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Phytoremediation of Chromium and Lead-Contaminated Soil Using Putative Raphanus raphanistrum

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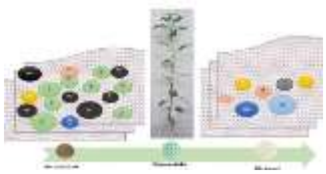
Abstract

Potentially toxic elements, including Chromium and Lead, naturally occur in the environment, however, human activities such as extensive farming, industrialization, and mining increase Potentially toxic element concentrations in soils. Hence, this study aimed to assess enhanced phytoremediation of Chromium and Lead-contaminated soils with putative mutant, Raphanus raphanistrum (wild radish). The putative plant was enhanced to phytoremediation of Chromium and Lead-contaminated soils. The soil physicochemical parameters pH, total organic matter, cation exchange capacity, and electrical conductivity determined were 5.20, 2.57%, 21.50 meq%, and 0.05 mS/cm, respectively. Raphanus raphanistrum seeds were treated with 0.00%, 0.25%, 0.50%, and 1.00% concentrations of colchicine to heighten growth and morphological development in enhanced phytoremediation of potentially toxic elements in soil. The treated Raphanus raphanistrum at 0.50% colchicine removed 226.69±1.22 mg/Kg and 236.95±0.82 mg/Kg of Chromium and 880.49±1.46 mg/Kg and 518.80±0.81 mg/Kg Lead in the first (M_1) and second (M_2) generations respectively. At the same treatment level, the putative plant hyperaccumulation potentially toxic elements at M_1 and M_2 generations absorbed 68.60% and 22.00% of Chromium and Lead, respectively. The plant bioaccumulated high amounts of metal elements, Chromium and Lead, capable of causing potential environmental and health concerns. This study finding contributes significantly to phytoremediation techniques in ecological restoration and recommends putative R. raphanistrum for Chromium and Lead polluted soil decontamination.

Graphical Abstract



Keywords: *Agriculture, bioremediation, potentially toxic elements, mutation, and colchicine.*

INTRODUCTION

Potentially toxic elements (PTEs) pollution is a global environmental challenge that impacts ecosystems in many ways. These pollutants often drain from industrial effluents and other anthropogenic activities to the environment, consequently into food chain. PTEs, Arsenic (As), Cadmium (Cd), Chromium (Cr), and Lead (Pb) have health and ecological impacts widely discussed in scientific symposiums and conferences as reported by Okereafor *et al.* (2020). According to Fei *et al.* (2020), agricultural and industrial activities are among the key drivers of PTEs pollution in soils. The long-term use of metal-containing fertilizers and pesticides leads to an increase in formation of toxic metal complexes in agricultural soil, then into food production therein (Kelepertzis, 2014). Also, dumpsites, sewage, and industrial wastes loaded with high levels PTEs spread to the environment through irrigation, erosion, and flooding (Opaluwa *et al.*, 2012). High amounts of PTEs such as Cr and Pb within an environment are commonly found in soil, sediment, air, and water which contribute to human health threats, especially children exposed are likely to acquire neurotoxic deficits and morphological deformities during growth as reported by Rahman *et al.* (2019). Additionally, PTEs pollution has a major negative effect on the microbial ecology of the soil which affects the number, variety, and bioactivity of soil microbes (Xie *et al.*, 2016).

Another focal source of PTEs contamination in developing countries is electronic waste. A study conducted at Lagos Alaba International e-waste Market showed high PTEs concentrations in surrounding soil and water above World Health Organization (WHO) tolerable limits (Olafisoye *et al.*, 2013). Similarly an environmental and human health assessments in Guiyu, a top e-waste dismantling and processing centers in China showed that surrounding communities were adversely impacted from dust, putting residents' health at high risk (Leung *et al.*, 2008). In addition, landfills and open dumpsites receiving unsorted wastes from construction work, manufacturing industries, municipalities, and households contained PTEs that eventually end up in the soils and get spread by erosion and metal mobility (Gworek *et al.*, 2016).

