyxiation in plants bring down their potential for hyperaccumulation. As a result, improvement strategies are utilized to improve the phytoremediation of arsenic in plants. Like other PTMs, several different plants have been used in the phytoremediation of arsenic in contaminated soil. However, the difficulties associated with asphyxiation in plants bring down their potential for hyperaccumulation. As a result, improvement strategies are utilized to improve the phytoremediation of arsenic in plants. (Sharma, Jha, *et al.*, 2021). Precipitation, electrocoagulation, film separation, bio char, adsorption, and nanotechnology are some of the additional approaches that can be utilized in the process of removing arsenic from the environment. Although a number of these choices come with a high cost and significant environmental repercussions that need to be taken into account before the implementation of the technology (Alka *et al.*, 2021).

2.4.4 Chromium

Chromium (Cr) is a naturally occurring element found in water, groundwater, and soil. It exists in different environmental forms from geogenic and anthropogenic sources (Tumolo *et al.*, 2020). Although Cr is an essential micronutrient for many plants and animals; it is one of the toxic metals that pose a health hazard to the ecosystem. It causes cancer in the lung, liver, and kidney and injures the stomach, including epidermal sensitivity and irritation. Also, Cr is reported to have caused toxicity in other species of plants, animals, and bacteria (DesMarias *et al.*, 2019; Kimbrough *et al.*, 1999). However, Cr in low quantity is commonly used in modern medical practice and dental implants. It serves as resistance to corrosion; however, in high concentrations, Cr can be very toxic and carcinogenic (Achmad *et al.*, 2017). Cr is one of the most widely distributed minerals in the earth's crust and occurs in various oxidation states that form compounds of halides, oxides, and sulfides (Shekhawat *et al.*, 2015). Chromium levels in the environment depend not only on its use, mobility, and distribution but also on its chemical speciation. Cr mainly exists in three states that are Cr (0), Cr(III), and Cr(VI). The latter, the hexavalent form, is the most soluble, mobile, and toxic to humans, animals, and plants (Ertani *et al.*, 2017).

Anthropogenic pollution in agricultural soil is induced by the use of synthetic fertilizers and other agrochemicals such as pesticides. Cr contamination in agricultural soil has deleterious effects on human health and plants including carcinogenicity. Cr causes morpho-phytotoxicity such as Chloris, root deformation, and constrains growth (Kayode *et al.*, 2022). Chromium potentially affects the leaf area, the rate of photosynthesis, delays conduction and transpiration in the stomata, and CO_2 regulation in plants. These actions subsequently lead to the production of reactive oxides including hydrogen peroxide (H_2O_2) and superoxide radical (O_2) that causes protein oxidation and reduced membrane stability index. It also reduces endogenous nitric oxide (NO) production in plants (Singh *et al.*, 2019). Chromium, particularly hexavalent chromium, Cr(VI) is commonly found in aquatic and terrestrial ecosystems and has significantly increased as a result of human activities. The increased levels of Cr in the environment have affected the life in the ecosystems, especially soil, plants and human health. For instance, Cr inhibits chlorophyll biosynthesis by obstructing enzymatic activities and enhances oxidation stress that results into retarded plant growth, wilting of leaves, and chlorosis in the plants (Sharma *et al.*, 2020). Chromium also impact plant from other anthropogenic sources of Cr such as industrial emission, smelting, mining and river sediments (Gan *et al.*, 2019). Chromium is reported to have serious impacts on the soil microbial organisms. Cr reduced the growth rate and the population of bacterial in soils as results of increasing Cr doses. It also affects the metabolic activities of soil microbes and lowers CO_2 generation due to reduced microbial respiration (Eze *et al.*, 2018). Although some microbes have shown potential Cr remediation capacity, it is lethal to other microbes in the environment. Chromium pollution in potentially toxic metals contaminated river sediments showed that Cr increased concentration of in sediments led to significant changes in the microbial composition and function (Pei *et al.*, 2018).

In humans, bioaccumulation of Cr causes teratogenesis and mutagenicity; and environmental and work-related exposure to Cr(VI) can lead to toxicity in several bodily organs including respiratory cancer, renal diseases, reproductive disease, especially in the male, and stomach ulcers (Sharma, Sodhi, *et al.*, 2021). In many instances, people are exposed to Cr through the soil, drinking water, and contaminated foods grown from Cr-polluted soils. The health risk and impacts of Cr on humans come as people are chronically exposed to minimum doses of Cr through various exposure routes (oral, dermal, and nostril). The health effects of Cr in the human range from dermal irritation to cancer, and DNA impairment (Poonia *et al.*, 2021; Tumolo *et al.*,, 2020).

A review of Cr in arable soil in China showed that anthropogenic activities significantly increased Cr concentrations in agricultural soils across China. Some areas were abandoned due to Cr pollution (Zhang *et al.*, 2016). Because of its health and ecological risks, several techniques have been employed to remove Cr from portable water/rivers, industrial wastewater, and contaminated soils. Some recently used conventional methods include membrane technology, electrical coagulation, and ion exchange techniques (Mia *et al.*, 2020). Nano-composite materials including Iron-sulfide and humic acid yielded better results in Cr remediation from polluted soils. The nano-composite successfully reduced Cr concentration in soil samples and improved soil physical-chemical parameters, microbial activities, and micro ecological diversity (Tan *et al.*, 2020). Plant microbial fuel cell technique has proved efficient in detoxifying Cr (VI) in contaminated soil. This system increased soil pH from acidity to neutral including the electrical conductivity making it a promising remediation technique for Cr(VI) decontamination in soil (Guan *et al.*, 2019). Furthermore, several environmentally friendly bioremediation approaches of Cr using microorganisms, plants, or combined plant-microbe techniques are lately being explored through various research (Guo *et al.*, 2021). The use of plant, phytoremediation to remediate Cr from terrestrial and aquatic environments is widely practiced. Several plant species have shown the potential to reduce Cr concentration in wetlands, and aquatic macrophytes in hydroponic experiments in which they assimilate, absorb, and precipitate Cr from aquatic ecosystems (Malaviya *et al.*, 2020). Similarly, several terrestrial plants, over 60 species were tested in Cr phytoremediation studies. The experimental plants accumulated a high amount of Cr from soil roots and shoots; using bioconcentration and translocation factors, the plants showed different potentials for phytoremediation, that is, phytostabilization and phytoextraction with a few showing hyperaccumulation capacities (Sajad *et al.*, 2020). Phytoextraction is a potential eco-friendly technology that is deemed promising to remove toxic metals and lessen human exposure to Cr in the environment (Ranieri *et al.*, 2020). Phytoremediation experiment with Malabar Spinach plant showed high efficacy of hyperaccumulation of Cr (VI) in polluted soil (Adiloğlu *et al.*, 2021).

2.5 Phytoremediation

The mobilization of potentially toxic metals by humans has increased their accumulation in the environment, where they are passed to agricultural products. Food polluted with potentially toxic metals impairs human health. As a result, bioremediation involving plants (phytoremediation) and soil microorganisms to lower the concentration of potentially harmful metals and their effects on the environment has gained increased attention to lessen the associated health implications (Ali *et al.*,, 2013). Phytoremediation is an eco-friendly and cost-effective technique. It is used to extract, sequester, and mineralize pollutants. It involves four technological approaches: phytoextraction, Phytostabilization, Phytofiltration, and phytovolatilization, (Nwoko, 2010). This study involves phytoextraction, which enhances plants' capacity to uptake potential toxic metals from soil and transfer them to aboveground biomass. It aids in mitigating ecotoxicity associated with several toxic metals. However, getting suitable plants for phytoextraction that tolerate high concentrations of toxic metals in the environment is challenging, except through genetic breeding (Koźmińska *et al.*, 2018).

Literature showed that plants of the Brassicaceae genera, family, and species including *Brassica napus, Brassica carinata, Brassica juncea, and Brassica oleracea* have high prospective phytoremediation capacity (Roy *et al.*, 2020). Other species identified include *Thlaspi caerulescens, N. caerulescens, N. praecox, Noccaea, N. caerulescens, and N. goesingense* are effective hyperaccumulators of toxic metals-cadmium, nickel, copper, zinc, etc. (Chaney *et al.*, 2005; Krämer, 2010; Pollard *et al.*, 2014). There are about 400 hyperaccumulating plants dominated by the families Asteraceae, Brassicaceae, Caryophyllaceae, etc. (Vara Prasad *et al.*, 2003). Phytoextraction has the prospect of recycling macronutrients harvested in plants, and their biomass can be an easily manageable energy source (Vara Prasad *et al.*,, 2003). Many plants are capable of uptaking metals from soil. Still, hyperaccumulators are plants that uptake a minimum of 100 mg/g (0.01% dry weight) of Cd, As, and other trace metals, or 1000 mg/g (0.1% dry weight) of Cobalt (Co) Copper (Cu), Cr, Nickel (Ni), and Pb and 10, 000 mg/g (1% dry weight) of Manganese (Mn) and Nickel are rare (Reeves, 2000; Watanabe, 1997). Apart from the Brassica commonly cited, studies on *Helianthus annulus L*, Zea mays L, and *B. napus*, are promising hyperaccumulators (Vamerali *et al.,*, 2010).

Brassicaceae is an economically essential crop family that includes fodder crops, oilseed plants and vegetables, organic fertilizers, and biofuels. Many species of Brassicaceae are resistant to stressed environments, and agrochemicals use such that they are wildly used as model species (Warwick, 2011). There are over 3000 species from more than 300 genera of the mustard family; amongst them is the most used, *Arabidopsis thaliana*. This simple angiosperm has paved the way for understanding the growth and development of plants (Meinke *et al.*, 1998). Numerous studies have been conducted to traceably establish the origin of the mustard family, with many indicating Eurasia as the family origin before it was spread to the Northern Hemisphere and other parts of the world. The ancestors of Brassicaceae are thought to have originated from the Northeastern Mediterranean and later spread to Asia and Europe, as evidenced by the biogeographic events in these areas (Arias *et al.*, 2014). *Brassica napus* are deliberated to have formed around 5000-10,000 mya, most likely originating from the interspecific hybridization of the genotypes *Brassica rapa* and *Brassica oleracea* (Iniguez-Luy *et al.*, 2011). It is one of the most cultivated medicinal crops in Eurasia and Saharan Africa; and also has attracted substantial commercial value from its enriched oilseed potential to produce cooking oil and renewable energy (Saeidnia *et al.*, 2012).

Raphanus raphanistrum (wild radish) is a widely known distributed weed, and it is understood to belong to the brassica plants, though with some disputes about any link (Yamagishi, 2017). It has been reported as a troublesome weed for cereals in some parts of Australia, growing sporadically from a multitude of seeds produced seasonally and interfering with production yields (Cheam *et al.*, 1995; Piggin *et al.*, 1978). It has herbicidal resistance to some agrochemicals used worldwide, including Acetolactate, chlorsulfuron, and metsulfuron-methyl (Costa *et al.*, 2014; Smit *et al.*, 2001; Yu *et al.*, 2012). Wild radish observed some dormancy in the soil seed bank by physical restrictions such as climatic conditions. The siliques provide additional adaptive mechanisms maximizing endurance during dormancy (Tricault *et al.*, 2018). Wild radish also contains healthy and nutritious bioactive compounds comprising phenolic and hydroethanolic extracts with supplementary antioxidant potential that can be added to the human diet (Iyda *et al.*, 2019; Turan *et al.*, 2012). The bioactive compounds in *Raphanus raphanistrum* have long been used for their medicinal values. Several studies have reported different uses of wild radish in different areas around the world to treat different health conditions (Jbilou *et al.*, 2006; Lim *et al.*, 2019).

Some plants have shown effectively enhanced hyperaccumulation of potentially toxic metals phytoextraction potential from soils, sediment, and water in previous studies such as *Brassica juncea (L.) Czern* in soil *((Ebbs et al., 2008); Brassica juncea* and *Brassica napus* copper uptake in hydroponic *(Feigl et al., 2015)*; *Helianthus annuus L.* in soils (Favas *et al.*, 2019); maize (*Zea mays L),* in soil (Almaroai *et al.*, 2012); and *Pisum sativum L,* in soil (Chaturvedi *et al.*, 2021; Sumiahadi *et al.*, 2018). Even though phytoremediation is greener, plant-based technology has some limitations. However, microflora and other rhizoid bacteria have the potential to enhance bioremediation, but recombinant DNA-transgenic biotechnology approaches have yielded promising results to address Phyto limitations (Dowling *et al.*, 2009; Pilon-Smits *et al.*, 2002). Genetic modification is vital to enhance phytoremediation. In eastern Spain, *Nicotiana glauca R.*, following Agrobacterium-mediated transformation, increased its tolerance and double-fold accumulation to Cd and Pb

(Gisbert *et al.*, 2003). Such potential hyperaccumulating plants can be further exploited to enhance the phytomining of essential elements for biological and environmental purposes (Rascio *et al.*, 2011).

Although not commonly used in phytoremediation, the induced mutation is the artificial irradiation of mutagenesis of organisms to form variants for intended purposes, including an increase in food production and resistance to pests and other environmental conditions (Micke *et al.*, 1990; Oladosu *et al.*,, 2016; Sigurbjörnsson, 1971). There are different methods of induced mutation, including physical irradiation and the use of chemicals. Transformation exposes specific dormant traits in plants to improve growth, yield, and tolerance (Mullainathan *et al.*, 2013). The basis of mutagenesis is the creation of desired genotypes. However, it occurs naturally, induced chemical and physical mutation to enhance the targeted characteristics of cultivating materials (Oladosu *et al.*,, 2016). The former has advantages: low cost, high variation density, and suitably applicable to many crops (FAO/IAEA., 2018).

Since its wide acceptance, thousands of variants have been produced through induced mutagenicity primarily to breed plant varieties that can adapt to the stressed environment through gene modification or dormancy break (Ahloowalia *et al.*,, 2004). Mutation can be natural or induced; induced mutation can also be physical or chemical using gamma rays and neutron or Ethyl Methyl sulfonate (EMS) and N-Nitroso-N-Methyl urea (NMU). The latter proved effective in a comparative induced mutagenic study of chickpeas (Kharkwal, 1998; Wani, 2017).

2.5.1 Enhanced Phytoremediation

On the other hand, phytoextraction is plants' ability to uptake and translocate toxic metals to aboveground parts. It helps to reduce and limit ecotoxicity in soil, remediates contaminated environment, and increases potentially toxic metals tolerance (Koźmińska *et al.*,, 2018). Studies have shown that species of plant Brassicaceae are active hyperaccumulators (Chaney *et al*., 2005; Krämer, 2010; Pollard, Reeves, & Baker, 2014). In addition to toxic metal removal, phytoextraction improves phytomining and metal recycling (Vara Prasad & de Oliveira Freitas, 2003). The addition of chelators can further enhance phytoextraction. Chelating agents are chemical reagents that enhance the bioavailability of potentially toxic metal uptake in plants through a soluble complex formation that is quickly taken, washed out, or stabilized (Leštan *et al.*, 2008). The use of a chelating agent is among several abatement technologies, including soil washing, excavation, toxic metals stabilization, and the use of plants, which have been employed to cleanse toxic metals contaminated soils and waters. Nonetheless, due to the high financial cost and resources associated with the above remediation techniques, more efforts and research are being devoted to alternative technologies, including bioremediation (phytoremediation), that are less costly and environmentally sustainable according to Ali *et al.*, (2013). This technology can improve via many approaches, including genetic engineering and plant breeding which increases the bioavailability of potentially toxic metals in soil (de Mello-Farias *et al.*, 2011). Nevertheless, the remediation of potentially toxic metals in contaminated soil is a challenge to all. The challenges continue to increase due to amplified geological transformation and anthropogenic activities that constrain plant growth, performance, and yield (Chibuike *et al.*, 2014). Phytoremediation is promising, but it is yet to be practiced on a large scale in places mostly faced with potentially toxic metals pollution either resulting from intensive agricultural, industrial processing, and mining activities (Dyer, 2018; Mwegoha, 2008). Many studies are devoted to finding a lasting solution to PTMs decontamination from the environment using enhanced phytoremediation techniques that will be cascaded from the lab to the field to bioremediate contaminated soils, ensuring food production in cleanup soils (Patra *et al.*, 2020).

Enhanced phytoremediation involves improving selected plants' growth and their capacity to withstand metals/metalloids toxicity, as well as increasing their strength to absorb and store the metals in their biomass. It is geared to overcome challenges such as retarded growth in phytoremediation

trials. There are several methods of enhancement including chemicals or chelators in addition to contaminated soils and modification of plants (Gavrilescu, 2022; Hasan *et al.*, 2019). However, many studies have focused on chemical amendments such as biochar, organic acid, and other empirical factors to select hyperaccumulating plants, but the use of molecular technology to increase plant resistance, growth, and remediation provides a better alternative, especially amid the global climate crisis. This involves modification of the plant's genome using artificial nucleases to enhance phytoremediation (Sarma *et al.*, 2021). This process can also be improved through genetic engineering, where plant genes are modified by editing the DNA through the addition/removal of precise genes for plant development to enhance the phytoremediation of the organic compounds, metals, and metalloids in polluted soils (Gao *et al.*, 2021). Phytoremediation can also be enhanced through the simulated construction of plants' communities. A Community of plants with different physiological activities induces synergy to complement the growth and is resistant to different environmental conditions. This approach can as well enhance the phytoremediation of plants in polluted soils (Sha *et al.*, 2019). Meanwhile, Brassicaceae plants have generally shown high potential for phytoremediation of potentially toxic metals in soils. Some plants referred to as hyperaccumulators are predominantly of the Brassicaceae family. These plant's potential can be further enhanced through transgenic approaches that will develop suitable traits such as tolerance, resilient, and effective adaption in transgenic Brassicaceae compared to their wild ancestors which will make way for efficient phytoremediation (Agnihotri *et al.,*, 2019). Several transcription factors have been used to improve plants' resistance to biotic and abiotic stresses that undermine plant growth, yield, and productivity. Transgenic plant development involves vector construction, transgenes integration, and transformation (Low *et al.*, 2018). Transformation approaches involve biological, chemical, and physical bombardment procedures in plant breeding. Chemical priming is a promising technique to increase plants' tolerance to various environmental stresses (Nguyen *et al.*, 2018).

2.6 Knowledge Gap

This research investigates the possibility of enhanced phytoremediation of Cr and Pb-contaminated agricultural soils from Uasin Gishu County, Kenya using chemically treated wild *B. napus* and *R. raphanistrum*. From the literature, several studies have been done, and others are ongoing on phytoremediation of industrial polluted soil using different plant species. These studies target a wide range of potentially toxic metals within the environment spread due to human activities, but very few have focused on agricultural soils. Moreover, where agriculture is concerned, little has been done to enhance plants for phytoremediation of PTMs in contaminated soil using colchicine. Therefore, this work focuses on the possibility of enhancing the selected Brassicaceae plants to be used on a viable scale to phytoremediation polluted agricultural soils in one of the active extensive farming areas in Moiben, Uasin Gishu County, Kenya. The study results will positively aid to decontaminate Cr, Pb, and other PTMs with similar chemical properties from the environment.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Introduction

This chapter describes the study's location, as well as the methods and procedures that were utilized to carry out the research following the specified research objectives. These include the areas for sampling, the soil sampling methods, the preparation and treatment of the soil samples, the experimental design, the process for treating the plants, the data collection method, the laboratory analysis procedure and techniques, reagents used, apparatuses, analytical instruments, and the statistical analysis of the results.