Bioremediation, lowering PTEs concentrations in the environment using plants and microbes has gained increased attention (Ali *et al.*, 2013). Brassicaceae are highly cited promising phytoremediators and economically essential crops that include fodder crops, oilseed plants and vegetables, organic fertilizers, biofuels. They are also reportedly resistant to stressed environments and agrochemicals (Warwick, 2011). There are over 3000 brassica species including *R. raphanistrum*, that is a widely distributed weed, though some disputes exists (Yamagishi, 2017). *R. raphanistrum* is reported as a troublesome weed with herbicidal resistance to some agrochemicals used globally and observes some temporary dormancy in soil seed banks. The siliques provide additional adaptive mechanisms maximizing endurance during dormancy (Tricault *et al.*, 2018). *R. raphanistrum* also contains healthy and nutritious phytochemicals comprising phenolic and hydroethanolic extracts with supplementary antioxidant potential that can be added to the human diet (Iyda *et al.*, 2019).

Induced mutation is the artificial irradiation of mutagenesis of plants to form variants for intended purposes, including increasing food production and resistance to pests and other environmental conditions (Oladosu *et al.*, 2016). Though mutagenesis may occur naturally, induced mutations enhance the targeted characteristics of cultivating materials at the anticipated time (Oladosu *et al.*,, 2016). The former has advantages: low cost, high variation density, and suitably applicable to many crops (FAO/IAEA., 2018). The chemical reagent, colchicine is widely used in plant to improve growth characteristics, increase biomass, and support environmental stress resistance. This depends on several factors, among them the concentration dose of colchicine, exposure duration, and explant materials (Eng *et al.*, 2019). Several studies have reported that colchicine enhancement technique improved plant growth, and physical and biological resistance among others (Chen *et al.*, 2022; Mwathi *et al.*, 2020). *R. raphanistrum* is a member of Brassicaceae wildly distributed in the environment. It is a weed, resistant to numerous herbicides, and reduces loss in crop yield (Kebaso *et al.*, 2020).

MATERIALS AND METHODS

Research design

The soil samples were collected, crushed, filtered, sieved at 2mm mesh wire, and transferred into polyethylene vessels for physicochemical properties analysis (Vandenhove *et al.*, 2009). 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 soil pH of the samples according to Kome *et al.* (2018). The soil's total organic matter was determined using Walkley-black (W-B) method (Walkley and Black, 1934). The oxidizable amount of the organic matter was quantified with a standard amount of chromate in the presence of sulfuric acid. This measures the soil's exchangeable acidity (Al^{+3}) in centimole pe r kilogram of the soil sample.

Thousands of matured seeds of the *R. raphanistrum* were garnered, stratified, labelled, and spread on trays to sun dry in the glasshouse at 25^0C to 45^0C after which they were then transferred to the lab and kept at room temperature. For the phytoremediation trial, soil samples were randomly collected, spiked, and placed in pots in a completely randomized design (CRD) in the glasshouse. It involved two sets, one set consisting of M_0 planted seeds and the other set consisting of M_1 planted seeds totaling 16 pots, four treatments, and four replicates. The experimental pots were equal in size, filled with 3kg of PTEs spiked soils. Each pot was spiked with 300ml of 3000 mg/Kg concentration of Lead from lead nitrate $Pb(NO_3)_2$, and 300ml of 1000 mg/Kg potassium dichromate ($K_2Cr_2O_7$) respectively, according to the techniques proposed by Arshad *et al.* (2016). The pots were well labeled and filled with soil before randomly assigning treated seeds of *R. raphanistrum* (Chen *et al.*, 2020). To determine the PTEs: Cr and Pb concentrations in soil samples about 0.5g of each soil sample was digested by gradually adding 9mL, 1mL, and 4mL of concentrated analytical grades HNO_3, HCl, and $HClO_4$, respectively in an ultra-clean and dry inert polymeric reaction vessels under the fume hood according to Kamunda *et al.* (2016). The mixture was left 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). After 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 analyzed using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (Agilent 7900) according to the method proposed by Helaluddin (2016). Similarly, the plant biomass including roots, stems, leaves, and seeds was measured to evaluate the potential of colchicine-enhanced phytoremediation of the putative *R. raphanistrum*.

Quality control and assurance (QC/QA) of the validated analytical method were routinely practiced to ensure the reproducibility of the results (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). The instrument was calibrated with standard solutions and certified reference materials (CRM) ISE 800, Cat Clay. 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. The standard solutions were analyzed with the CRM for the elements and values plotted in the control chart to devise the standard operating procedure (SOP**)**. During analysis, CRM was included and run before every batch of samples, and results were presented in micrograms per kilogram (µg/Kg) or part per billion (ppb). The actual concentrations of Cr and Pb in the samples in mg/Kg were determined using equation one (Eqn. 1) as proposed by Kingston *et al.* (1998):

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

Eqn (1)

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

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). PTE concentrations in soil and uptake in putative mutant *R. raphanistrum* 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). This process was repeated for every consecutive planting seasoning, from M_2 and M_3 in the glasshouse. Each trial lasted for about three months. 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 *R. raphanistrum* and its control were assessed through growth rate, height, and leave broadness as suggested by CHEN *et al.* (2018). The surviving *Raphanus raphanistrum* in every generation was planted in contaminated soils, harvested, and tested for their hyperaccumulating capacities of Cr and Pb as in similar studies by Das *et al.* (2015).