3.2 Materials

3.2.1 Study location

The research was carried out in Kosyin, Kaprobu, Moiben, Naiberi, and Ziwa in the county of Uasin Gishu in Kenya (Figure 3.1). The equator runs through the middle of this East African nation, which also features a variety of landforms, the majority of which are dry and semi-arid lands, and a coastal strip that runs along the Indian Ocean. There is not much precipitation experienced in the country (Ingham, 2020). Uasin Gishu is located at Longitude 4° 50´ east, 35° 37´ west; and Latitudes 0° 03´South and 0° 55´ north. It is bordered by Trans Nzoia, Elgeyo-Marakwet, Baringo, Kericho, Nandi, and Kakamega counties in the North, East, South, West, South-West, and North-West, respectively. The total land area covers about 3,345.2 square kilometers on a high plateau. It is about 1500 to 2700 meters above sea level; it is relatively calm with an annual mean temperature below 21^0C. The area receives about 1000 to 1250 millimeters of rainfall per annum (MoALF, 2017). It is located in the rich highlands of western Kenya, which are primarily inhabited by farmers (Lomurut, 2014). Uasin Gishu County experiences two seasons, predominantly wet and dry seasons: the months of March through October, which are considered to be the rainy seasons, are followed by November through February, which are considered to be the dry seasons (Daniel *et al.*, 2018). Additionally, this region is a significant contributor to the production of dairy goods, most notably milk, in Kenya. Approximately, 70 %, 20 %, and 10 % of the total production are devoted, respectively, to farmers whose primary focus is on subsistence, semi-marketable, and marketable agriculture. (Kembe *et al.*, 2016). Uasin Gishu is one of the counties in Kenya that is included in the group of places that are generally known as the "breadbasket" of Kenya due to its high agricultural output and contributions to the country's overall food security. (MoALF, 2017).

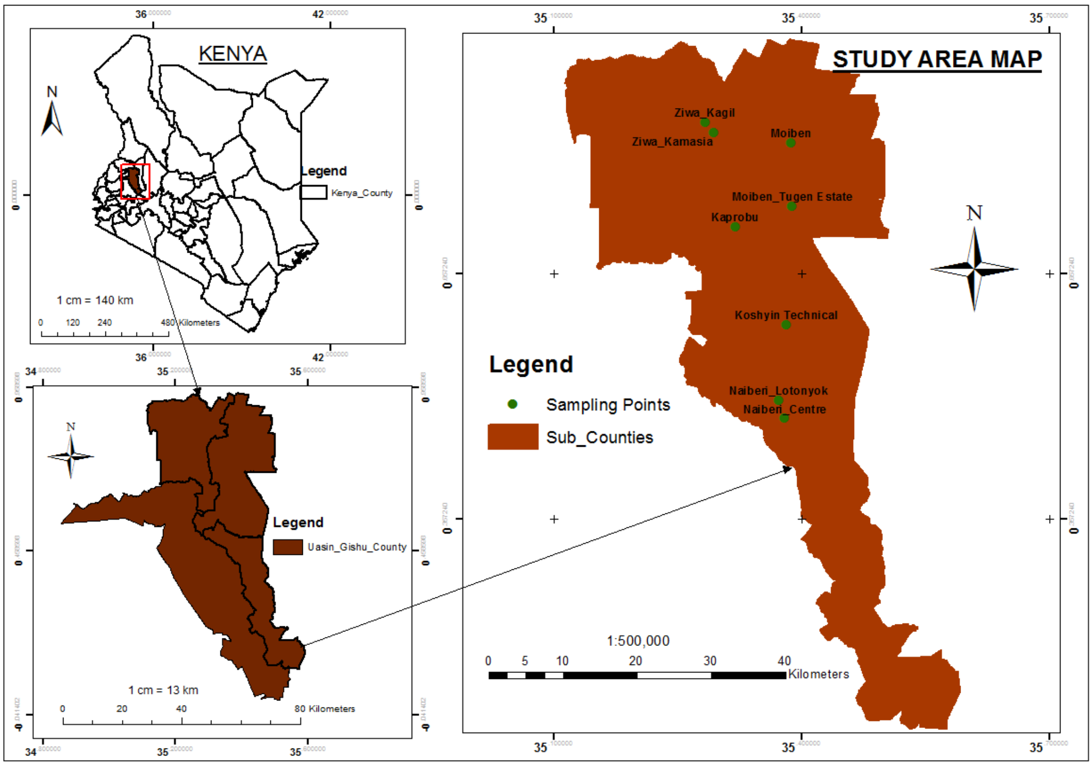

Figure 3.1: Study Area Map (Source: Author, 2022)

3.2.2 Geology and Geochemistry of Uasin Gishu County

Uasin Gishu County consists of different landforms. The lands comprise different types of soil, such as Ferralsols, Nitisols, and Regosols (Ngunjiri *et al*., 2019). The county is separated into three physiographic regions: upper highlands, upper midlands, and lower highlands. These regions affect the climatic condition of the county and play a key role in land-use patterns and socio-economic activities of the regions. The areas are predominantly made of volcanic rocks with no identified industrially exploitable minerals. The soils generally consist of loam and clay soils of different colorations suitable for growing varieties of crops such as maize, wheat, and potatoes. There are six main rivers in the area, these include Sosiani, Kipkaren, Kerita, Nderugut, Daragwa, and Sambu all of which drain into Lake Victoria (GoK, 2019). Assessment of PTMs around Uasin Gishu County agroecological zones showed a high variation of the metals above their natural levels. For example, Cd notably exceeded the WHO standard of PTMs in soil; hence these increased variations are attributed to intensive agricultural practices in the area (Akenga *et al.*, 2016).

3.2.3 Population and socioeconomics activities

Uasin Gishu County is one of the forty-seven counties of Kenya. It is divided into six major administrative districts called sub-counties that are further subdivided into wards, locations, and sub-locations. The sub-counties are Ainabkoi, Kapseret, Kessess, Moiben, Soy, and Turbo. The soils of Uasin Gishu contain a good amount of land capital and different agroecological resources (Kitonga *et al.*, 2018). The present population of the county stands at 1.2 million people according to GoK (2019). The county has about 2995 square kilometers of arable lands with an average of 5-hectare rural landholding per person. Many residents of the county practice farming consisting of 2-10 acres for both crops and livestock. The primary means of subsistence in the county of Uasin Gishu are a diverse mix of farming, commerce, and employment activities (formal and casual). The agricultural industry is made up of both small and large-scale businesses that specialize in the cultivation of a variety of crops including maize, wheat, and sorghum; and the raising of livestock. The farmers have a major reliance on agriculture that is fed by rain, and the expenses of agrochemical amendments represent a significant portion of the total cost of production. Farmers get together to form memberships in cooperative organizations that are officially registered, and these societies are involved in the production and distribution of agricultural commodities (County, 2013).

3.2.4 Laboratory reagents, apparatus, and equipment

During the field survey, soil samples were gathered with the assistance of a hook, spade, shovels, a tape measure, various-sized paper, polyethylene bags, and GPS. Mortar and pestle, 2mm sieve, beakers, volumetric flasks, graduated cylinders, micropipettes, hotplates, Sartorius analytical balance (A200s), pH meter (Milwaukee), microwave-assisted digestion (MAD) system (MAR6), microscope, Atomic Absorption Spectrophotometry (SOLAAR S series' AAS), and Inductively Coupled Plasma Mass spectrometry (Agilent 79000s). The laboratory reagents used include distilled water, colchicine, Potassium dichromate, and Lead nitric. Other reagents include Hydrochloric acid, Nitric acid, Perchlorate acid, Sulfuric acid, gibberellic acid (GA3), and potassium nitric. All lab reagents were procured and purchased from Kobian (Kenya) Ltd.

3.3 Methods

3.3.1 Research design

The study adopted a “Land Use/Cover Area frame statistical soil Survey-Lucas” field survey technique to collect soil samples from the selected farms in the study areas within Uasin Gishu County, Kenya. The soil samples per farm were collected, packaged, and transported to the biotechnology lab for further preparation and processing before analysis (Orgiazzi *et al.*, 2018). A similar procedure was followed to collect the wild seeds of the experimental plants. In addition, a completely randomized design (CRD) was used in the glasshouse for the phytoremediation trials of the selected plants with different treatments. The pots were well labeled and filled with soil before randomly assigning treated seeds of *B. napus* and *R. raphanistrum* (Chen, Yang, *et al.*, 2020).

3.3.2 Field survey

3.3.2.1 Soil Sampling

Soil samples were randomly collected before and after the harvesting seasons from the selected farms in Kosyin, Kapropbu, Moiben, Naiberi, and Ziwa in Uasin Gishu County, Kenya. Two farms in each area were selected for the soil sampling survey. Ten soil samples were taken from randomly apportioned areas in each farm, which is two samples per area at a depth of 30 cm below the surface unpolluted with organic matter. This study utilized a variety of research designs, including multiple surveys and experimental techniques (Kirk, 2012; Kothari, 2004). At the selected site, a 20x20 square meter (m^2) quadrant was delineated and soil samples were picked from each corner. The samples were mixed to make a composite sample. From the composite, duplicate samples were subsampled by quartering as proposed by Tarafdar *et al.* (2019). The sampled farms within the sampling locations grow different crops, comprising maize, wheat, and vegetable farms (Munjeb *et al.*, 2018; Pennock *et al.*, 2007). The canopy of the topsoil was not sampled, hence it did not make parts of the composite soil samples (Chaoua *et al.*, 2019; Ebong *et al.*, 2020; Yang *et al.*, 2018). Before subsampling, the sample quartering guaranteed the homogeneity of the soil samples to ensure that they were thoroughly mixed and truly representative of the selected sites (Schumacher *et al.*, 1991; Vandenhove *et al.*, 2009; Zhang *et al.*, 2013). About 2Kg of homogenized soil sample were collected at each point per farm for the survey. The samples were transported to the lab and subjected to other pretreatment before analysis for the parameters of interest (Mirzaei *et al.*, 2020). After the survey, Cr and Pb recorded relatively higher concentrations in the soil and this was found in Moiben. Hence, the study focused on these two PTMs and chose Moiben for sampling soil for possibility of enhanced phytoremediation. A similar procedure as in the survey was followed to sample about 250Kg of soil from Moiben for the phytoremediation trial of PTMs in the glasshouse. In Moiben, soil samples were also collected from non-agricultural land to evaluate the source of increased Cr and Pb concentrations in the soil.

3.3.2.2 Identification of *Brassica napus* and *Raphanus raphanistrum*

Following the literature survey, it was ascertained that Brassicaceae plants comprised significant numbers of prospective phytoremediation species for the decontamination of potentially toxic metals. A botanist consulted from the Department of Wildlife, School of Natural Resource Management at the University of Eldoret to identify locally available Brassicaceae species. A field survey on selected farms around Eldoret, including those within the study areas was conducted to identify and collect seeds of the available Brassicaceae species. Four different Brassicaceae species were identified, these include *R. raphanistrum* (wild radish), *B. napus* (canola), *B. oleracea* (kale), and wild Georgia Southern collards (Sukuma wiki) were collected from wheat and maize farms where they have grown as postharvest weeds. The plants were taken to the herbarium and sorted; with consideration mainly on the basics of consumption and biomass level of the plant organs including roots, stems, and leaves. These criteria were used to select the suitability of the plant for phytoremediation purposes. *R. raphanistrum* (wild radish) and *B. napus* (canola) were selected, as they are less consumable by the local communities and contained sizeable biomass.

Thousands of matured seeds of each plant species were randomly garnered while still green and fully raped within the siliques. They were taken to the biotechnology laboratory, stratified, labeled, and spread on trays for sun drying in the glasshouse at a temperature ranging from 25^0C to 45^0C. The seeds were constantly weighed at regular intervals until consistent weights were recorded. The seeds were then transferred to the lab and kept at room temperature. A portion of each seed was randomly taken to measure the morphometries through a microscope. The parameters measured included the longitudinal length (length), transverse length (width), perimeter, and area in millimeters. This was followed by a germination test for each species to break temporary dormancy in these wild plants.

3.4. Lab experiment design

All soil samples were transported to the lab from the field in sealed polyethylene bags and air-dried in a glasshouse until constant weights were observed. The samples were crushed, filtered, sieved using a 2mm mesh wire, and transferred into polyethylene vessels for physicochemical parameters and PTM analysis (Vandenhove *et al.*,, 2009). The total PTMs: As, Cd, Cr, Hg, and Pb concentrations in soil samples were determined. About 0.5g of each sieved soil sample were digested by gradually adding 9mL, 1mL, and 4mL of concentrated analytical grades HNO_3, HCl, and $HClO_4$, (if suspicious of Hg presence) respectively to the soil sample and transferred into ultra-clean and dry inert polymeric reaction vessels under the fume hood according to Kamunda *et al.* (2016) method. The mixture was left under the fume hood for 5-10 minutes to allow a complete reaction of the acid solution before sealing the vessels. The sealed vessels were placed on the rotor into a microwave-assisted digester (MAD). Upon complete digestion, the digests were cooled and filtered. The filtrates were transferred into 250mL volumetric flasks and filled to the mark using deionized water. The samples were ready and injected into the Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (Agilent 7900) to analyze for As, Cd, Cr, Hg, and Pb according to the method proposed by Helaluddin (2016). Quality control and assurance (QC/QA) of the validated analytical procedures and methods were routinely practiced to ensure the repeatability and reproducibility of the results (Magnusson *et al.*, 2014). Approved laid-down steps, and standard operating procedures (SOP) were practiced to minimize errors in analytical results. The study adopted Bureau Internationale des Poids et Mesures (BIPM) uncertainty criteria to calibrate the instrument ICP-MS (Andersen *et al.*, 2013; Andersen, 2018); and method validation of the instrument (ICP-MS) was carried out on quantitative tests for the impurity of the selected metals considering selectivity, specificity, the limit of quantification, linearity, accuracy, and precision (Magnusson *et al.*,, 2014). After every tenth sample run, certified reference material and a blank were run to safeguard the validated calibration and ensure contaminant-free samples (Kamunda *et al.*,, 2016). These procedures provided quality data that were reproducible to ensure that the findings reported are truly representative of the levels of PTMs in soils and plant biomass and can be reproduced (Gholizadeh *et al.*, 2015). The validation method of the ICP-MS for trace metals analysis in the potting soil and plant biomass was conducted, keeping in mind acceptance levels of environmental pollutants (van Zonen *et al.*, 1998; Voica *et al.*, 2012).

The instrument was calibrated using prepared standard solutions and certified reference materials (CRM). Stock solutions of multiple elements comprising 10 µg/L (10ppm or 10mg/L) were prepared. From the stocks, 0.00ppb, 10ppb, 20ppb, 30ppb to 100ppb were prepared each in a 100ml flask for calibration before running sample analysis. The standard solutions were analyzed with CRM for the elements and values plotted in the control chart for devising the standard operating procedures (SOP**)**. During analysis, CRM was included and run before every batch of samples. The results were presented in micrograms per kilogram (µg/Kg) or part per billion (ppb). To determine the actual concentrations of the PTMs in the soil in mg/Kg, equation one (Eqn. 1) was used as proposed by Kingston *et al.* (1998):

$$Sample\ mgKg^{-1} = \frac{(readings\ \mu gKg^{-1}\ x\ df)}{wt.sample\ (mg)}\ x\ \frac{1mgKg^{-1}}{1000\mu g}\ \textbf{......................Equation1}$$

From Eqn. 1, df is the dilution factor and readings are the results from the ICP-MS.

3.4.1 PTMs assessment in soils

3.4.1.1 Ecological risk characterization

To evaluate the likely ecological risks related to potentially toxic metal levels in the soil samples from the study areas, Hakanson's ecological risk index was adopted (Hakanson, 1980). The pollution index, Eqn. (1) was originally used to evaluate potentially toxic metals physiognomies and environmental patterns in sediments. This quantitative method assesses possible contamination effects of toxic metals in the ecosystem based on preindustrial background levels of PTMs in soil. It catalogs and isolates single and multiple contaminants' effects on a given environment different from probable hazards (Wang *et al.*, 2013). To date, various indexes are used to characterize ecological risk because of anthropogenic-induced environmental degradation. The following equations, Eqn. (2) to Eqn. (4) were used to estimate ecological risk in the selected areas emanating from agricultural activities; Geo-accumulation Factor and Ecological Risk index were adopted to compute the risk levels (Ali, Malik, *et al.*, 2013; Odukoya, 2015).

$$I_{geo=log_2}\left({C_n}/{1.5xB_n}\right) \qquad \textbf{Equation 2}$$

$$\mathrm{E}RI = \sum E(i) = \sum Ti\ x\ C_f \qquad \textbf{Equation 3}$$

$$\mathbf{Eri} = \sum \mathbf{E(i)} = \sum \mathbf{Ti\ x} \frac{\mathbf{Ci}}{\mathbf{Coi}}\ \mathbf{,\ where}\ \frac{\mathbf{Ci}}{\mathbf{Coi}}\ \mathbf{is\ Cf,\ hence\ ERI} = \sum \mathbf{Ti\ x\ Cf} \qquad \textbf{Equation 4}$$

From Equations 2, 3, and 4, Cf =Contaminator Factor of the individual element; I_{geo} is the Geo-accumulation factor; ERI is the Ecological Risk Index, C_n is the metal concentration in soil at the sampling sites; B_n is metal concentration in the background (preindustrial) soil; and T_i is the toxic response factor in soil (Hakanson, 1980; Turekian *et al.*, 1961; Weissmannová *et al.*, 2017).

3.4.1.2 Health risk characterization

Human health risks, that is, carcinogenic and non-carcinogenic were evaluated using the USEPA algorithm of risk assessment (USEPA, 2011). The main indirect exposure routes to a pollutant (PTMs) in agricultural soils are incidental ingestion, inhalation, dermal, and food. For each potentially toxic metal, chronic average daily intake (ADI) is often calculated. In this study, incidental ingestion, ADIing, Eqn. (5) and dermal contact, ADIdermal, Eqn. (6) exposure levels of the PTMs (As, Cd, Cr, & Pb), carcinogenicity risk, Eqn. (8) and non-carcinogenicity risk, Eqn. (10) were calculated (Johnbull *et al.*, 2019; USEPA, 2011; Zhang *et al.*, 2020). The respective exposure factors are enlisted in Appendix II as adapted from the USEPA screening and risk assessment guides (Agency, 2011; USEPA, 2011).

$$\boldsymbol{ADI_{ing} = \frac{C_{soil}\ x\ InR\ x\ EF\ x\ ED}{Bw\ x\ AT}\ x\ 10^{-6}}$$ …………………………………….. **Equation 5**

$$\mathbf{ADI_{dermal}}\ \boldsymbol{\frac{C_{soil}xSAxFExAF_{soil}xABSxEFxED}{BW\ x\ AT}\ x\ 10^{-6}}$$ ………………………… **Equation 6**

From equation 7 and equation 8, C_{oil} is the concentration of the contaminant (PTMs) in the soil and plant (mg/kg) and is the PTMs ingestion rate from soil (mg/day). ADI_{ing} and ADIdermal, are the chronic average daily intake for incidental soil ingestion, and dermal contact measured in mg/day. *EF* is the exposure frequency (day/year), *ED* is the exposure duration (year), and *BW* is the average body weight (kg). *AT* is the average time (day), *SA* is the skin surface area (cm^2), AF_{soil} is the soil adherence factor (mg/cm^2), *ABS* is the dermal absorption factor (unitless), *FE* is the dermal exposure ratio (unitless), and *CF* (10^{-6}) is the conversion factor.

The carcinogenic risk was computed based on an incremental chance of cancer occurrence over the lifespan of an exposed individual to potentially toxic metals. The likelihood for such an individual to acquire generic cancer was estimated as in Eqn. (8). The integrated cancer effect occurs from exposure to more than one carcinogen computed according to Eqn. (9). An acceptable target carcinogenic risk (CTR) value lies between 1.0E-04 and 1.0E-06; hence, CTR above 1.0E-04 require further chemical analysis, whereas CTR value below this requires no further action (Johnbull *et al.*,, 2019).

The non-carcinogenic risk potential for each PTM was computed using the Hazard Quotient as in Eqn. (9). The HQ is the estimate of the adverse effects of each PTM on human organs. Therefore, the hazard index (HI) is estimated in Eqn. (10) is the cumulative sum of the HQ through the exposure pathways (incidental ingestion and dermal contact). In general, HI or HQ ≤ 1 is considered within an acceptable limit, that is, no significant risk of non-carcinogenicity. On the other hand, HI or HQ > 1 means that there is a significant risk of non-carcinogenicity (USEPA, 2011; Zhang *et al.*,, 2020).

$$\mathbf{CR = ADI}\boldsymbol{x}\mathbf{SF} \quad \textbf{Equation 7}$$

$$\mathbf{CTR = \sum CR} \quad \textbf{Equation 8}$$

$$\boldsymbol{HQ = \frac{ADI_{chronic}}{RfD}} \quad \textbf{Equation 9}$$

$$\mathbf{HI} = \sum \boldsymbol{HQ} \quad \textbf{Equation 10}$$

From equations, 7, 8, 9, and 10, CR is the cancer risk (CR) and HQ is the hazard quotient of each PTM through various exposure routes (Appendix I), and SF is the cancer slope factor as indicated in Appendix II. ADI is the average daily intake, RfD is the reference dose as indicated in Appendix II, CTR is the cumulative target risk, and HI is the hazard index (DoE, 2011; USEPA, 2009).