Results

Mean pH concentrations, total organic matter in percentage (TOC %), CEC, and electrical conductivity in soils as illustrated in Table 1.

Table 1: ***Physicochemical parameters of agricultural and non-agricultural soil in Moiben***

Parameters	Amount in soil
pH	5.20±0.10
TOC (%)	2.57±0.01
Electrical Conductivity (mS/cm)	0.05±0.08
Cation Exchange Capacity (meq %)	21.50±1.00

Enhanced phytoremediation of *Raphanus raphanistrum*

Concentrations of PTEs were assessed in *R. raphanistrum* and soils from initial concentrations of Cr and Pb 274.55 mg/Kg, and 3985.64 mg/Kg in soil, respectively. The highest concentrations of PTEs were recorded in plant roots, followed by leaves, stems, and seeds. Equally, the concentrations of PTEs in the plant's organs were significantly different within trials except for Chromium concentrations in the stem in trials 2 and 3 (Table 2). At 0.50% colchicine dose treatment, roots absorbed more PTEs, which is the uptake of PTEs per plant compared to other organs. The different treatment doses affected the plant's organs in different ways in the trials, M_1, M_2, and M_3.

Table 2: Potentially toxic metals concentrations (mg/Kg) in plants (RR) organs

PTEs	Trial	Root (mg/Kg)	Stem (mg/Kg)	Leaf (mg/Kg)	Seed (mg/Kg)
	M1	108.05±56.58a*	5.20±0.26b**	9.41±0.20c**	14.52±0.49d**
Cr	**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*
	M1	812.06±0.65a*	12.31±0.15b*	51.74±0.74c*	4.39±0.36*
Pb	**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.*

Effects of colchicine dosage on PTEs concentrations in the plant's organs

Effects of colchicine dosage on potentially toxic elements uptake in the plant's biomass for the different planting periods, that is, M_1, M_2, and M_3 were assessed. Treatment doses, 0.00% 0.25%, 0.50% and 1.00% of colchicine in *R. raphanistrum* showed higher mean Cr concentrations in at 0.50% colchicine with 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. 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. A similar trend was observed in Pb uptake within all treatments in *R. 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 as shown in Table 3.

Table 3: Effect of colchicine dosage and Trial on the plant's biomass accumulated PTEs

			M_1	**M_2**	**M_3**
	PTEs	**Colchicine dosage**	**Mean±Std (mg/Kg)**	**Mean±Std (mg/Kg)**	**Mean±Std (mg/Kg)**
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.

Effects of colchicine dosage on plants' morphology

Effects of colchicine doses treatment on the plant morphology were assessed. An increase in Pb levels resulted in a positive insignificant correlation with plant height and leaf broadness in all trials (p>0.05), as presented in Figure 1. There was also a positive but not significant correlation between Cr and plant height and leaf broadness in M_1 and M_3 with a negative and not significant correlation observed in M_2.

In addition, a correlation analysis between the heights and leaf areas 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; 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 1.