3.4.1.3 Determination of soil physicochemical properties

Physicochemical parameters, including soil color, texture soil pH, organic carbon (OC), available nitrogen, available phosphorus (P), Exchangeable Aluminum (A^{+3}), and exchangeable acidity (H^+) (Table 3.1) were determined using conventional methods (Motsara, 2008; Yao *et al.*,, 2013). The soil pH and organic carbon play a pivotal role in potentially toxic metals and nutrient availability, distribution, and uptake by plants in soils (Solis *et al.,* 2005). These soil physicochemical parameters often indicate anthropogenic interference with the soil's natural parameters (Okalebo *et al.*, 2002). To determine the physical and chemical parameters of the soil, duplicate soil samples were air-dried, ground in a mortar and a pestle, and sieved through a 2mm mesh wire. The replicate samples were accurately weighed in an appropriate amount and analyzed.

Table 3.1:Physicochemical parameters of soil

s/n	parameter	Analytical method
1	Soil color	By inspection
2	Texture	Bulky density
3	pH	Potentiometer
4	Organic carbon	Walkley-black
5	Exchangeable Al^{+3}	Atomic Absorption spectrometry
6	Exchangeable H^+	Atomic Absorption spectrometry

In soil pH determination (1:5 soil: 0.1M$CaCl_2$), 1.00g of each soil sample was measured in replicates and added 5 mL of 0.1M $CaCl_2$ solution was in test tubes. The mixture was stirred for about 10 minutes and allowed to settle for 30 minutes. Then the mixture was shaken for 30 minutes and allowed to soak and pH was measured using a Desk pH meter (PHS-3D) potentiometer. The pH meter was calibrated using pH 4.0, pH 7.0, and pH 9.0 buffer solutions before measuring the pH of the soil samples according to Kome *et al.* (2018). The soil organic matter was determined using Walkley-black (W-B) method. The oxidizable amount of the organic matter was quantified with a standard amount of chromate in the presence of sulfuric acid. The residual chromate was determined at 600mm wavelength using atomic absorption spectrophotometer. The soil organic matter was calculated based on organic matter containing 58% carbon (Nelson *et al.*, 1983). The soil exchangeable acidity, Al^{+3}, and H^{+} were determined. A 10g sample of soil was weighed into a 50ml glass beaker and 25mL of 1M KCl was added. The suspension was stirred with a glass rod and allowed to stay for 30 minutes before filtering clearly. 1-2ml Phenolphthalein indicator was added dropwise, titrated with 0.1M NaOH to the first constant pink color of the endpoint, and calculated using equation 11 as described by Okalebo *et al.*, (2002)

$$\boldsymbol{Ex.acidity\ (cmol\ (+)) = (mlNaOH\ sample - mNaoH\ blank)x}\ \mathbf{10}$$ **--Equation 11**

This measures the soil's exchangeable acidity (Al^{+3}) in centimole per kilogram of the soil sample.

3.4.1.4 *In Vitro* Bioaccessibility of potentially toxic metals

Human exposure to PTMs in the environment is a major public health concern because of their associated adversity. High concentrations of PTMs in agricultural soils are a recipe for health exposure through dermal and incidental ingestion; hence, PTMs assessment in soil has gained more attention in research. In recent times, i*n vitro* bioaccessibility (IVB) methods are applied to estimate the relative bioavailability of PTMs in contaminated soils (Li *et al.*, 2015). IVB was tested using the United States Environmental Protection Agent's (USEPA) method (USEPA, 2017). Potential toxic metals bioaccessibility measurements were conducted as outlined by USEPA methods 1340 (Paltseva *et al.*, 2018); 1.0g of each soil sample was weighed in duplicates and transferred to high-density polyethylene (HDPE) bottles containing 100 mL extraction fluid: 0.4 mol/L glycine (reagent-prepared in deionized water). The pH was adjusted to 1.50 ± 0.05 by adding 0.5% HCl; the samples were heated at 37°C before extraction (Obrycki *et al.*, 2016). The extraction was completed by centrifuging the samples at 30 rpm for one hour. About 40 mL aliquot of the supernatant was filtered using a 0.45µm cellulose filter before PTM analysis using ICP-MS. The Bioaccessibility (BioAC%) of the metals: As, Cd, Cr, and Pb in soils was determined using Eqn. 12 (Li *et al.*,, 2015).

$$\boldsymbol{BioAC = \frac{C_{extractable}}{tCon} x100\%} \quad \text{...} \quad \textbf{Equation 12}$$

From Equation 12, $C_{extractable}$ is the concentration of the extractable PTMs and tCon is the total concentration of the PTMs in the soil samples.

The measurement of bioavailability and bioaccessibility of potentially toxic metals: As, Cd, Cr, and Pb in soils is a crucial pollution assessment tool. They are analyzed following the physicochemical parameters (Motsara, 2008; Yao *et al.*,, 2013). The bioavailability of toxic metals in soil is the amount of As, Cr, Cd, and Pb available for uptake that can physiologically enhance bioaccumulation or cause supplementary effects in the organisms from the sum of available As, Cd, Cr, and Pb present in the soil (Kim *et al.*,, 2015).

3.4.1.5 Determination of germination rates of *Brassica napus* and *Raphanus raphanistrum*

The seeds were sorted and pretreated including drying and stratifying. A few of the seeds were arbitrarily selected to study the morphometries using a digital microscope to measure: longitudinal length, transverse length, perimeter, and area in millimeters. The Brassicaceae seeds with their pods were measured every 24hrs for fourteen (14) days until a consistent dry weight was achieved; then, they were mechanically removed from the siliques and kept in paper bags at 20-25^{0}C before the experiment. The individual seed of each species was measured in five replications (Tables 4.5 and 4.6). It was subdivided into experimental groups comprising 100 seeds/species and treated with different priming techniques, and chemical germination agents (CGA) to induce laboratory germination. The laboratory test materials and reagents included 100 by 15 mm Petri dishes, doubled-layer tissue papers, wash bottles, 70% ethanol, 0.25 mg/L of gibberellic acid (GA3), 0.1% potassium nitrate (KNO_3), concentrated hydrochloric (HCl) and sulfuric (H_2SO_4) acids.

The priming techniques' efficiency to break dormancy in the selected Brassicaceae seeds was measured using equations 13, 14, and 15 to calculate the Germination Index (GI), Time for 50% germination of seedlings, and Mean Germination Time, respectively (Ali *et al.*, 2012). The germination index (GI) was used to compute daily germination counts for the 14-day trial, adopting the Association of Official Seed Analyst method as indicated in Eqn. 13 (Isely, 1965).

$$\mathbf{GI} = \frac{\textbf{No. germinated seeds (GmS)}}{\textbf{Days of firts count(TsD)}} + - - - + \frac{\textbf{No.of germinated (GmS)}}{\textbf{Days of final count}} \qquad \textbf{Equation 13}$$

The time required for 50% germination of the seedlings (T_{50}) was calculated using Eqn. 14 (Coolbear *et al.*, 1984). However, the percent of the seedling germinated at half of the experimental time, that is, in 7 days, was calculated.

$$(\boldsymbol{T_{50}}) = \boldsymbol{t_i} + \frac{\left(\frac{\boldsymbol{N}}{\boldsymbol{2-n_i}}\right)(\boldsymbol{t_j}-\boldsymbol{t_i})}{\mathbf{1}!} \qquad \textbf{Equation 14}$$

From equation 14, N is the final number of germinated seeds, t_i is the start time, n_i is the number of seeds that have germinated at t_i, and n_j is the final number of seeds germinated at t_i (final time of MGT, respectively when $n_i < N/2 < n_j$.

Similarly, the mean germination time (MGT) was computed according to Eqn. 15, where n is the number of germinated seeds or emerging seedlings on day D, where D is the sum of days counted from the start of germination (Ellis *et al.*, 1981):

$$\mathbf{MGT} = \sum(\mathbf{D_n}) / \sum \mathbf{n} \qquad \textbf{Equation 15}$$

As indicated in Table 4.7, twenty (20) seeds of each crop were placed in 50 mL beakers and presoaked in distilled water H_2O for 24 hrs before treatment with chemical germination agents. The seeds were removed from the water and desiccated for 2-3 hours (hrs.) before treatment. The seeds were then transferred to Petri dishes disinfected with 70% ethanol and filled with double-layer soft tissues. The seeds were unselectively divided into five (5) groups, that is, 20 seeds per petri dish (5 replicates), and treated with 10 mL of GA_3, KNO_3, HCl, H_2SO_4, and H_20 in separate setups. Hydro priming using distilled H_2O was the control treatment for all induced germination in these trials.

3.4.1.6 Phytoremediation *Brassica napus* and *Raphanus raphanistrum*

3.4.1.6.1 Enhanced phytoremediation putative mutants of *Brassica napus* and *Raphanus raphanistrum*

Abiotic factors, such as salt stress, suppress plant growth and productivity. However, research mostly in plant breeding and biotechnology has shown that some species of Brassicaceae, through genetic modification, have become stress-tolerant over time as discussed by Salah (2018). Some Brassicaceae have shown extra-economic and medicinal values with enriching genetic diversity and distribution around the globe.

These characteristic traits have enhanced the conservation, breeding, and management of Brassicaceae species according to El-Esawi (2017).

This objective was achieved through the enhancement of selected wild Brassicaceae species, *B. napus* and *R raphanistrum* using colchicine. The plants were enhanced through chemical treatment of the seeds in different doses of prepared colchicine solutions as described by Nura *et al.* (2017). Colchicine is a commonly used mutagenic agent that boosts plant growth, and development increases biomass and reinforces its resistance to different stress factors in the environmental (Le *et al.*, 2020; Viana *et al.*, 2019). Chemical modification in plants enhances genetic variations, plant growth, and morphological improvements as stated by Viana *et al.*, (2019).

In this procedure, a stock solution of 1.00% colchicine was prepared, from which the desired working concentrations (percentage) were prepared as indicated in Eqn. 16. The working solutions' concentrations of colchicine prepared were 0.00%, 0.25%, 0.50%, and 1.00% colchicine as in Eqn. 16.

$$\mathbf{Concentration\%\ (w/v)} = \frac{\mathbf{Weight\ solute_{gram}}}{\mathbf{Volume\ of\ Solution_{mL}}}\mathbf{x100\%} \qquad \textbf{Equation 16}$$

From the 1000s of harvested and dried seeds of *B. napus* and *R. raphanistrum* seeds, about 500 seeds each were presoaked for 8-10 hours in distilled water and drained for about 30 minutes to 1 hour at room temperature. The soaked seeds were divided into four groups including the control (0.00%), minimum dose (0.25 %), medium dose (0.50%), and maximum dose (1.00%).

In the colchicine chemical modification experimental setup, each group of 500s (M_0) seeds per plant was apportioned into four batches, 100 seeds per treatment per species, and the remaining seeds were kept in the glasshouse. The apportioned seeds were transferred into separate 250ml bottles before adding 200 ml of each varying concentration, 0.00%, 0.25%. 0.50%, and 1.00% for six (6) hours with alternating shaking according to Mullainathan *et al.,* (2013) procedural steps. The seeds were rinsed with deionized water to remove excess reagent before planting. The treated M_1 seeds and the controls, which were treated with 0.00% concentrations of colchicine, were sown in thirty-two (32) experimental pots, two (2) seeds per pot comprising *Brassica napus* and *Raphanus raphanistrum* accordingly. The experiment was setup in a complete randomized design and each treatment dose was replicated four times. The M_2 seeds harvested from M_1 and the seeds from the wild plants from untreated seed pots (M_o) were allowed to dry in the glasshouse. The dried seeds were then planted in sampled soil from Moiben in the pots experiment.

The pots were regularly moistened with rain water and monitored in a controlled environment as they grew, and weekly measured data was recorded as done in previous studies (Vandenhove *et al.,*, 2009; Xu *et al.,* 2016). The soils and plant biomass were analyzed using ICP-MS. Each plant's morphometries, including height and leave's broadness, were evaluated through analysis of variance (ANOVA) to test the treatment significance. All experimental analyses of enhanced Brassicaceae in phytoremediation of the selected potentially toxic metals: Cr and Pb were conducted using ICP-MS according to Yang *et al.,* (2018), and Helaluddin (2016).

3.4.1.6.2 Phytoremediation: Soil treatments and setup

Evaluation of phytoremediation to decontaminate potentially toxic metals, Cr, and Pb in agricultural soils collected from Moiben was conducted at the glasshouse, University of Eldoret. The 4 x 8 pot experiment was carried out in a completely randomized design (CRD) as described by Sale (2015). There were two sets, one set consisting of M_0 planted seeds and the other set consisting of M_1 planted seeds. There were 32 pots, 16 per species, with four treatments and four replicates. The experimental pots were an equal size, filled with 3kg of agricultural soil from Moiben, and spiked with the PTMs. The soil in each pot was spiked with one hundred milliliters of 3000 parts per million (mg/Kg) concentration of Lead from lead nitrate $Pb(NO_3)_2$, according to the techniques proposed by Arshad *et al.* (2016). Lead nitrate stock solution was prepared by weighing 7.992g of Pb $(NO_3)_2$ analytical grade reagent in a 1000-mL volumetric flask. 50mL of Nitric acid was added before diluting to the 1000mL mark with deionized water. A similar preparation procedure was carried out to make the stock solution for Cr. About 1g of potassium dichromate ($K_2Cr_2O_7$) analytic grade was dissolved into 1000 ml of deionized water to prepare 1000 mg/L (mg/Kg) of Cr. To get the desired working solutions, Eqn. 17 was used.

$$\mathbf{C_1V_1 = C_2V_2} \qquad \textbf{Equation 17}$$

C_1= the initial concentration of the stock solution, V_1= the volume of the stock solution, C_2=the concentration of the desired solution, and V_2=the volume of the desired solution.

The solution was diluted to the desired concentrations of PTMs after spiking the soil at 274.56 mg/Kg and 3985.64 mg/Kg for Cr and Pb respectively. The spiked soils were allowed to stay for 72 hours before planting *B. napus* and *R. raphanistrum* treated with varying doses of colchicine, 0.00%, 0.25%, 0.50%, and 1.00% to enhance their phytoremediation potential to uptake PTMs as explained in section (3.3.1.6.1).

The PTMs concentrations in the soil were measured before and after each trial. Similarly, the plant biomass including roots, stems, leaves, and seeds was measured to evaluate the potential of colchicine-enhanced phytoremediation of the treated *B. napus* and *R. raphanistrum*. One-way Analysis of variance (ANOVA) was used to compare the means. The means were separated using Fisher's Least Significant Difference (LSD) at p=0.05 as proposed by Glaz *et al.* (2020). This process was repeated for every consecutive planting season, from M_2 and M_3 in the glasshouse. Each trial lasted for about three months for *R. raphanistrum* and four months for *B. napus*. The same procedure was followed from M_2 to get the M_3 without further treatment also as in M_2 according to the method proposed by Al-Naggar *et al.* (2015). The procedures followed are also proposed by Khursheed *et al.* (2017). The modified populations of every generation (M_1, M_2, and M_3) of each Brassicaceae species and its control were assessed through growth rate, height, and leave broadness as suggested by CHEN *et al.* (2018). The surviving Brassicaceae of *Raphanus raphanistrum and Brassica napus* in every generation were planted in contaminated soils, harvested, and tested for their hyperaccumulating capacities of Cr and Pb as in similar studies by Das *et al.* (2015).

3.5 Statistical analysis

The collected data were analyzed using descriptive statistics; the results are presented in tables, graphs, and charts from Microsoft Excel and SPSS version 23.0 (Diana, 2013; George *et al.*, 2016). Potentially toxic metal concentrations in soil and uptake in mutated plants were determined using analysis of variance (ANOVA), single factor, and students t-test (Assaad *et al.*, 2015).

Generally, statistical significance was tested at $p<0.05$, except otherwise specified as proposed by Benjamin *et al.* (2018). Experimental pots containing the two plant species, *Brassica napus*, and *Raphanus raphanistrum*, and four replicates of each plant were studied in a controlled environment using a complete randomized block design (Rady *et al.*, 2019; Serek *et al.*, 1994).

CHAPTER FOUR

RESULTS

4.1 Introduction

This chapter presents analytical results from the potentially toxic metals concentrations in soils within the study area and the associated health and ecological risks, selected physicochemical parameters measures, *in vitro* bioaccessibility, and the enhanced phytoremediation efficiency experimental study of *R. raphanistrum* and *B. napus* to decontaminate PTMs polluted soils.

4.2 Potentially toxic metals concentrations in agricultural soils

Potentially toxic metals assessed in this study are As, Cd, Cr, Hg, and Pb. The mean concentration of each PTM analyzed in the soil is provided in Table 4.1. The maximum mean concentrations of the elements in agricultural soil samples in the study areas, 6.39±0.10 mgkg^{-1} As, 0.13±0.02 mg/kg^{-1} Cd, 48.19±0.06 mg/kg^{-1} Cr, and 35.89±0.01 mgkg^{-1} Pb as presented in Table 4.1. At all sites, As and Cr were above the USEPA's agricultural soil regulatory standards (USEPA, 2002).

Table 4.1: Potentially toxic metals concentrations in soil (Cs, mg/kg) collected from surface soils, Uasin Gishu, Kenya

Site	As	Cd	Cr	Pb	Hg
Kaprobu	5.68±0.04	0.12±0.02	26.76±0.08	24.84±0.05	ND
Moiben	5.63±0.00	0.12±0.01	48.19±0.06	35.89±0.01	ND
Ziwa	6.39±0.10	0.13±0.02	27.65±0.01	33.29±0.02	ND
Kosyin	2.99±0.02	0.06±0.06	14.31±0.02	16.46±0.03	ND
Naiberi	5.04±0.05	0.08±0.01	25.46±0.01	29.55±0.02	ND

Relatively, Cr and Pb recorded higher concentrations compared to As and Cd, particularly in Moiben; whereas the concentrations of Hg were not detected (ND) by the Inductively Coupled Plasma Mass Spectrometry using the adopted method as indicated in Table 4.1.

However, Pb concentration levels in the soil samples were within the recommended values of USEPA standards in agricultural soils. The concentration of Pb in non-agricultural soil from the same location showed no significant difference. In addition, Cd and Pb concentration levels in soil samples from Moiben compared to other international standards including Tanzania, Canada, and China showed that the two PTMs were within allowable limits. Furthermore, the concentrations of As and Cr were more than the regulatory standards of the World Health Organization (WHO) as indicated in Table 4.2 (Cepa, 2007; Kinuthia *et al.*, 2020; Mee, 2018).

Table 4.2: Potentially toxic metals regulatory standards for agricultural soils, adopted from He et al. (2015) and (Kinuthia et al., 2020a)

Regulator/Country	As	Cd	Cr	Hg	Pb
Australia	20	3	50	1	300
Canada	20	3	250	0.8	100
China	20-40	0.3-0.6	150-300	0.3-1.0	80
Kenya	NG	NG	NG	NG	NG
Tanzania	1	1	100	2	200
USEPA	0.11	0.48	11	1	200
WHO	-	0.003	0.10	0.08	0.10

4.2.2 Ecological Risk Index of PTMs in Moiben

The ecological risks assessed focused on the geo-accumulation factor (Igeo) and ecological risk index (ER_I**)**. The calculated results in the study areas are summarized and presented in Table 4.3. The Igeo showed extreme contamination of As, Cr, and Pb in the study area (5< *Igeo,* extremely high) (Appendix III); but Cd was low (0< *Igeo*≤1, moderate**)** according to Muller (1969). Hakanson's indexing method as in equations 2 and 3 was used to assess the ecological contamination. Given that, the cumulative effects of Igeo, ER_I was low in the study area, that is, ER_I < 40=low as proposed by Hakanson (1980).

Table 4.3: Ecological risks, Igeo and ERI computed results of the study areas

	Igeo				**ER_I**			
Site	**As**	**Cd**	**Cr**	**Pb**	**As**	**Cd**	**Cr**	**Pb**
Kaprobu	8.52	0.17	40.14	37.26	3.79	3.47	0.59	17.75
Moiben	8.44	0.19	72.28	53.84	3.75	3.72	1.07	25.64
Ziwa	9.59	0.19	41.47	49.93	4.26	3.88	0.61	23.78
Kosyin	4.49	0.09	21.46	24.69	1.99	1.80	0.32	11.76
Naiberi	7.55	0.12	38.18	44.32	3.36	2.44	0.57	21.10

The results are interpreted based on ecological risk standards as indicated in Appendix III. Though the selection of reference soil values differs significantly, with some studies considering means of PTMs contained in sediments and shale; while other studies adopt recognized national soil standards and environmental screening levels (Wang *et al.*,, 2013; Weissmannová *et al.*,, 2017). However, the Hakanson procedure followed preindustrial levels of potentially toxic metals in soil and was considered in this study.

The single factor contamination estimates, that is, the geo-accumulation factor of the metals were all above threshold values (Appendix III) for all elements (As, Cr, and Pb) in the study area; ranging from low to extremely high contamination. The ecological index was with the highest value, 25.64 (low) in Moiben as indicated in Table 4.3. These values present a very high geo-accumulation factor.

4.2.3 Health risk characterization

The health risk focused primarily focused on the target cancer risk (CTR) and non-cancer risk (HI) levels in soil samples collected from the selected farm in Moiben, Uasin Gishu County. The results are summarized and presented in Table 4.4. The human health risk was assessed according to the United States Environmental Protection Agency (USEPA) guidelines for potentially toxic metals monitoring and assessment protocols (USEPA, 2011). The Target Cancer Risk (CTRs) and Hazard Index (HI) were computed for the PTMs via incidental ingestion, and dermal exposure (Johnbull *et al.*,, 2019).

Table 4.4: Target Carcinogenic Risk (CTR) Non-carcinogenic (HI) risks of selected PTMs in the study area

Sample Area	Total Cancer Risk		Total Non-Cancer Risk	
	Children	**Adult**	**Children**	**Adult**
MOIBEN	1.865E-05	1.584E-05	0.184	0.039

4.2.4 Cancer risk assessment

The CTR ranged from 1.354E-05 to 1.865E-05 and 1.584E-05 to 1.167E-05 for children and adults, respectively. On the general basics, the USEPA method assumes the carcinogenic risk, that is, the occurrence of less than one cancer possibility in about 1000,000 people (1.0 E-06) is negligible and possibilities above 1.0E-04 is significantly high and require environmental remediation (Gu *et al.*, 2016; USEPA, 2011; Zhang *et al.*,, 2020).

4.2.5 Non-carcinogenic risk characterization

Similarly, the HI measured were all below unity, indicating non-carcinogenic risk to exposed humans in the study areas at present. However, these results are not warranties that the present low health risk levels will not ever change as the PTM concentrations are slightly above the geogenic levels (Hakanson, 1980). Continuous unfriendly environmental human activities affect the concentration levels of PTMs in soils and subsequently will affect the health risk index.

4.3 Physicochemical parameters

4.3.1 Physicochemical parameters of agricultural and non-agricultural soils in Moiben

Total concentrations for selected PTMs, Cr, and Pb in agricultural and non-agricultural soils from the farm in Moiben were relatively high. A student *t-test* computed for Cr levels in the area between agricultural and non-agricultural soils showed that there was no significant difference, ($t = -0.13$, p-value = 0.9014), it was found that non-agricultural soil contained 48.23 mg/kg compared to 48.19 mg/kg in agricultural soil. For Pb, the concentrations were high but not significant, ($t = -0.75$, p-value = 0.4961). The PTMs recorded in non-agricultural soil was 36.63 mg/kg while agricultural soil recorded the lowest, 35.96 mg/kg as shown in Figure 4.1. The high concentrations in the sampled soils are possibly due to human activities and the geochemistry of the study areas (Mbene *et al.*, 2017).

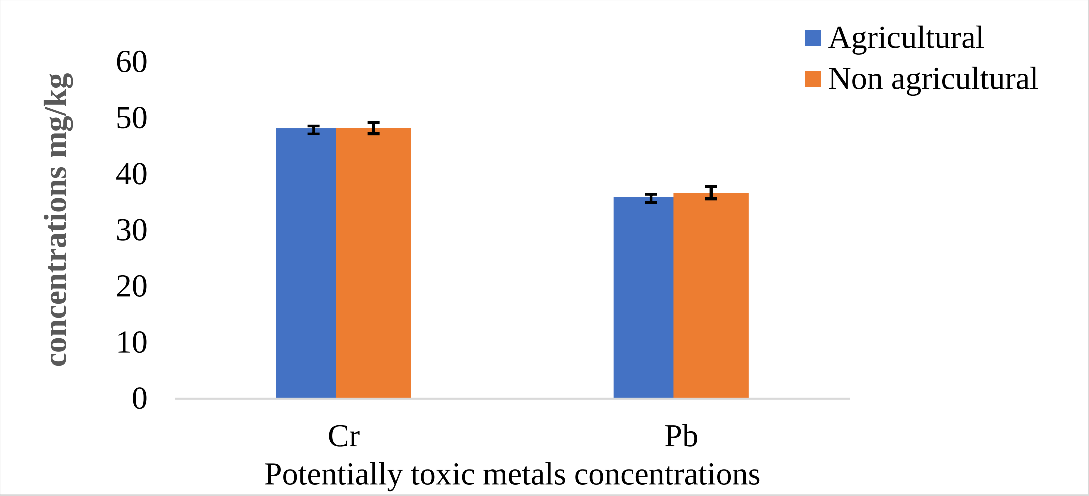

Figure 4.1: Mean total concentrations of potentially toxic metals in Moiben agricultural soil

The mean levels of potential hydrogen (pH) were high (5.39±0.01) in non-agricultural soil as compared with agricultural soil (5.20±0.10) with a significant difference (t=-3.27, p=0.0307). Similarly, organic matter in percentage (OC %) was significantly high (t=55.11, p<0.0001) in non-agricultural land (1.38±0.01) compared with agricultural land (1.83±0.01). Levels of Al (Cmol+kg-1) and H^+ (Cmol+kg-1) did not differ between agricultural and non-agricultural soils as illustrated in Table 4.5.

Table 4.5: Physicochemical parameters of agricultural and non-agricultural soil in Moiben

Parameters	Agricultural	Non-agricultural	t-test	p-value
pH	5.20±0.10	5.39±0.01	-3.27	0.0307*
OC (%)	1.83±0.01	1.38±0.01	55.11	0.0000*
AL (Cmol+kg-1)	4.33±2.08	6.00±1.00	-1.25	0.2794
H (Cmol+kg-1)	3.00±1.00	2.33±0.58	1.00	0.3739

** Represent a significant difference*

4.4 Bioaccessibility of potential toxic in soils

In vitro bioaccessibility of the potentially toxic metals, Pb and Cr in agricultural soil samples collected from Moiben was assessed. The percent *in vitro* bioaccessibility in the soil sample of the PTMs was determined using the USEPA (USEPA, 2008). The results in percentage (%) of the *in vitro* bioaccessibility computed are summarized and presented in Table 4.6.

Table 4.6: In vitro bioaccessibility (%) of Cr and Pb in Moiben.

***In vitro* Bioaccessibility (IVB) of PTMs (%)**		
Site	**Cr**	**Pb**
Moiben	0.77%	11.88%

Soil physicochemical properties affect *in vitro* bioaccessibility of selected PTMs in agricultural soil as reported by Lake *et al.* (2021). The results were correlated to the physical-chemical parameters in soil samples from the study area. In the correlation study, the *in vitro* bioaccessibility of Pb (%) and Cr (%) were compared to the soil pH, percent organic matter (OC), available aluminum (AL^{+3}) (Cmol+kg^{-1}), and available hydrogen (H^+) (Cmol+kg^{-1}) in Moiben. The physicochemical parameters of soil that were significantly correlated (Appendix IX) with the *in vitro bioaccessibility* include, Pb (%) and pH (r=-1, p=<0.0001) with a linear equation of y=-0.1x +12.39 and $R^2 = 0.75$, Pb (%) and OC (%) (r=-1, p=<0.0001) with a linear equation of y = -1x + 13.70 and $R^2 = 0.75$. Equally, the following physicochemical parameters were significant with Chromium: Cr (%) and pH (r=-1, p=<0.0001) with a linear equation of y = -0.1x + 1.28 and $R^2 = 1$, Cr (%) and OC (%) (r=-1, p=<0.0001) with a linear equation of y = -1x + 2.59 and $R^2 = 1$.

Table 4.7: Pearson correlation coefficients for physicochemical parameters and *in Vitro* bioaccessibility of potentially toxic metals

Physicochemical parameters	Cr (%)	Pb (%)	pH	OC (%)	AL (Cmol+kg-1)	H (Cmol+kg-1)
Pb (%)	1.00					
pH	-1.00*	-1.00*				
OC (%)	-1.00*	-1.00*	1.00			
AL (Cmol+kg-1)	0.96	0.96	-0.96	-0.96		
H (Cmol+kg-1)	0.50	0.50	-0.50	-0.50	0.24	1.00

From Table 4.7, there was no correlation between both the available aluminum (Al^{+3}) (Cmol^{+}kg^{-1}) and hydrogen (H^{+}) (Cmol^{+}kg^{-1}) and the PTMs concentration, percentage of Pb and percentage of Cr in the study area. That is, a change in either AL^{+3} or H^{+} does not affect the *in vitro* bioaccessibility of the Pb and Cr. However, there were strong negative correlations between *in vitro* bioaccessibility (Pb and Cr) and physicochemical parameters (pH and OC) in the study area. This means an increase in one or all of these parameters, soil pH and OC reduces the bioaccessibility of the PTMs, Pb, and Cr. The low *in vitro* bioaccessibility of the PTMs is due to a slightly increasing pH and OC in the soil. That is, other physical-chemical parameters such as soil texture and aggregates including other anthropogenic activities have interfered with *in vitro* bioaccessibility of the PTMs in the study area (Fernández-Landero *et al.*, 2021; Guo *et al.*, 2022).

4.5 Chemical priming of seeds

Seeds of *R. raphanistrum* (wild radish) and *B. napus* (canola) were collected from farms within the study areas around Uasin Gishu County during postharvest seasons (Appendix VIII). The *B. napus* seeds appeared more spherical, rounded, and narrowly curved at the apexes. They have light brown skins that easily peel off when they are outside of the siliques. They weighed, about 0.03g compared to the *R. raphanistrum*. The *B. napus* seeds are easily removed from the siliques when they are matured and dried. Unlike the *Brassica napus*, the *Raphanus raphanistrum* seeds weighed heavier, they are dark brown, and more oval in shape. The *Raphanus raphanistrum* seeds were tightly held within the siliques and required aid to be removed. The seeds of both species are displayed in Figure 3.2.

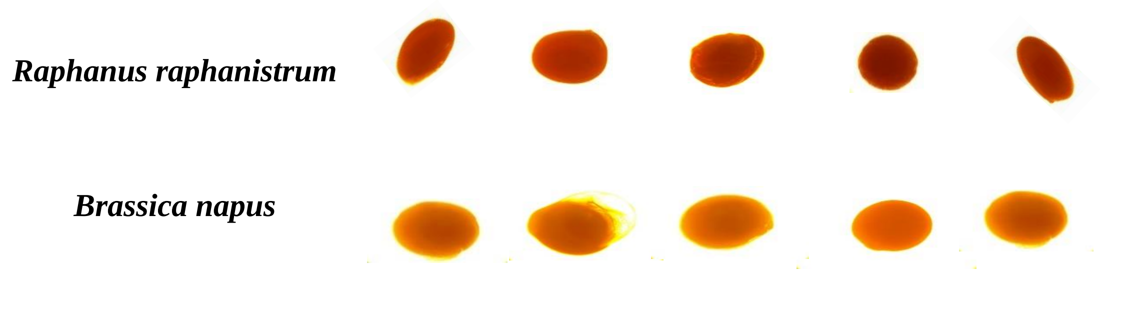

Figure 4.2: Images of Brassicaceae seeds (Source: Author, 2022)

The morphometric characteristics of the *Raphanus raphanistrum* seed, including its length, width, perimeter, and area were measured and the results are summarized and presented in Table 4.8. The mean seed weight, length, width, perimeter, and area for *Raphanus raphanistrum,* were 0.06 ±0.02gram, 1.51±0.01millimeter, 1.18±0.01 millimeter, 4.25±0.04 square millimeters, and 1501.81±33.11 square millimeters, respectively.

Table 4.8: Morphological characteristics of Raphanus raphanistrum (RR)

Species	**Morphological Characteristics**				
Radish (RR)	**Weight (gram)**	**Length (mm*)**	**Width (mm)**	**Perimeter (Sq.mm)**	**Area (Sq.mm)**
RR1	0.06 ±0.00	1.51±0.01	1.28±0.02	4.36 ± 0.04	1614.51 ± 32.16
RR2	0.06 ±0.01	1.50±0.01	1.17±0.03	4.28 ± 0.04	1512.45 ± 33.19
RR3	0.05 ±0.02	1.50±0.01	1.17±0.02	4.22 ± 0.04	1472.29 ± 34.18
RR4	0.05 ±0.00	1.57±0.01	1.16±0.01	4.31 ± 0.04	1523.47 ± 31.16
RR5	0.05 ±0.01	1.48±0.01	1.13±0.01	4.11 ± 0.04	1386.36 ± 35.17
Means	0.06 ±0.02	1.51±0.01	1.18±0.01	4.25 ± 0.04	1501.81 ± 33.11

*mm= millimeter

Similarly, the measured physical parameters of *Brassica napus* are summarized and presented in Table 4.9. The results include 0.03±0.02 gram, 0.94±0.02 millimeter, 0.88±0.04 millimeter, 2.88±0.07 square millimeter, and 701.68±36.77 square millimeters for the weight, length, width, perimeter, and area, respectively.

Table 4.9: Morphological characteristics of *Brassica napus*

Species	Morphological Characteristics				
Canola (BN)	**Weight (gram)**	**Length (mm)**	**Width (mm)**	**Perimeter (sq. mm)**	**Area (sq.mm)**
BN1	0.03 ± 0.01	0.88 ± 0.02	0.72 ± 0.04	2.56 ± 0.05	559.72 ± 32.78
BN2	0.03 ± 0.02	0.99 ± 0.01	0.93 ± 0.03	2.94 ± 0.07	729.95 ± 37.76
BN3	0.03 ± 0.00	0.98 ± 0.02	0.90 ± 0.05	2.99 ± 0.08	768.39 ± 37.77
BN4	0.03 ± 0.01	0.93 ± 0.01	0.94 ± 0.02	2.94 ± 0.07	741.62 ± 36.76
BN5	0.03 ± 0.00	0.91 ± 0.02	0.90 ± 0.06	2.88 ± 0.08	708.71 ± 38.77
Mean	0.03 ± 0.02	0.94 ± 0.02	0.88 ± 0.04	2.88 ± 0.07	701.68 ± 36.77

When the seeds are compared side by side (Figure 4.3), morphometries of the species showed a wider difference in perimeters, lengths, and weights, with *Raphanus raphanistrum* showing dominance in all physical characteristics measured compared to *Brassica napus*.

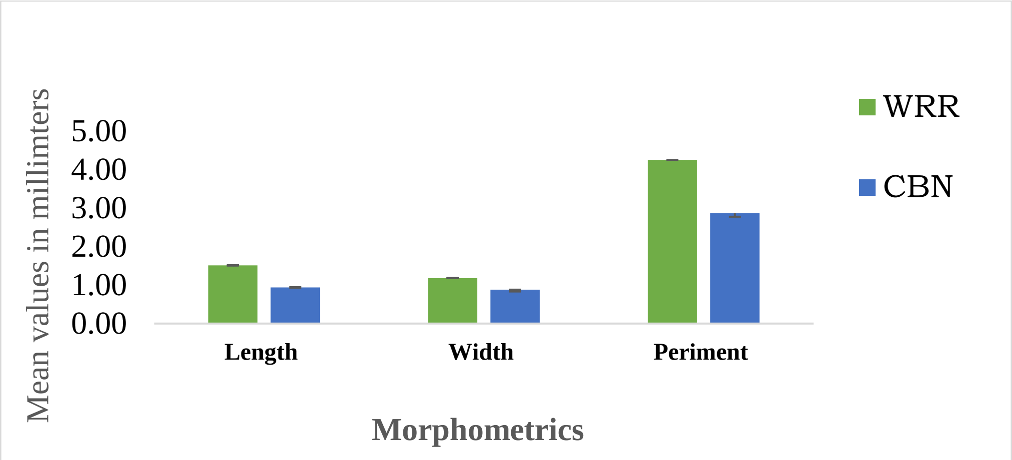

Figure 4.3: Comparative morphometric characteristics in canola (BN) and wild radish (RR)

From the experiment, treatments of *Brassica napus* seeds with chemical agents performed well with germination rates 90, 80, 30, 0, and 0 for KNO_3, GA3, chilling, HCl: H_2SO_4, and H_2O, respectively (Table 4.10). Similarly, treatment of *Raphanus raphanistrum* with the same chemical agents gave the following results: 70, 45, 10, 0, and 0 for GA3, chilling, KNO_3, HCl: H_2SO_4, and H_2O, respectively.

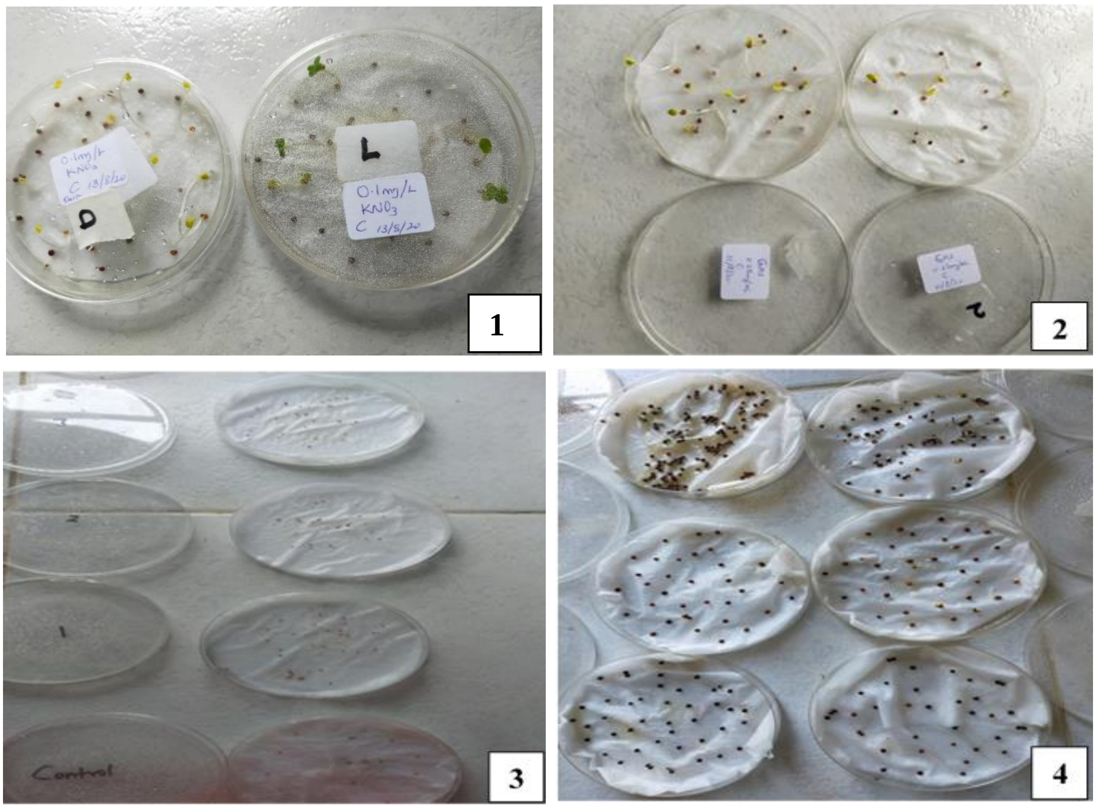

Plate 4.1: Images of KNO_3, GA3, HCl/H_2SO4, and H_2O: 1, 2, 3, & 4 respectively in germination trial.

The germination rates were determined using the germination index (GI) and the mean germination time (MGT). The results showed that GA3 and KNO3 are the most effective germination agents for the selected plants, *B. napus* and *R. raphanistrum* *(Yang et al., 2020).*

Table 4.10: Results of priming of *Brassica napus* and *Raphanus raphanistrum* using various induced germination agents

			Brassica Napus					*Raphanus Raphanistrum*				
Treatment	**Time**	**D**	**T_g**	**GP**	**T_{50}**	**GI**	**MGT**	**T_g**	**GP**	**T_{50}**	**GI**	**MGT**
H_2O	24hrs	10	0	0	0	0	0	0	0	0	0	0
$HCl:H_2SO_4$	0.5Hrs	10	0	0	0	0	0	0	0	0	0	0
Chilling	72Hrs	10	6	30	20	6.55	1.67	9	45	25	10.94	1.11
GA3	24hrs	10	16	80	50	19.43	0.63	14	70	60	23.02	0.71
KNO_3	24hrs	10	18	90	55	19.26	0.56	2	10	10	2.77	5

D=numbers of days

T_g=total seeds germinated, Day=numbers of days of trial, GP=germination percent, T_{5o}= percent germination at half of the trial day, GI=germination index, MGT=mean germination time.

4.6 Phytoremediation

4.6.1 Enhanced phytoremediation of *Brassica napus* and *Raphanus raphanistrum*

Concentrations of potentially toxic metals were assessed for *B. napus* and *R. raphanistrum* in the plant materials and soils. The metals, Cr and Pb had initial soil concentrations of 274.55 mg/Kg, and 3985.64 mg/Kg, respectively. The highest concentrations of PTMs were recorded in the plant roots for all except Cr in the *B. napus* leaf and seeds of M_1 and Pb in the leaf of *R. raphanistrum* followed by leaves, stems, and seeds. Equally, the concentrations of PTMs in the plants' organs were significantly different within generations apart from Chromium concentrations in *Raphanus raphanistrum* stem in the second and third generations (Table 4.11).

Table 4.11: Potentially toxic metals concentrations (mg/Kg) in plants (BN and RR) organs

PTM	Plant species	Trial	Root (mg/Kg)	Stem (mg/Kg)	Leaf (mg/Kg)	Seed (mg/Kg)
Cr	BN	M1	67.12±0.15a**	11.65±0.13b*	150.25±0.13c*	12.32±0.10d*
		M2	67.86±0.54a**	28.90±0.65b*	59.60±0.33c*	36.91±0.45d*
		M3	136.23±1.66a*	5.20±0.26b**	9.41±0.20c**	14.52±0.49d**
	RR	M1	108.05±56.58a*	5.20±0.26b**	9.41±0.20c**	14.52±0.49d**
		M2	139.49±0.39a*	36.66±0.46b*	49.52±0.37c*	11.27±0.19d*
		M3	38.28±0.80a*	8.82±0.44b*	5.96±0.62c*	48.29±0.26d*
Pb	BN	M1	1024.16±1.57a*	546.42±0.77b*	47.35±0.17c*	1.75±0.06*
		M2	674.13±0.37a*	30.73±0.49b*	23.46±0.14c*	1.15±0.07*
		M3	709.41±0.58a*	15.96±0.50b*	21.93±0.89c*	2.90±0.45*
	RR	M1	812.06±0.65a*	12.31±0.15b*	51.74±0.74c*	4.39±0.36*
		M2	240.81±0.87a*	14.37±0.23b*	261.83±0.72c*	1.80±0.21*
		M3	476.37±14.72a*	6.68±0.41b*	13.49±0.53c*	2.42±0.58*

**a,b, c means showed significant differences within rows, and means followed by a single asterisk (*) and double asterisks (**) showed significant and not significant differences within plants' parts at p=0.05 in columns.*

At 0.50% colchicine dose treatment, the roots of both plants absorbed more PTMs, that is, the uptake of PTMs per plant compared to other organs. The different treatment doses affected the plants' organs varying in the different generations, M_1, M_2, and M_3.

4.6.2 Bioconcentration of PTMs in Plant biomass

The bioaccumulation Factor (BCF) of the PTMs in the plant was computed to evaluate the phytoremediation potentials of *B. napus* and *R. raphanistrum* against the uptake of Cr and Pb in the enhanced phytoremediation study. BCF is used to assess plant capacity to concentrate PTMs in its different biomass including roots, stems, leaves, and seeds (Raskin *et al.*, 1994). It measures the phytoremediation potential of PTMs of the plants entirely including phytostabilization and plant biochemical activities that bind PTMs in soils (Takarina *et al.*, 2017). The PTMs bioconcentration factors in *B. napus* and *R. raphanistrum* were computed using the equation, Eqn 21:

$$\boldsymbol{BCF = \frac{C_{shoot}}{C_{soil}}} \text{ --} \textbf{Equation 21}$$

From Eqn. 21, BCF is the measure of bioconcentration factor, and C_{shoot} and C_{soil}, are the PTM concentrations (mg/Kg) in the plant shoot and soil, respectively.

The bioconcentration factor results were computed and tabulated in Table 4.12 where the BCF showed that the plants are promising hyperaccumulators that can be used in the phytoremediation of potentially toxic metals in polluted soils. The results showed that both species, *B. napus* and *R. raphanistrum* have more affinity for Cr and Pb decontamination from the polluted soil, hence hyperaccumulation potential according to Madanan *et al.* (2021). However, the plants are more likely to remove high among of Cr compared to Pb as presented by the cumulative bioconcentration factor of the plants in Table 4.12.

Table 4.12: BCF of the different organs of BN and RR

PTM	BN			RR		
	Root	Stem	Leaf	Root	Stem	Leaf
Cr	2.41*	0.42	1.99*	2.84*	0.70	0.66
Pb	0.91	0.23	0.04	0.77	0.02	0.16

**plant with hyperaccumulation potential of PTM*

4.6.3 Effects of colchicine dosage on PTMs concentrations in the plants' organs

Effects of colchicine dosage on potentially toxic metals uptake in the plants' biomass for the different planting periods, that is, M_1, M_2, and M_3 were assessed. Treatment doses of colchicine in both plants were 0.00% 0.25%, 0.50% and 1.00%. In *B. napus* as presented in Table 4.13, the higher mean concentration of Chromium, 241.35±0.22 mg/kg was recorded in the planted species treated with 0.50% dosage of colchicine and the lower mean concentration in plants that received 0.00% in M_1, with a significant difference ($F_{0.05\,(3,\,12)}$ =2783.90, p=0.0001). A Ronald Aylmer Fishers Post hoc test, Least Significance Difference (LSD) showed that significant differences were found within all treatment levels, 0.00%, 0.25%, 0.50%, and 1.00%. In M_2, the higher mean concentration of chromium, 210.05±0.44 mg/Kg was recorded in the plants treated with 0.50% dosage of colchicine, and the lower mean concentration, 138.73±3.13 mg/Kg in plants that received 0.00% treatment of colchicine. A similar trend was observed in M_3 in *B. napus*. A generally comparable trend was observed in the treatments M_1, M_2, and M_3, where a statistically significant difference was observed in Chromium absorption within all generations ($p<0.05$). Similarly, higher mean concentrations of Pb, 1619.67±1.99 mg/Kg, 729.47±0.83 mg/Kg, and 750.19±0.69 mg/Kg found in M_1, M_2, and M_3 respectively were recorded in plants treated with 0.50% dose colchicine. The lower mean concentrations, 484.19±0.63 mg/Kg, 422.05±1.23 mg/Kg, and 454.64±9.02 mg/Kg were recorded in plants that received 0.00% for M_1, M_2, and M_3, respectively. In M_1, the mean concentration has a significant difference, ($F_{0.05\,(3,\,12)}$ =659900.00, $p<0.0001$). M_2 and M_3 also followed a similar trend, with significant ($p<0.05$) between and within means of all treatment dosages as indicated in Table 4.13.

Table 4.13: Effect of colchicine dosage and Trial on the plant's biomass accumulated PTMs

			M_1	M_2	M_3
Plant	**PTM**	**Colchicine dosage**	**Mean±Std (mg/Kg)**	**Mean±Std (mg/Kg)**	**Mean±Std (mg/Kg)**
BN	Cr	0.00%	105.53±0.67a*	138.73±3.13b*	60.41±0.44c*
		0.25%	188.13±0.42a*	193.28±1.67b*	106.33±0.47c*
		0.50%	241.35±0.22a*	210.05±0.44b*	165.36±1.42c*
		1.00%	181.71±1.06a*	209.16±1.05b**	116.19±3.50c*
	Pb	0.00%	484.19±0.63a*	422.05±1.23b*	454.64±9.02c*
		0.25%	627.600.86a*	525.42±0.67b*	565.13±3.22c*
		0.50%	1619.67±1.99a*	729.47±0.83b*	750.19±0.69c*
		1.00%	883.821.03a*	555.56±0.86b*	624.08±1.72c*
RR	Cr	0.00%	103.70±0.42a*	119.21±1.28b*	53.09±0.77c*
		0.25%	180.67±1.39a*	196.35±0.72b*	65.63±0.88c*
		0.50%	226.69±1.22a*	236.95±0.82b*	101.35±1.18c*
		1.00%	191.38±0.75a*	225.26±0.85b*	70.18±3.99c*
	Pb	0.00%	248.53±1.75a*	334.59±0.55b*	305.35±13.78c*
		0.25%	438.46±0.75a*	394.18±0.99b*	376.49±7.72c*
		0.50%	880.49±1.46a*	518.80±±0.81b*	498.96±14.45c*
		1.00%	663.29±0.68a*	418.920.47b*	381.77±9.70c*

a,b,c, Means followed by the different letters in the same row are significantly different at p=0.05 between seasons, whereas means followed by a single asterisk (*) and double asterisks (**) showed significantly and no significant differences within seasons at p=0.05, respectively.

In *R. raphanistrum*, Chromium higher mean concentrations uptake were recorded in plants treated with colchicine at 0.50% and had a significant difference ($p<0.05$) for all within generations, 0.00%, 0.25%, 0.50%, and 1.00% and across, M_1, M_2, and M_3 for all treatments. At 0.50% colchicine treatment, 226.69±1.22 mg/Kg, 236.95±0.82 mg/Kg, and 101.35±1.18 mg/Kg of Cr were recorded for M_1, M_2, and M_3, respectively. While at 0.00% colchicine dose, 103.70±0.42 mg/Kg, 119.21±1.28 mg/Kg, and 53.09±0.77 mg/Kg Chromium were recorded for M_1, M_2, and M_3, respectively. Also, a statistically significant difference ($p<0.05$) was recorded during the experimental trials of Chromium uptake in *Raphanus raphanistrum* as presented in Table 4.13. A similar trend was observed in Lead uptake within all treatments in *Raphanus raphanistrum*. At 0.50% colchicine dose, 880.49±1.46 mg/Kg, 518.80±0.81 mg/Kg, and 498.96±14.45 mg/Kg Pb were recorded at M_1, M_2, and M_3, respectively. In addition, lower uptake of Pb was observed in plants treated with a 0.00% dose of colchicine in *Raphanus raphanistrum*. There was a significant difference ($p<0.05$) at 0.50% colchicine treatment within all generations as presented in Table 4.13.

4.6.4 Effects of colchicine dosage on plants' morphology

Effects of colchicine doses treatment on potentially toxic metals uptake and plant morphology were assessed in both species, *R. raphanistrum* and *B. napus*. An increased in Cr and Pb concentrations uptake in *B. napus* led to negative and not statistically significance correlations with the height and leaf broadness within all generations, M_1, $M_{2,}$ and M_3 ($p>0.05$) as indicated in Table 4.14.

In *R. raphanistrum*, an increased Pb level resulted in a positive but not significant correlation with plant height and leaf broadness in all generations ($p>0.05$), as presented in Table 4.14. At the same time, Cr resulted in a positive but not significant correlation between plant height and leaf broadness in M_1 and M_3. In addition, a negative and not significant correlation was observed in M_2 as presented in Table 4.14.

Table 4.14: Correlation of plant height and leaf broadness to PTMs concentration and trial

	PTM	M1	M2	M3
BN	**Cr**	-0.15 (0.5436)	-0.11 (0.6762)	-0.12 (0.6471)
	Pb	-0.07 (0.9052)	-0.09 (0.1210)	-0.15 (0.7204)
RR	**Cr**	0.06 (0.8093)	-0.12 (0.6326)	0.38 (0.1175)
	Pb	0.28 (0.1015)	0.45 (0.1390)	0.19 (0.2845)

Means numbers in parenthesis are p values

Furthermore, a correlation analysis between the heights and leaf areas of the *B. napus* and *R. raphanistrum* treated with different doses (0.25%, 0.50%, and 1.00%) of colchicine against the control (0.00%) was evaluated. The results showed statistically no significant difference ($p>0.05$) in plant heights and leaf broadness for all *B. napus* across all generations and within all treatment doses of colchicine as illustrated in Figure 4.4. For *R. raphanistrum*, a similar trend was recorded in heights, however, there was significant different between treatment doses 0.50% and 0.00% ($p= 0.0283$), $r2=0.998$ and between treatment 1.00% and 0.00% ($p=0.0355$), $r2=0.997$ in leaf broadness, Figure 4.4.

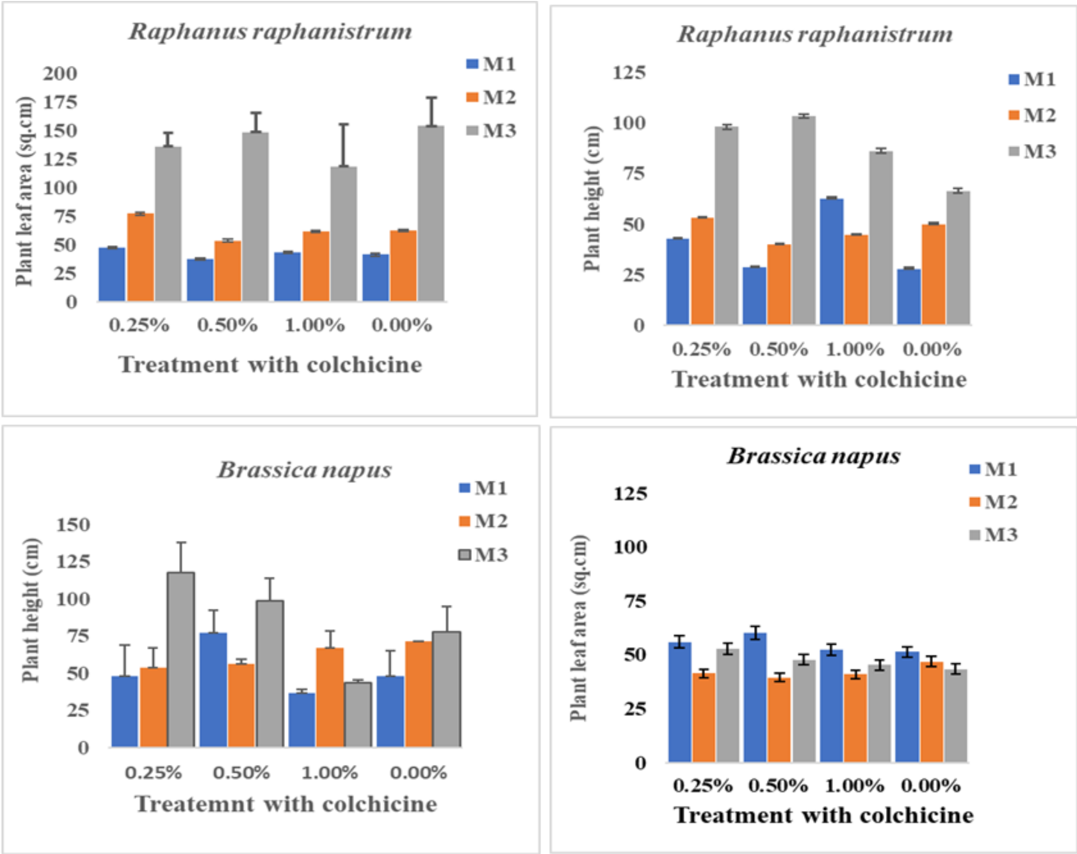

Figure 4.4: Heights (cm) and leaf broadness (sq. cm) of BN and RR in the Trials

4.6.5 Efficiency of enhanced *Brassica napus* and *Raphanus raphanistrum*

Percentage efficiency in potentially toxic metals removal was estimated at the optimal colchicine dosage, 0.50% for all treatments in all generations. This was calculated as the total mean concentration of the metals (mg/Kg) in each plant divided by the initial concentration in the soil multiplied by 100. The average Cr removal efficiency for *B. napus*, 74.88% was not significantly different from *R. raphanistrum* ($p>0.05$) but recorded a higher removal efficiency than *R. raphanistrum*, 68.60%. In M_3 generation, *B. napus* had high percentage Cr removal efficiency of 60.22%, significantly different from that of *R. raphanistrum*, 36.92% ($\chi^2 = 5.4454$, d.f.=1, p= 0.0196).

The average percentage of Lead removal efficiency was higher but not significantly different in *B. napus*, and lower in *R. raphanistrum* (χ^2=2.3935, d.f.=1, p= 0.1218) as illustrated in Figure 4.5. In the M_1 generation, the percentage of Lead removal by *B. napus*, 40.64% was high and significantly different (χ^2 = 5.7569, d.f.=1, p= 0.0164) compared to that of Raphanus raphanistrum, 22.09%.

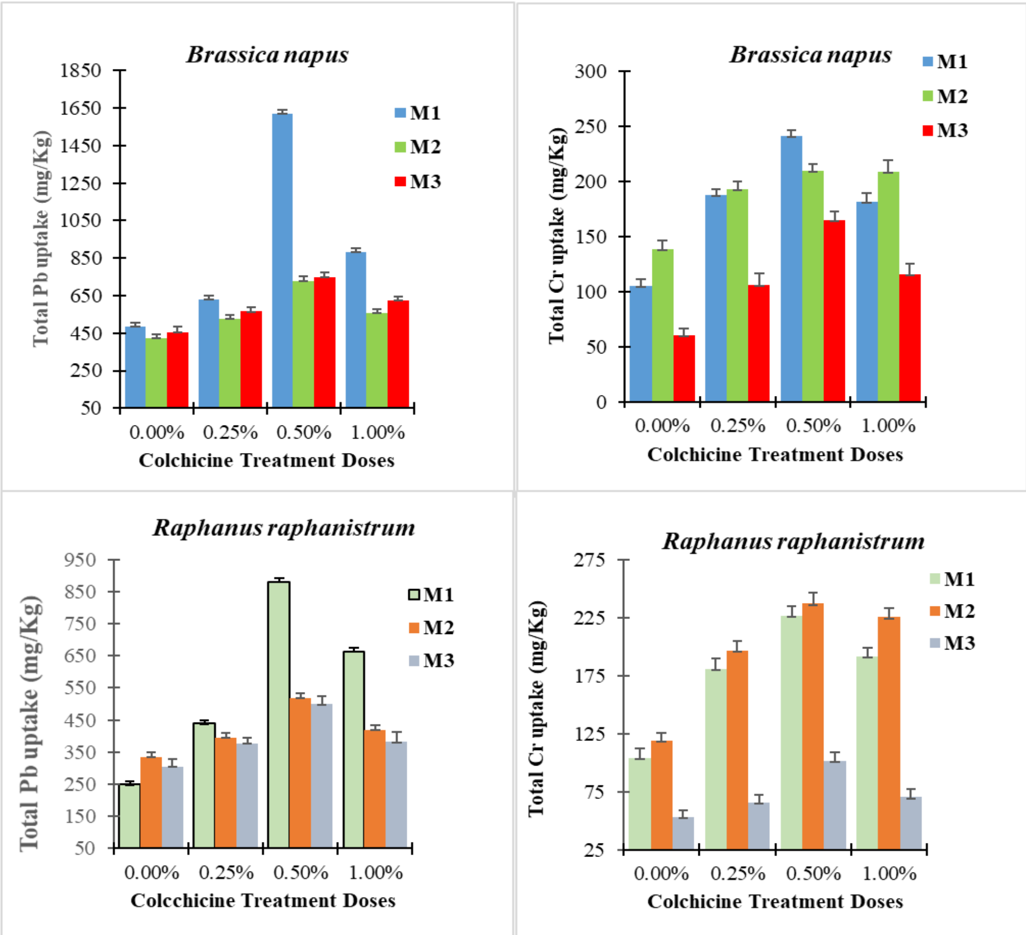

Figure 4.5: Total PTMs uptakes per enhanced plants Brassica napus and Raphanus raphanistrum

CHAPTER FIVE

DISCUSSION

5.1.1 Potentially toxic metals in soils in the study area

The PTMs results presented in Table 4.1, showed that the potentially toxic metals–As, Cr, Cd, and Pb concentrations (mg/kg) levels in soils from the study areas are within a similar range of a review study on potentially toxic elements (PTMs) monitoring in East Africa agroecosystems. In that study, the elements reviewed focused on Hg, Cu, Cd, Zn, Pb, and Cr which are most probably sourced from the use of agrochemicals as reported by Munishi *et al.,* (2021). A similar study on Cd, Pb, Zn, Cu, Cr, As, Hg and Ni in agricultural soils in Kenya also reported comparable concentrations of the metals as reported by Mungai *et al.,* (2016). However, the mean concentration of Pb in all areas, 28.72±7.59 mg/kg was higher than the background values recommended by Hakanson and Bowen's preindustrial standards as presented in Appendix IV. Other metals, that is, Cd and Hg were lower and below minimum threshold values respectively as presented in Table 4.2. These concentration levels in soil compared to a similar study in Baltimore in the United States reported by Qi *et al.* (2020), showed that Moiben recorded higher levels of As and Cr and lower levels for Cd, and Pb except for Hg which was not detected.

Concerns over increasing levels of potentially toxic metal concentrations in agricultural soils have long been investigated to understand the health and ecological risks of PTMs and the possible transfer from soil into foods as discussed by Holmgren *et al.* (1993). As found in this study, increased Pb concentrations in agricultural soil are mostly associated with the use of agrochemicals, and the combustion of leaded fuel in vehicular and industrial wastes (Musa *et al.*, 2017; Shi *et al.*, 2019; Tóth *et al.*, 2016). Soil health is crucial to sustainable agriculture, ecosystem services delivery, and human health. It enhances soil nutrients, microorganisms, carbon, and structure management and improves high productivity that supports food security (Chu *et al.*, 2019; Kibblewhite *et al.*, 2008). Increased PTMs levels in agrarian soils lead to PTMs bioaccumulation in soil and food crops, hence leading to ecological and health risks according to Chen *et al.* (2016).

On the other hand, potentially toxic metals including As, Cr, Cd, and Pb contaminate agricultural soils, synergistically and antagonistically reducing macro and micronutrient bioavailability to different crops and vegetables according to Khan *et al.* (2019). Several potentially toxic metals remediation techniques have been developed to minimize human exposure and improve soil quality for agricultural purposes, for example, phytoremediation, soil washing, excavation, electro kinetics, and metals binding (Awa *et al.*, 2020). However, the levels of the metals from the study areas were within the range of some previous findings and standards as shown in Table 4.2. Correspondingly, the concentrations of As and Cr at all sampling sites were above the USEPA allowable limits of 0.110mg/Kg and 11.00mg/Kg, respectively; whereas Cd and Pb, did not exceed the regulatory standard, 0.48mg/Kg and 200mg/Kg, respectively (USEPA, 2002). The high levels of Cr are probably due to the use of various fertilizers, both organic and inorganic in the local markets. A study by Kinaichu (2020) on the levels of Cd, Cr, and Pb of bio-slurry (cow dungs and chicken droppings) and inorganic fertilizers (DAP, CAN, UREA, and NPK) in Kenya showed the highest detectable amounts of Cr in the inorganic fertilizers compared Cd and Pb in CAN, DAP UREA, and NPK, respectively. In addition, the elevated levels of Arsenic in agricultural soils in the study areas were not further investigated, especially source apportionment of the PTMs. However, elevated levels of Arsenic in farm soils are as results of application of pesticides, fertilizers and use of As-polluted irrigated water in farming as reported by Wang *et al.* (2019); and Dahal *et al.* (2008). An elevated amount of Arsenic in agricultural soil poses several health problems. Furthermore, research in Taiwan conducted by Lee *et al.* (2021) found that increased levels of Arsenic in agricultural soils were associated with the prevalence of Parkinson's Disease (PD) in that country. Agricultural soil plays an important role in food production and safety as it provides the means of food composition. Therefore, increased levels of potentially toxic metals in agricultural soils pose threat to PTMs translocation and bioaccumulation in food crops (Chen *et al.*,, 2016). This is typically common with Cd and Pb in cereal crops such as maize, rice, and wheat grown in contaminated agricultural soils (Feng *et al.*, 2021; Zhang, Li, *et al.*, 2018). Hence, prolonged exposure to PTMs through incidental ingestion via water, food, and vegetables or dermal to contaminated dust and soil, especially in children (As, Cd, and Pb) can probably lead to serious health consequences such as cancer, vital organs malformation, and retarded growth (Pruvot *et al.*, 2006). In Kenya, several studies have attributed the high levels of PTMs in food to the increased levels in the soil. Among them, a study reported by Mongi *et al.* (2020) on cocoyam produced in the Lake Victoria basin indicated high levels of PTMs above the WHO levels in food and was related to the high levels of the PTMs in soils. In addition, research by Tenai *et al.* (2016) found PTMs (As, Cd, Cr, and Pb) in Lesser Flamingos tissues from Lakes Nakuru, Elementaita, Crater, and Oloidien as a result of high PTMs concentrations in the Lakes' water and sediments resulting from agricultural runoff from the surrounding areas. A similar study on Lakes Naivasha, Elementataita, Nakuru, and Bogoria that investigated PTMs comprising of As, Cd, Hg, and Pb acknowledged human activities as the primary cause of PTMs pollution in the Lakes. The results further indicated that As and Hg may pose probable non-carcinogenic health risks to residents via ingestion from Lakes Elemenntaita, and Bogoria according to Yang *et al.* (2017).

Also, the potential ecological risk per site computed values is disproportionate to the single factor results (Appendix III) and was generally high at the Pb level, but had low ER_I, <40 as discussed by Pan *et al.* (2016). This is true for many agricultural-induced soil pollutions compared to industrial, smelting, and mining-induced soil pollutions, even though, these categorized contaminations are anthropogenic as discussed by Wang *et al.,* (2013). Therefore, this level of potentially toxic metal pollution in soil calls for timely intervention to control, monitor, and manage through sustainable measures and avoid further Pb contamination of the soil. This will lower the likely probable harm of the potentially toxic metals in the study areas as reported by Abuduwaili *et al.* (2015).

Increased potentially toxic metal concentrations in agricultural soil pose a health risk and lower soil quality according to Golui *et al.* (2019). Similarly, harmful to the environment is the use of inorganic fertilizers and pesticides in agriculture. These amendments pollute the environment and subsequently affect food quality (Gajić *et al*., 2018). Exposure to potentially toxic metals such as Hg, Cd, and Pb over a long period causes health consequences including carcinogenicity, mutagenicity, and teratogenicity as well as endocrine disruption and behavior change in children (Ali *et al*., 2013). With different background values (Appendix IV), environmental risk indices including $I_{geo,}$ and ER_I are widely used to evaluate and monitor potentially toxic metals pollution and toxicity in environmental matrices (Bahloul *et al.*, 2018; Golui *et al.*,, 2019; Hakanson, 1980; Rostami *et al.*, 2021).

5.1.2 Health and Ecological risk characterization

5.1.3 Health risk characterization

The CTR results are less than the minimum reference standards for children and adults in the study areas according to the USEPA environmental health assessment protocol. Therefore, the potentially toxic metals in the study areas are unlikely to pose a cancer risk to humans at present. Similarly, the HI for non-carcinogenic risk was less than 1, indicating no significant non-carcinogenic risk to exposed individuals including children and adults in the study areas per the USEPA assessment protocol used (USEPA, 2011). The finding is similar to a recent PTM study in agricultural soils from Iran. The study by Kharazi *et al*., 2021 reported that PTMs (Pb, As, Cd, and Hg) concentrations were lower than recommended standard. But reported high CTR and HI on the contrary (Kharazi *et al.*, 2021). This result is parallel to a contemporary health and ecological risks study of contaminated agricultural soils conducted by Zhang *et al.*, (2022) in China. Although the findings are dissimilar in terms of the empirical results, this could be due to the difference in physicochemical parameters of the distinct geographical locations and the anthropogenic activities in land-use changes (Zhang *et al.*,, 2020). In Africa, a study on PTM contamination in agricultural soil in Malawi showed low to moderate ecological risks as was found in this study (Mussa *et al.*, 2020). Additionally, a case study on eight PTMs (Pb, Cd, Cu, Cr, Ni, Hg, As, and Zn) in agricultural soils from selected areas in Kenya was found to be near toxicity threshold values in line with the USEPA regulatory standards.

The ecological risk index of PTMs showed increased levels of anthropogenic impacts in agricultural soils primarily due to increasing unsustainable agricultural techniques and amendments, urbanization, and industrialization (Mungai *et al.*,, 2016). As the PTMs, Cd, and Pb levels found in this study, a similar previous study in the study area

(Moiben), found that PTMs concentrations in agricultural soils showed relatively high levels of the contaminants in soil samples but within WHO allowable standard limits as was reported by Akenga *et al.* (2020).

5.1.4 Ecological risk

The ecological risk indices including Geo-accumulation (I_{geo}) factor and ecological risk index (ER_I) were used to evaluate the anthropogenic footprint of PTMs in agricultural soil in the study areas. With different background values (Appendix IV), environmental risk indices are generally used to assess and monitor potentially toxic metal pollution and their probable toxicity in environmental matrices (Bahloul *et al.*,, 2018; Golui *et al.*,, 2019; Hakanson, 1980). The indices also are used to assess the intensity of human activities on the presence and spread of the PTMs in surface soils as discussed by Barbieri (2016). The results of the geo-accumulation factor ranged from 21-72mg/kg Cr and 24.69-53.84 mg/kg Pb as found in Moiben, which recorded relatively the highest Igeo compared to others in the study area.

The Igeo indicated that the pollution of Cr and Pb ranged from low to extremely high contamination levels. The below 40 ecological risk index in the study area indicates low ER_I as presented in Table 4.2. These results showed that there are Cr and Pb contaminations in the study areas that required remediation efforts to minimize the levels of PTMs in soil and their subsequent transfer into the food chain as suggested by Dogra *et al.* (2020). These results are similar to the geo-accumulation study of PTMs in agricultural soils. as reported by Rostami *et al.*, (2021) which found that Cd and As were moderately contaminated and showed a low ecological risk for the PTMs. It further stated that the contamination levels of the PTMs are a result of anthropogenic activities mainly agrochemicals, such as pesticides and fertilizers used in agriculture. This argument is supported by a study report conducted in the soil near a fertilizer production plant in Egypt. The study found that elevated levels of PTMs, Cu, Cd, Pb, and Zn in the sampled soils were above the national background and WHO levels; also the Igeo and Contaminated factor showed high contamination degree according to Mohamed *et al.* (2014) and Ullah *et al.* (2020). A related study reported that Cd and Pb in agricultural soil from Thall, Dir-Kohistan recorded low to moderate Igeo contamination with higher ER_I, particularly from Cd which exceeded 40 in the area.

Also comparable to the finding in this study is an assessment report of PTMs in agricultural soil in Morocco. According to Oumenskou *et al.* (2018), the elevated levels of PTMs found in soils are an indication of the human activity's impacts on the soils in the study area. Similarly, in Ghana, PTMs assessment in soils from various human activities including vehicle overhauling, oil exchange, and spraying fields showed alarming levels of the metals which were followed that the ecological risks, $ER_{I,}$ and Igeo of the Pb, As, and other PTMs were heavily contaminated in the surface soils as reported by Asamoah *et al.* (2021). According to a study reported by Wanjala *et al.* (2020) in Ortum, Kenya on selected PTMs, Cr, Pb, As, and Cu among others in soils from various environmental matrices showed that the Igeo ranged from 0.4 to 4.92 mg/Kg and $ER_{I,}$ 19.69 were moderately contaminated and posed low ER_I pollution, respectively. A similar finding was reported from the sediments in Lake Naivasha, Kenya indicating low to moderate contamination of the sediments which also confirmed human interferences with the environment as reported by Maina *et al.* (2019). This finding was recently supported via research conducted by Njogu *et al.* (2021) in which PTMs (Pb and Cd) concentrations in soil, sediment, and food samples in the Lake Naivasha basin reported high values as a result of increased agricultural activities in the lake's basin.

5.2 Physicochemical parameters of soil

5.2.1 Soil pH

Soil pH plays a pivotal role in potentially toxic metals and nutrient availability, distribution, and uptake by plants in soils (Solis *et al.*,, 2005). Table 4.5 and Table 4.1 summarizes the physicochemical parameters and the PTM concentrations in the study area, respectively. The agricultural soil was found to be acidic, pH of 5.20 with relatively high concentrations of Pb, 35.97±0.41 mg/Kg, and Cr, 48.19±1.51 mg/Kg. The pH and PTMs concentrations were slightly higher in a non-agricultural (neutral) soil in the same area, that is, pH 5.39, Pb 36.03±1.14 mg/Kg, and Cr 48.23±0.46 mg/Kg. Thus, it is widely accepted that low pH enhances increased potentially toxic metal concentrations, but on the contrary, this study found that the PTM concentration in the neutral soil, with a slightly high pH, had higher Pb concentrations with no significant difference, Table 4.4. Though it is difficult to conclude what led to these differing results, however, other studies have reported similar contrary results in pH-PTMs concentrations in soils (Khaledian *et al.*, 2017; Mao *et al.*, 2019). A student t-test conducted found that there was no significant relationship between the pH, Cr, and Pb in the agricultural and neutral soils collected from the study areas. This could be due to the kind of farm practice, the areas are largely involved in mixed cropping, maize and wheat farming with the use of inorganic fertilizer and other amendments that subsequently leach nitrogen and increases phosphorus, and potassium hence increasing soil pH as stated by Lv *et al.* (2020). The low soil pH is also accredited to agrochemicals use, particularly sulfur-containing. Ammonium-based fertilizers and carbamate (urea). These chemical substances including atmospheric depositions and precipitation of acidifying gases increase soil acidity (Goulding, 2016).

The high concentration levels of PTMs in the study areas could also be a result of intense anthropogenic activities, mainly agriculture including fertilizer and pesticide applications (Shan *et al.*, 2013; Su *et al.*, 2022). This lowers the soil pH and increases the bioavailability of PTMs in crops. High intake of bioavailable PTMs from the soil into crops and vegetables increases the ecological and health risk of the consumers including humans, animals, and the ecological communities (Ali *et al.*, 2019; Lian *et al.*, 2019).

5.2.2: Organic Matter

The biochemistry of soil organic matter is an important, complex, and dynamic soil physicochemical parameter that contributes greatly to the functioning of the soil environment and the welfare of the soil ecosystems. It contains mostly carbon, the constituent backbone of living matter. The biogeochemistry, that is, the composition, distribution, and interaction of organic and inorganic matters, for example, PTMs are to a larger extent regulated by soil organic matters (Ondrasek *et al.*, 2019). In this study, the soil organic matter, 1.84 % in agricultural soil was slightly higher than 1.38% in non-agricultural soil with low soil pH, 5.20 and 5.39, respectively. This is similar to a study by Enya et al., (2020); the authors reported that soil pH plays important role in the regulation and distribution of soil organic matter in contaminated soils. A similar study report was also described by Cao et al., (2019), using *B. napus* to test soil physicochemical parameters on the bioavailability of PTMs, Cd, and Pb. The result showed that PTMs (Cd and Pb) concentrations in the rape oilseed strongly correlated to the soil organic matter, available phosphorus, and potassium in agricultural soil as reported by Cao *et al.* (2019). Also, a study report on the bioavailability of Cd and Pb in Maize from an agricultural field indicated that synergic effects of combined soil pH and organic matter were strongly correlated to the PTM uptake (Hou *et al.*, 2019).

In the forest, a study conducted in Poland reported that physicochemical parameters, such as pH, and Nitrogen were highly impacted in roadway forested areas, subsequently increasing the sodium concentration that antagonistically increased soil pH and organic matter concentration (Kupka *et al.*, 2021; Łyszczarz *et al.*, 2021). A study report on the disturbed cultivated (anthropogenic impact) and undisturbed lands in Cameroun showed a significant increase in the available nitrogen, potassium, phosphorus, pH, and cation exchange capacity within the forest soil than in cultivated soil. This is in line with our finding, except for soil organic matter which indicated an opposed trend (Tellen *et al.*, 2018); a parallel result was also reported in Ethiopia in a comparative physicochemical parameters study on forest and agricultural soils which showed low pH in agricultural land than forestland with a significant difference as reported by Assefa *et al.* (2020). A similar trend was reported in Nigeria where the natural forest indicated the highest levels of soil chemical parameters (organic matter, cation exchange, phosphorus, and total nitrogen) than arable land, plantations, and farmlands reported by Olorunfemi *et al.* (2018). On the contrary, it was found that anthropogenic and natural phenomena affect the biogeochemistry of soil. The report further highlighted that human impact acidified areas had a high level of exchangeable Al^{+3} than the natural site, contrary to our finding as stated by Pavlů *et al.* (2021). In summary, land-use change including anthropogenic activities such as crop farming, animal grazing, deforestation, and mineral extraction rigorously affects soil physicochemical properties. Moreover, these parameters can be used to measure human impacts on the serene environment.

5.3 *In vitro* bioaccessibility

The relative bioaccessibility of the potentially toxic metals, Cr and Pb varied in the study area. The results indicate high bioaccessibility of Lead than chromium, Pb>Cr. The results showed that *in vitro* bioaccessibility of PTMs in the agricultural soil varies across the study areas and is related to physicochemical properties (Fernández-Landero *et al.*,, 2021; Guo *et al.*,, 2022). The Bioaccessible amounts of Pb and Cr were 11.88% and 0.77%, respectively. Humans and ecological risk research conducted by Louzon et al., (2020) on the bioaccessibility of PTMs in soil reported similar results on As, Cd, and Pb (Louzon *et al.*, 2020). These results are comparable to another study on Pb in agricultural soils (Misenheimer *et al.*, 2018). The Bioaccessible amounts of Cr were far lower than their total concentrations in soil, 48.19±1.51 mg/Kg. However, Cr bioaccessibility amounted to the lowest in this study. There are few literature reports on Cr bioaccessibility in agricultural soils. The bioaccessibility and total concentrations of Cr as reported in this study are similar to some studies in agricultural soils (Wang, Wei, *et al.*, 2021; Xie *et al.*, 2018).

The likely effect of PTM's total concentrations and *in vitro* bioaccessibility (IVBA) concentrations of each metal in the soils was studied employing correlation analysis. The relationship between the physicochemical parameters and *in vitro* bioaccessibility was significant for Pb except for Al^{+3} and H^+ as indicated in Appendix IX. Similarly, the correlations between the physicochemical parameters and *in vitro* bioaccessibility of PTMs in soils from the sampling location were significant and strongly related. Similar studies have reported a strong correlation between the physicochemical parameters and percent bioaccessibility of PTMs in agricultural soils; in Baoji

Northwestern China (Ai *et al.*, 2019), Naples, Italia (Agrelli, Caporale, *et al.*, 2020); and Tharsis (Spain) agricultural soils influenced by acid mine drainage closed to a historic mining site (Fernández-Caliani *et al.*, 2019).

Soil pH is fundamental in soil bioaccessibility assessment, and a study on soil microbial and PTM availability was conducted. The results showed that among the soil physicochemical parameters, the soil pH was the dominant factor to distinguish different land use activities and the PTM contamination in soil (Xiao *et al.*, 2022). An *in vitro* bioaccessibility of selected PTMs in the soil also indicated that in addition to the soil pH, the geochemistry and geology of the soil sample including the composition of the bioaccessibility extracting solutions influenced the bioaccessible amount of PTMs in soil (Fernández-Landero *et al.*,, 2021). The total concentration of the PTMs in soils also influenced the results of PTMs bioaccessibility, a study conducted by Soltani *et al.* (2021) reported that the bioaccessibility of PTMs in soils is generally explained by the total concentrations of PTMs in the soils. These study reports agreed with our finding that the total concentration and physicochemical parameters, soil pH, and OC percentage of PTMs in soil influence the bioaccessibility of PTMs in soil from the study areas. Other physical parameters of soil that also influence bioaccessibility of PTMs in soil are the particle size, aggregate and the soil class, for example, sand, loam, or clay (Wang, Xue, *et al.*, 2021). Several studies have compared and reported *in vitro* bioaccessibility and the health risk of PTMs in soil. The results are mostly parallel, that is, increasing bioaccessibility often results in increased health risk of PTMs in the soils as reported by other studies (Cao *et al.*, 2020; Liu *et al.*, 2019).

5.4 *Brassica napus* and *Raphanus raphanistrum* seeds germination

Various seed priming techniques were used to induce germination in *Brassica napus* and *Raphanus raphanistrum*. Priming is an experimental procedure, a less expensive, and maneuverable technique that helps to improve seed germination and enhance early seedling emergence, and stem formation against environmental stress conditions (Ashraf *et al.*, 2018). Seeds priming techniques have a great influence on the overall performance of plants. It improves the physiology of the plants, that is, the biochemistry and phenotypic characteristics including heights, growth, yields, and development quality (Zulfiqar, 2021). Different seed priming procedures have been used to enhance seed preservation, cultivation, and viability. Seed dormancy and vigor significantly impact plant development and evolutionary characteristics (Rao *et al.*, 2019). There are several means of seed priming, including osmo-priming, hydro-priming, chemical priming, physical priming, Nano-priming, and hormonal priming (Lutts *et al.*, 2016; Nawaz *et al.*, 2013). Modern technological method of priming, cold-plasm (CP) is gaining attention. This new technique involves the production of low-frequency charged particles at low pressure from various kinds of reactors to which seed samples are exposed directly or indirectly within specified times (Shelar *et al.*, 2022). It is affected by several other factors including temperature, aeration, photosynthesis, prime time, and characteristics of the seed(Waqas *et al.*, 2019). Seeds priming techniques are used to develop traits in plants for different physiological and environmental stress factors. It is lately used by plant breeders to develop drought resistance crop seeds in arid and semi-arid areas amidst the global climate crisis as an alternative low-cost for drought tolerance seed production to mitigate food insecurity (Marthandan *et al.*, 2020). This technique in combination with other agronomic practices such as proper spacing also helps to improve the harvest time, yields, and productivity of crops and boost the economic returns for farmers (Farooq *et al.*, 2020).

5.4.1 Chemical priming

There are several chemical germination agents used to prime seeds including KNO_3, H_2O, HCl, and H_2SO_4 using different concentrations for different seeds depending on the secondary dormancy and stress resistance. Halo priming using 0.1M of KNO_3 used for the two species was very effective for *B. napus* compared to *R. raphanistrum* which agreed with similar studies report (Abdollahi *et al.*, 2012; Omidi *et al.*, 2011). Also, used were H_2SO_4 and HCl on the same crops. The seeds were exposed to the acid solutions for a shorter time compared to KNO_3, that is, 5 minutes and 30 minutes, respectively. Hydrochloric and sulfuric acids blocked germinations in *B. napus* and *R. raphanistrum* contrary to the halo-chemical, KNO_3, and this is also true for other related studies (Rincón-Rosales *et al.*, 2003). However, a similar study using the same acids, HCl and H_2SO_4 showed high germination rates in other species of Brassicaceae contrary to this study (Barmukh *et al.*, 2008; Bhoyar *et al.*, 2010; Kanmegne *et al.*, 2017; Rincón-Rosales *et al.*,, 2003). On the contrary, the use of acid to improve seed germination is commonly practiced. The acid, for example, sulfuric acid can be used to remove seed husks and enhance germination in seeds compared to manual cleaning and flaming. A study report showed that acid cleaning increased seed germination efficiency and cleaning (Pedrini *et al.*, 2019).

5.4.2 Hormonal priming

Gibberellic acid (GA3) is a plant hormone used to enhance germination in plants. Hormonal priming using GA3 that stimulates diverse metabolic synthesis in plants was used to induce laboratory seed germination, extensive growth, and flowering as reported by several studies (Gupta *et al.*, 2013; Schwechheimer, 2008). A single concentration dose, 0.25mg/L, of GA3 was applied to induce germination in *Brassica napus* and *Raphanus raphanistrum*. The results, 80% and 70%, germination for BN and RR, respectively were achieved. In this experiment, GA3 was the most promising and effective priming agent, confirming similar studies on GA3 to enhance germination in Brassicaceae (Bojović *et al.*, 2010; Chauhan *et al.*, 2006; Li *et al.*, 2010). Gibberellic acid enhanced seed germination in many different seed types that show secondary dormancy traits. GA3 enhances seed germination through the inducement of growth by lowering the physical barriers in the closed environment (Tuan *et al.*, 2018). A study by Tsegay *et al.* (2018) agreed with our finding using a similar concentration of GA3 to induce germination in maize, *Pisum sativum*, and *Lathyrus sativa* seeds germination was effective. The hormone enhanced germination percent, shortened the average germination period, and increased growth in the crops. The long-term beneficiary effects of GA3 priming on seeds have also been reported. According to Ma *et al.* (2018), *L. chinensis* seed primed with a single GA3 treatment has a worthy growth-promoting, such as increased germination, biomass (fresh and dry weight), and plant height in subsequent seasons. The effects lasted though in certain plant species, and at specified concentrations, GA3 slows the germination of some seeds (Ghodrat *et al.*, 2012).

5.5 Phytoremediation

5.5.1 Effects of enhanced *Brassica napus* and *Raphanus raphanistrum* in PTMs uptake

In this study, selected Brassicaceae plants were modified to ameliorate their efficacy to decontaminate PTMs polluted soils. There are several enhancement techniques, but chemical modification using colchicine was used to induce growth, development, and resistance in *Brassica napus* and *Raphanus raphanistrum* as reported by Nedjimi (2021). The different treatment doses, 0.00%. 0.25%. 0.50% and 1.00% colchicine in *Brassica napus* and *Raphanus raphanistrum* for PTM removal in contaminated agricultural soil were assessed. The treated plants turned to absorb more PTMs at the medium dosage (0.50%) of colchicine compared to the minimum and maximum doses, 0.25%, and 1.00%, respectively. An increased PTM absorption trend as a result of colchicine treatment was observed from minimum to medium before the plants' absorption trend turned to reduce absorption with a high concentration dose of 1.00%. This showed that high-concentration dosages of colchicine are possibly toxic to treated plant growth as was `reported by Hansen *et al.* (1996). As found and reported in this study, plants generally strive to grow and develop in stress conditions, such as in potentially toxic metals-contaminated soils when treated with minimum to medium dosages of colchicine (Abello *et al.*, 2021; Kara *et al.*, 2018). Colchicine treatment enhances increased growth in plants' root hairs, leaves, and biomass. This improves the plant's potential to uptake potentially toxic metals, mostly in the biomass, especially in the roots as was reported by Feng *et al.* (2019). A similar growth pattern was observed in this study with both plants, *Brassica napus* and *Raphanus raphanistrum* across all planting trials. A correlation study analysis (Table 4.12) showed that there was no positive significant relationship between the concentrations of PTMs removed from soil to the morphology, height, and leaf broadness in the treated plants. This is similar to studies that reported comparable results on plant height and leaf areas treated with a low dose of colchicine (Zahedi *et al.*, 2018). Colchicine has also been used in crossing the breeding of Brassicaceae. The treatment of *B. junea* x *B. oleracea* with 0.05 to 0.25% resulted in a successfully fertile and partly established allohexaploid as reported by Mwathi *et al.*, (2020). Similarly, treatment of *Raphanus sativus L*. with different doses and durations of colchicine from 0.05 to 2.00% and from 1 to 12 hours, respectively. The results showed that treatment with 1.0% colchicine for 1 hour was effective to produce breeds, thus with a significant reduction in the leaf and root width as reported by Kim *et al.* (2022).

Colchicine concentrations of 0.50% and 1.00% showed significant morphological enhancement in the leaf areas of *Raphanus raphanistrum* by enhancing their potential bioaccumulation of potentially toxic metals, especially for Chromium in the leaf, root, and stem (Table 4.8). This finding corresponds to several similar study reports of colchicine-induced putative plants in phytoremediation using the Brassicaceae family of plants. A similar study using red-flesh radish with similar concentration doses of colchicine was comparable in morphological and phytoextraction characteristics of the treated plants as was reported by Chen *et al.* (2021). Also, other species of Brassicaceae including *Lepidium sativum* and L., *Aethionema*, L, used in different communities for food, medicine, and ornaments have shown similar results of PTMs uptakes (Aqafarini *et al.*, 2019; Manzoor *et al.*, 2018). The results also agree with the finding by Rodiansah *et al.* (2020) on *Setaria Italica (L.) Beauv,* when treated with different doses of colchicine increased leaf broadness, that is, length, width, and diameter with little change in the plant height.

In terms of the different plants' organs: roots, stems, leaves, and seeds, the roots uptake and store more PTMs than other parts of the plants. This phenomenon is fundamental, mainly in potentially toxic metals phytoextraction studies as discussed by Rezvani *et al.* (2011). The roots are the primary lines of PTM extraction in hyper-accumulating plants in most phytoremediation studies. Overall, Chromium was the most absorbed metal in both plants, with about 87.91%, 76.51%, and 60.23% percent absorption efficiency in *Brassica napus* against 82.57%, 86.30%, and 36.92% percent absorption efficiency in *Raphanus raphanistrum* for planting trials: M_1, M_2, and M_3, respectively as presented in Appendix V. This finding is similar to an assisted phytoremediation study report of ryegrass multiple PTMs decontamination experiment, in which a triple voltaic electrical current treatment of ryegrass increased the plant's roots and shoots potential to uptake more Pb, Cd, and Zn from the PTMs contaminated soils in a greenhouse study as by Keshavarz *et al.* (2021).

From the results of colchicine, treated plants, at 0**.**50% enhanced *Brassica napus* and *Raphanus raphanistrum* PTMs uptake compared, the latter removed more Cr in M_2 than M_1 and M_3, while the former, removed more Cr and Pb in M_1 than M_2 and M_3. The result is similar to others on colchicine enhancement in plant breeding (Chen *et al.*, 2021; Manzoor *et al.*, 2018).

Even though there are several species of chromium in the environment, the results presented herein focus on non-radioactive, total Chromium in soil that is naturally occurring on the earth and induced by anthropogenic activities (Jin *et al.*, 2018; Ranieri *et al.,*, 2020). This study is similar to a phytoremediation study conducted by Tabinda *et al.* (2018) on Chromium and Copper (Cu). In their research report, the former was efficiently removed with a higher percentage. Another study also that agreed with this report on Cr removal efficiency in phytoremediation, including the addition of fungi to enhance plant PTMs uptake, is reported in several pieces of literature (Hussain *et al.*, 2018); Shehata *et al.* (2019).

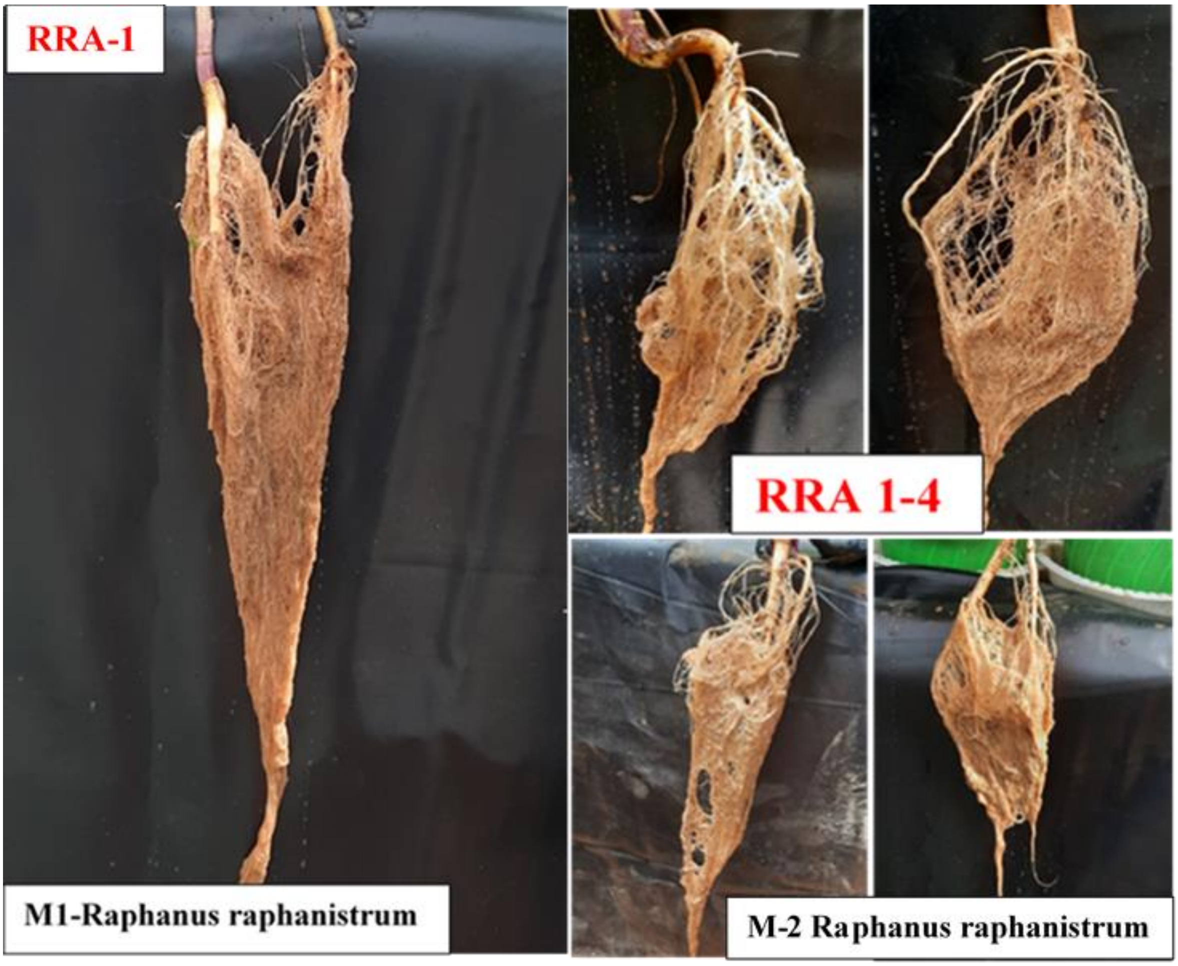

Plate 5.1: Root masses of treated M1 and M2 *Raphanus raphanistrum* 0.50% colchicine

In the phytoremediation of PTMs from the soil, the roots played critical roles comprising of providing the surface area for the biochemical activities, storage, and channeling of the PTMs from ground to shoot. It forms the soil-plant interface where complex biogeochemical activities occur, including phytoaccumulation, phytoextraction, and translocation of PTMs to other parts of the plant. Sometimes, the plants biodegrade, stabilize or volatilize the PTMs in soil (Tangahu *et al.*, 2011). This biochemistry involves chelation formation, a molecular compound that provides bonding for metal ions in which the ligand binds through the exchange of the donor atoms. Plant’s capacity to signal and synthesize various metal chelators including Phytochelatin, metallothioneins, or ferritin, the more that plant can decontaminate PTMs from soils. Synthesis of the chelates enhances the plant's PTM uptake, storage, and resilience to metal polluted soils environment. This process occurs at the interface of the soil-plant barrier in soil; the process involves in vivo/in vitro chemical activities as discussed in Kumar *et al.* (2016) research report. Though synthetic chelators are available, phytochelatins are oligomers formed by phytochelatins synthase in plants, fungi, and algae where they enhance PTMs decontamination. This induces more secretions of chelates, hence hyperaccumulation of PTMs. The plant’s response to potentially toxic metal stress, mostly as an antioxidant is the most effective mechanism for PTMs tolerance in plants (Kumar *et al.*, 2019). This complex process is also considerably affected by other processes such as plant species, soil pH, PTMs bioavailability, and enhancement techniques (Awa *et al.*,, 2020). According to Gul *et al.* (2020), enhanced phytoremediation of Pb in a pot experiment yielded high results especially in the root when synthetic chelators, EDTA, ammonium nitrate, and nitric acid were applied in the soil. Acidic soil (pH 0.1-pH 6.5) enhances PTMs' bioavailability and then phytoremediation. Soil pH is critical to PTMs' biogeochemistry; it provides the ideal conditions for chemical reactions including redox and potentiometric reactions to take place. Low soil pH increases the solubility of PTMs in soil, consequently increasing PTMs' bioavailability and phytoremediation (Yan *et al.*, 2020; Yuan *et al.*, 2021). The report also agrees with the finding by Poursattari *et al.* (2022) in EDTA-enhanced soil where *Brassica napus* removed high concentrations, more than 98% of Pb in the soil. Likewise, EDTA-assisted phytoremediation of PTMs using *Bryophyllum laetiveriens* from contaminated garden sludge soil was about 2 to 6 more effective compared to the control, particularly in bioaccumulation of the PTMs in the roots as reported by Li *et al.* (2020).

Our study was aimed at PTMs decontamination in Moiben soil using enhanced plants, *Brassica napus*, and *Raphanus raphanistrum* to optimize their potential to uptake PTMs without selection as discussed by Gavrilescu (2022). It was observed that this led to the formation of elaborate root mass (Plate 5.1). The enhanced plants had higher uptake of PTMs and were optimum at 0.50% of colchicine for both species of the second generation. This is confirmation of the enhancement of phytoremediation of PTMs in polluted soil. This led to the high uptake of PTMs: Cr and Pb in soil, particularly in the M_2 generation at optimum treatment, 0.50% Colchicine. In our report of three planting trials, the overall root performed better than other plants' organs. It was found that at this treatment level that in *Brassica napus*, 92.41% of the total removed Pb was found in the root followed by 3.19% in the leaf. For Cr in the same plant, 35.11% of the absorbed was found in the root followed by 30.84% in the leaf. As for *Raphanus raphanistrum*, 58.87% of the total absorbed Cr was found in the root followed by 49.52% in the leaf; however, 50.42% of total absorbed Pb was found in the leaf closely followed by 46.42% in the roots. This finding agrees with enhanced phytoremediation studies' reports in which plant treatment led to root improvement and subsequently PTMs decontamination (Luo *et al.*, 2016). This finding also corresponds to a study by Perotti *et al.* (2020), where enhanced hairy *Brassica napus* root removed about 98% Cr from the solution. Similarly, our finding agrees with a study by Pino-Vallejo *et al.* (2021). In their study report of *Raphanus raphanistrum* phytoremediation of Pb from wastewater sludge mud, the roots efficiently absorbed about 16.40% Pb more. Moreover, it was reported that chemically treated *Raphanus raphanistrum* among other plants, was effective in

PTMs (Pb, Cd, Ni, Zn, and Cu) decontamination in soil. In environmental management practices, these plants can be used to produce diesel fuel, *Brassica napus* and biomass energy, and *Raphanus raphanistrum* from the high calorific biomass through what is termed Integrated phytoremediation (DalCorso *et al.*, 2019), after which the residues can be disposed of appropriately as hazardous wastes.

5.5.2 Enhanced phytoremediation of *Brassica napus* and *Raphanus raphanistrum*

Enhanced phytoremediation is a promising environmental technology that has drawn more research toward seeking hyperaccumulating plants. Chemical inducement, genetic engineering, biotechnology, and microbial use have increased incredibly in this regard. The phytoremediation potential of chemically induced plants, *Raphanus raphanistrum*, and *Brassica napus* was tested using empirical analysis. There is presently no single authorized conventionally agreed definition to characterize hyper-accumulating plants in phytoremediation studies. Scientists and research groups use different criteria to define hyperaccumulation according to Farooqi *et al.* (2022). A few criteria have well-defined hyperaccumulation in terms of specific metals absorption capacity, for example, Nickel (Ni), Lead, Chromium, and Cadmium, or some define it in terms of empirical computation, for example, Bioaccumulation Factor, Bioconcentration Factor, and Translocation Factor and other define it in term of the potential of plants use in phytoremediation studies (Alaboudi *et al.*, 2018; Deng *et al.*, 2018). A Bioconcentration factor less than one and a translocation factor value greater than one computed in soil-plant ratio indicate phytoextraction potential; whereas a bioconcentration factor greater than one and translocation factor greater than one shows bioaccumulation potential (Mellem *et al.*, 2012; Takarina *et al.*,, 2017). In a review study of two decades search of hyperaccumulators of PTMs in

China, a Brassicaceae, *Arabis peniculata* is enlisted among classified hyper accumulating plants in China for PTMs Cd, As, Cr, Pb, and Zn as its bioconcentration and translocation factors were greater than 1 as reported by Li *et al.* (2018).

The BCF results (Table 4.10) showed *B. napus* roots and leaves were encouraging potential hyperaccumulating species for phytoremediation and decontamination of Cr-polluted soils. *R. raphanistrum* showed a similar pattern. With regards to Cr, it was discovered that Pb, although more than 40 percent of it was removed, was not bioconcentrated and a little amount could be moved from the root to the shoot. The research universally is continuing to investigate and develop more methodologies at present. This finding is similar to the study report by Rosca *et al.* (2021), where *B. napus* showed poor translocation of Pb and Cd from root to shoots in the phytoremediation experiment. It also corresponds to a similar study on various plants including *B. napus* for phytoremediation of Pb and Ni where *B. napus* underperformed compared to others (Kaur, 2018). In addition, a similar study in a Mexico mining area reported that, local widely distributed tailings weed, *V. Campechiana* a hyperaccumulator of PTMs, Cr, Pb, and Cu as can bioaccumulate the PTMs in its biomass, roots, and leaves from the soils as reported by Santoyo-Martínez *et al.* (2020).

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Introduction

The study aimed to evaluate and improve phytoremediation of selected potentially harmful metals, such as Pb, Cd, Cr, Hg, and As, found in agricultural soils from Kaprobu, Kosyin, Moiben, and Ziwa located in Uasin Gishu County, Kenya that can be replicated to bioremediate other PTMs contaminated soils. The study analyzed the concentration levels of PTMs in the soils, as well as their *in vitro* bioaccessibility and physicochemical properties. In addition, the study found, improved, and evaluated two locally available Brassicaceae species, *B. napus*, and *R. raphanistrum*, as promising potential phytoremediation species of PTMs polluted soils; and from the study results, the following conclusions were drawn.

6.2 Conclusion

The PTMs concentrations in the soil samples from the study areas ranged from 0.08-0.13mg/Kg Cd, 2.99-6.39mg/Kg As, 6.46-35.89mg/Kg Pb, and 14.37-48.19mg/Kg Cr whereas Hg was not detected. The PTMs, As and Cr generally exceeded the USEPA regulatory standards of PTMs in agricultural soil, while Cd and Pb were within acceptable limits. Similarly, when compared with Tanzania's national/FAO standards, only As (1.0mg/Kg) was found to be high. However, when compared with the WHO Standards of PTMs in agricultural soils, As, Cd, Cr, and Pb in the study areas were above the allowable limits. Nonetheless, the environmental risk assessment indices, Geo-accumulation (Igeo), and ecological index (Eri) revealed that there are anthropogenic impacts of pollution in the study area. Whereas, there was a low chance of carcinogenic and non-carcinogenic risks to exposed individuals-children and adults from selected potentially toxic metals, Cr and Pb in the research study.

From the physicochemical parameters assessed, the soil pH and organic matter concentrations in the study areas were low. This low soil pH leads to soil acidity and subsequently reduces organic matter and increases PTM solubility. The lowering of soil pH can be naturally caused by soil geochemistry or induced by anthropogenic activities such as land-use change and the addition of nitrogen-based fertilizers. This may have been caused by the use of agrochemicals, mostly inorganic fertilizers and pesticides in farm amendments. This trend, if continues will aggravate potentially toxic metal levels in agricultural soils in the study areas. Moreover, the bioaccessibility of PTMs in soil was measured. The results showed that the bioaccessible amounts of the PTMs, Cr and Pb were low, 0.77% and 11.88%, respectively. It can be concluded from these values that the PTMs are less bioavailable for absorption in organisms upon exposure (Darko *et al.*, 2022). A strong association was found between the soil pH and organic matter on one hand and *in vitro* bioaccessibility, Cr, and Pb on the other hand. Notwithstanding this low bioaccessible amount changes with time, unsustainable land-use practices including excessive use of inorganic fertilizers will equally increase PTMs *in vitro* bioaccessibility in the study areas.

Germination test in the Brassicaceae seeds conducted using various priming agents showed that germination was effective in *B. napus* and *R. raphanistrum* when primed with 0.25mg/L of gibberellic acid (GA3) hormones and 0.1M of KNO_3 alkaline. Although chilling the seeds was partially efficiently breaking secondary dormancy and inducing germination in the Brassicaceae, it was not effective. Hydro priming, which utilized distilled water, was completely poor and ineffective.

Furthermore, it was found that the optimal treatment for both plants, *B. napus* and *R. raphanistrum* in enhanced phytoremediation was achieved at a concentration treatment dose of 0.50 % colchicine, especially in M_1 and M_2 generations. Therefore, a higher concentration dose of colchicine, 1.00 % resulted in a decreased level of PTM absorption in the plants. *B. napus* and *R. raphanistrum* showed promising potential for enhanced PTMs phytoremediation, particularly from contaminated soils. Enhanced *B. napus* and *R. raphanistrum* were able to remove up to 74.88% and 68.60% of Cr from the soil, respectively. Similarly, 44.0% and 22.00% of Pb were removed respectively by *B. napus* and *R. raphanistrum*. Treatment with colchicine enhanced morphological development in plant heights, root systems, and leaf organs. *B. napus* had a higher percentage of PTMs removed from soils compared to *R. raphanistrum*; nevertheless, the latter is empirically effective in PTMs removal when compared to the former, notably for Cr decontamination. The chemically modified *R. raphanistrum* has a hyperaccumulation potential compared to that of *B. napus*.

6.3 Recommendations

From the research finding, the following are recommended:

1. From this study's results, PTMs bioremediation in agricultural soils in the study areas as the present levels of As and Cr exceeded the USEPA regulatory standards in soils. In addition, it is recommended to continuously monitor the soils and pay keen attention to PTMs, Pb as its levels were relatively high compared to Cr even though it was found within the limits of USEPA regulatory standards.
2. In addition, from the enhanced phytoremediation trials, it is recommended to use 0.50% colchicine concentration treatment to enhance *B. napus* and *R. raphanistrum*, especially for the decontamination of PTMs polluted soils in the study areas and elsewhere.
3. This study found that enhanced *B. napus* and *R. raphanistrum* have promising potential in the phytoremediation of PTMs, Lead, and Chromium in polluted soils; however, it is recommended to conduct a study aimed at understanding the biogeochemical mechanism of the soil-plant interface where the biochemistry of PTMs uptake occurs in the phytoremediation.

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APPENDICES

Appendix I: Exposure factors used in the human health risk for rate model

Reference exposure risk assessment parameters used by the United States Environmental Protection Agency.

Parameters	Unit	Children (1-6years)	Adult
Lifetime (LT)	years		70
Average Time Carcinogenic	Days	25,550*LT=25550	365*LT=25550
Average Time Non-Carcinogenic	Days	365*ED=2,190	365*ED=8,760
Body Weight average	Kg	15	70
Dermal absorption factor (ABS)	Unit less	0.001 for all metals	0.03 for As
Dermal exposure ratio (FE)	Unit less	0.2	0.7
Exposure duration (ED)	Years	6	24
Exposure frequency (EF)	Days/year	180	350
Ingestion rate	mg/Kg	200	100
Inhalation rate	mg/Kg	7.6	20
Particular emission factor	m^3/kg	1.36 E-09	
Skin surface area (SA)	cm^2	2,800	3,300
Soil adherence factor (AF)	mg/cm^2	0.2	0.07
Conversion factor	Kg/mg	1.000 E-06	

NB: the United Nations Population Division projection of the life expectancy at birth for an average Kenyan is 66.669 years, that is, approximately ~70 years as used in this study (UNPD, 2019).

Appendix II: Oral Reference Dose and Cancer Slope Factors of Selected PTMs

Oral reference dose and cancer slope factor of selected PTMs use in carcinogenic and non-carcinogenic risks assessment according to the USEPA protocols.

PTMs	Oral Ref Dose	Inhalation Ref Dose	Dermal Ref Dose	Cancer slope factor (CSF) oral	CSF Inhalation	CSF Dermal Absorption
As	3.00 E-04	4.29 E-06	3.00 E-04	1.5	1.50 E + 01	3.66 E+00
Cd	1.00 E-03	2.86 E-06	2.50 E-05	-	6.30 E + 00	-
Cr	3.00E-03			5.00E-01		
Pb	4.00 E-03	3.50 E-03	5.25 E-04	8.50 E-03	4.20 E-02	8.50 E-06

Appendix III: Matrices of Contamination Factor (C_f), Contamination Degree (C_{deg}), and Ecological Risk Index (E_{ri})

Indices for ecological risk assessment: contamination (Cf), degree of Contamination (Cdeg), and Ecological risk (Eri).

I*geo* Index	C_f Index	Cd_{eg} Index	Eri Index	Risk status
Igeo ≤ 0	$C_f < 1$	$C_{deg} \leq 8$	$E_{ri} < 40$	**Low**
0< *Igeo*≤1	$1 < C_f < 3$	$8 \leq C_{deg} < 16$	$40 \leq E_{ri} < 80$	**Moderate**
1< *Igeo*≤2	$3 < C_f < 6$	$16 \leq C_{deg} < 32$	$80 \leq E_{ri} < 160$	**Considerable**
2< *Igeo*≤3	$6 \geq C_f$	$C_{deg} \geq 32$	$160 \leq E_{ri} < 320$	**Very High**
3< *Igeo*≤4		---	$E_{ri} \geq 320$	**Heavy to extreme**
4< *Igeo*≤5 5< *Igeo*			-------	**Extremely contaminated**

Appendix IV: Background levels of potentially toxic metals of selected

Soil preindustrial Background values of potentially toxic metals in the environment.

Metal	Geochemical Background (Czarnowska, 1996)	Preindustrial (Hakanson, 1980)	(Bowen, 1966)	Toxic Factor
As		15	6.0	10
Cd	0.18	1.0	0.06	30
Cr		90	100	2
Hg		0.25	0.03-0.8	40
Pb	9.8	7.0	10	5.0

Appendix V: Total PTMs uptake in percent (%) per *Brassica napus* and *Raphanus raphanistrum* in all trials

Table

PTM		Trial	initial	Total Uptake at 0.50%	% efficiency
Cr	*Brassica napus*	M1	274.555	241.348	87.90516
		M2	274.555	210.048	76.50489
		M3	274.555	165.357	60.22728
	Raphanus raphanistrum	M1	274.555	226.691	82.5667
		M2	274.555	236.946	86.30183
		M3	274.555	101.354	36.91574
Pb	*Brassica napus*	M1	3985.642	1619.67	40.63762
		M2	3985.642	729.474	18.30255
		M3	3985.642	750.193	18.82239
	Raphanus raphanistrum	M1	3985.642	880.494	22.09165
		M2	3985.642	518.804	13.01682
		M3	3985.642	498.956	12.51884

Appendix VI: NACOSTI Research Permit

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Appendix VII: Some pictorials of wild seeds collection

Field Assessment of *Raphanus Raphanistrum* (1,2) and seed BN (3) and RR (4)

Appendix VIII: Schematic diagram of experimental design and set-up

Schematic diagram of pot experimental design and set-up in the glasshouse

Appendix IX: Statistical summary analysis or Chromium

Statistical summary of chromium in phytoremediation

		Root	4	67.1237	0.14914	0.22%	67	67.3	0.3		Between g	51224.4	3	17074.8	1040764	0
T1	**BN**	Stem	4	11.649	0.12861	1.10%	11.5	11.8	0.3		Within gr	0.19687	12	0.01641		
		Leaves	4	150.25	0.1291	0.09%	150.1	150.4	0.3		Total (Co	51224.5	15			
		Seed	4	12.3249	0.10088	0.82%	12.2	12.4391	0.2391							
		Total	16	60.3369	58.4377	96.85%	11.5	150.4	138.9							
		Summary	Statistics								ANOVA	Table				
			Count	Average	Standard	Coeff. of	Minimum	Maximum	Range		Source	Sum of Sq	Df	Mean Squ	F-Ratio	P-Value
T2		Root	4	67.864	0.5438	0.80%	67.287	68.591	1.304		Between g	4065.5	3	1355.17	5218.99	0
	BN	Stem	4	28.9023	0.65281	2.26%	28.066	29.585	1.519		Within gr	3.11593	12	0.25966		
		Leaves	4	59.6005	0.33369	0.56%	59.199	59.981	0.782		Total (Co	4068.62	15			
		Seed	4	36.9135	0.45322	1.23%	36.292	37.379	1.087							
		Total	16	48.3201	16.4694	34.08%	28.066	68.591	40.525							
		Summary	Statistics								ANOVA	Table				
			Count	Average	Standard	Coeff. of	Minimum	Maximum	Range		Source	Sum of Sq	Df	Mean Squ	F-Ratio	P-Value
T3		Root	4	136.233	1.65533	1.22%	135.168	138.655	3.487		Between g	48200	3	16066.7	20862.8	0
	BN	Stem	4	5.19603	0.25792	4.96%	4.926	5.42492	0.49892		Within gr	9.24131	12	0.77011		
		Leaves	4	9.407	0.1957	2.08%	9.203	9.631	0.428		Total (Co	48209.2	15			
		Seed	4	14.521	0.48527	3.34%	14.043	15.069	1.026							
		Total	16	41.3392	56.6917	137.14%	4.926	138.655	133.729							
		Summary	Statistics								ANOVA	Table				
			Count	Average	Standard	Coeff. of	Minimum	Maximum	Range		Source	Sum of Sq	Df	Mean Squ	F-Ratio	P-Value
T1	**RR**	Root	4	108.051	56.5821	52.37%	23.214	138.655	115.441		Between g	29188.6	3	9729.54	12.15	0.0006
		Stem	4	5.19603	0.25792	4.96%	4.926	5.42492	0.49892		Within gr	9605.61	12	800.467		
		Leaves	4	9.407	0.1957	2.08%	9.203	9.631	0.428		Total (Co	38794.2	15			
		Seed	4	14.521	0.48527	3.34%	14.043	15.069	1.026							
		Total	16	34.2938	50.8555	148.29%	4.926	138.655	133.729							
		Summary	Statistics								ANOVA	Table				
			Count	Average	Standard	Coeff. of	Minimum	Maximum	Range		Source	Sum of Sq	Df	Mean Squ	F-Ratio	P-Value
		Root	4	139.494	0.39404	0.28%	139.261	140.084	0.823		Between g	37383.7	3	12461.2	91579.8	0
T2	**RR**	Stem	4	36.6553	0.46485	1.27%	36.133	37.184	1.051		Within gr	1.63284	12	0.13607		
		Leaves	4	49.5215	0.36773	0.74%	49.209	49.991	0.782		Total (Co	37385.4	15			
		Seed	4	11.2747	0.19415	1.72%	11.036	11.447	0.411							
		Total	16	59.2364	49.9235	84.28%	11.036	140.084	129.048							
		Summary	Statistics								ANOVA	Table				
			Count	Average	Standard	Coeff. of	Minimum	Maximum	Range		Source	Sum of Sq	Df	Mean Squ	F-Ratio	P-Value
		Root	4	38.2777	0.80442	2.10%	37.23	39.026	1.796		Between g	5370.77	3	1790.26	5540.2	0
T3	**RR**	Stem	4	8.82475	0.44405	5.03%	8.217	9.268	1.051		Within gr	3.87768	12	0.32314		
		Leaves	4	5.95784	0.61712	10.36%	5.27668	6.5558	1.27912		Total (Co	5374.65	15			
		Seed	4	48.294	0.25973	0.54%	48.015	48.634	0.619							
		Total	16	25.3386	18.9291	74.70%	5.27668	48.634	43.3573							

Appendix X: Statistical summary Lead analysis

Statistical summary of phytoremediation of Lead

								Lead								
		Summary Statistics									ANOVA Table					
			Count	Average	Standard	Coeff. of vari	Minimum	Maximum	Range		Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
		Root	4	1024.16	1.56867	0.15%	1022.34	1025.92	3.58072		Between g	2.78E+06	3	925178	1199703.21	0
		Stem	4	546.416	0.76784	0.14%	545.515	547.336	1.82045		Within gr	9.25407	12	0.771173		
T1	**BN**	Leaves	4	47.3478	0.17374	0.37%	47.1415	47.5594	0.41788		Total (Co	2.78E+06	15			
		Seed	4	1.75018	0.06477	3.70%	1.68158	1.83729	0.15571							
		Total	16	404.919	430.158	106.23%	1.68158	1025.92	1024.24							
		Summary Statistics									ANOVA Table					
			Count	Average	Standard	Coeff. of vari	Minimum	Maximum	Range		Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
		Root	4	674.132	0.36704	0.05%	673.768	674.571	0.803		Between g	1.29E+06	3	430556	4277272.29	0
T2		Stem	4	30.7318	0.49455	1.61%	30.064	31.254	1.19		Within gr	1.20793	12	0.100661		
	BN	Leaves	4	23.4643	0.13811	0.59%	23.341	23.624	0.283		Total (Co	1.29E+06	15			
		Seed	4	1.14675	0.06539	5.70%	1.091	1.235	0.144							
		Total	16	182.369	293.447	160.91%	1.091	674.571	673.48							
		Summary Statistics									ANOVA Table					
			Count	Average	Standard	Coeff. of vari	Minimum	Maximum	Range		Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
		Root	4	709.405	0.58353	0.08%	708.691	710.101	1.41		Between g	1.45E+06	3	484403	1221907.76	0
T3		Stem	4	15.96	0.5046	3.16%	15.54	16.655	1.115		Within gr	4.75718	12	0.396432		
	BN	Leaves	4	21.9288	0.88533	4.04%	21.106	22.791	1.685		Total (Co	1.45E+06	15			
		Seed	4	2.8995	0.45474	15.68%	2.556	3.569	1.013							
		Total	16	187.548	311.257	165.96%	2.556	710.101	707.545							
		Summary Statistics									ANOVA Table					
			Count	Average	Standard	Coeff. of vari	Minimum	Maximum	Range		Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
		Root	4	812.064	0.64794	0.08%	811.239	812.807	1.56738		Between g	1873910.00	3.00	624638.00	2238272.54	0.00
T1	**RR**	Stem	4	12.3052	0.15292	1.24%	12.0762	12.3893	0.31315		Within gr	3.35	12.00	0.28		
		Leaves	4	51.7362	0.73909	1.43%	51.2367	52.8221	1.58543		Total (Co	1873920.00	15.00			
		Seed	4	4.388	0.35613	8.12%	4.013	4.729	0.716							
		Total	16	220.123	353.451	160.57%	4.013	812.807	808.794							
		Summary Statistics									ANOVA Table					
			Count	Average	Standard	Coeff. of vari	Minimum	Maximum	Range		Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
		Root	4	240.811	0.86597	0.36%	240.099	241.929	1.83		Between g	237850.00	3.00	79283.30	232203.16	0.00
		Stem	4	14.3708	0.22986	1.60%	14.145	14.634	0.489		Within gr	4.10	12.00	0.34		
T2	**RR**	Leaves	4	261.825	0.72055	0.28%	261.312	262.844	1.532		Total (Co	237854.00	15.00			
		Seed	4	1.79775	0.20936	11.65%	1.517	1.978	0.461							
		Total	16	129.701	125.924	97.09%	1.517	262.844	261.327							
		Summary Statistics									ANOVA Table					
			Count	Average	Standard	Coeff. of vari	Minimum	Maximum	Range		Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
		Root	4	476.368	14.721	3.09%	459.821	494.814	34.9929		Between g	659680.00	3.00	219893.00	4044.09	0.00
		Stem	4	6.67675	0.40761	6.10%	6.07963	6.94917	0.86954		Within gr	652.49	12.00	54.37		
T3	**RR**	Leaves	4	13.4864	0.53364	3.96%	12.6889	13.8168	1.1279		Total (Co	660332.00	15.00			
		Seed	4	2.42425	0.58024	23.93%	1.617	2.999	1.382							
		Total	16	124.739	209.815	168.20%	1.617	494.814	493.197							

Appendix XI: Similarity Report

Turnitin Originality Report

PHYTOREMEDIATION OF POTENTIALLY TOXIC METALS CONTAMINATED AGRICULTURAL SOIL USING PUTATIVE Brassica napus AND Raphanus raphanistrum IN UASIN GISHU COUNTY, KENYA by Salia Sheriff

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