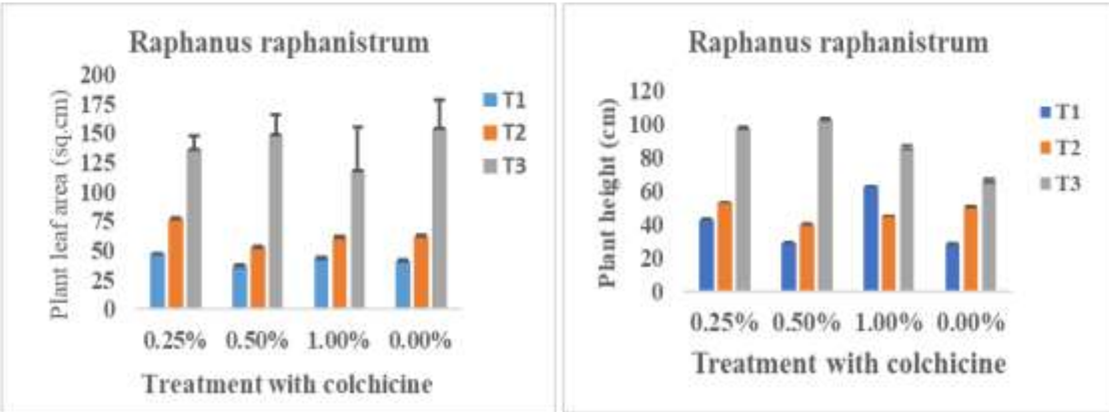


Figure 1: Heights (cm) and leaf broadness (sq. cm) of BN and RR in the Trials

Percentage efficiency in PTEs removal was also estimated at the optimal colchicine dosage, 0.50%. This was calculated as the total concentration of metals (mg/Kg) in the plant divided by the initial concentration in the soil multiplied by 100. The average Cr removal efficiency for *R. raphanistrum* was 68.60%. In M_3, the highest percentage of Cr removal efficiency of *R. raphanistrum* is 36.92% (χ^2 = 5.4454, p= 0.0196). The mean percent Pb removal efficiency was lower but not significantly different in *R. raphanistrum* (χ^2=2.3935, p= 0.1218). In M_1, the percentage of Pb removal by *R. raphanistrum* was 22.09%.

DISCUSSIONS

Soil Physicochemical properties

Soil pH plays a pivotal role in PTEs and nutrient availability, distribution, and uptake by plants in soils (Solis *et al.*, 2005). Table 1 summarizes the physicochemical properties of soil. The soil was found to be acidic, pH of 5.20. On the other hand, the biochemistry of soil organic matter is an important, complex, and dynamic soil property that contributes greatly to the function of the soil environment and the welfare of the ecosystems. Organic matter contains mostly carbon which is the constituent backbone of living matter and it regulates PTEs to some extent (Ondrasek *et al.*, 2019). In this study, soil organic matter, 2.57 % was slightly higher and as reported, the bioavailability of Cd and Pb in Maize from an agricultural field indicated synergic effects of combined soil pH and organic matter were strongly correlated to the PTEs uptake (Hou *et al.*, 2019). This finding is similar to a study by Enya et al., 2020; they reported that low soil pH enhanced the regulation and distribution of soil organic matter in contaminated soils. Hence, land-use change activities such as crop farming, animal grazing, deforestation, and mining rigorously affect soil physicochemical properties use to measure human impacts on the environment.

Effects of enhanced *Raphanus raphanistrum* in PTEs uptake Phytoremediation

The putative mutant plant, *R. raphanistrum* was enhanced to ameliorate its efficacy of PTEs decontamination in polluted soils as it induces growth, development, and resistance as reported by Nedjimi (2021). *R. raphanistrum* was effective to absorb more PTEs at 0.50% dose of colchicine compared to doses, 0.25%, and 1.00%, respectively. An increased PTEs absorption trend as a result of colchicine treatment was observed from low to medium before a downward trend in absorption in some treated with a dose of 1.00%. As found and reported in this study, plants generally strive to grow and develop in stress conditions, such as in PTEs 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 which improves the plant's potential to uptake PTEs, 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 *R. raphanistrum* across all planting trials. A correlation analysis showed that there was no positive significant relationship between the concentrations of PTEs 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 the cross-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, treatment with 1.00% for an hour evidently proved effective to produce tetraploid breeds, thus with a significant reduction in the leaf and root width as reported by Kim *et al.* (2022). This finding corresponds to several similar studies on colchicine-induced putative plants in phytoremediation. A similar study using red-flesh radish with similar 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 for different purposes have shown similar results of PTEs 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 which resulted in increase in 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 PTEs than other parts of the plants. This phenomenon is fundamental, mainly in PTEs

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phytoextraction studies as discussed by Rezvani *et al.* (2011). The roots are the primary lines of PTE extraction in phytoremediation plants in most studies. Overall, Cr was the most absorbed metal in the putative plant, *R. raphanistrum* plant, with about 82.57%, 86.30%, and 36.92% percent absorption efficiency in M_1, M_2, and M_3. This finding is similar to an assisted phytoremediation study of ryegrass multiple PTEs 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 PTEs contaminated soils in a greenhouse study by Keshavarz *et al.* (2021).

From the results of colchicine treatment, 0.50% enhanced *R. raphanistrum* PTEs uptake, it removed more Cr in M_2 than M_1 and M_3, **Figure 2**. The result is similar to other studies on colchicine enhancement in plant breeding (Chen *et al.*, 2021; Manzoor *et al.*, 2018).

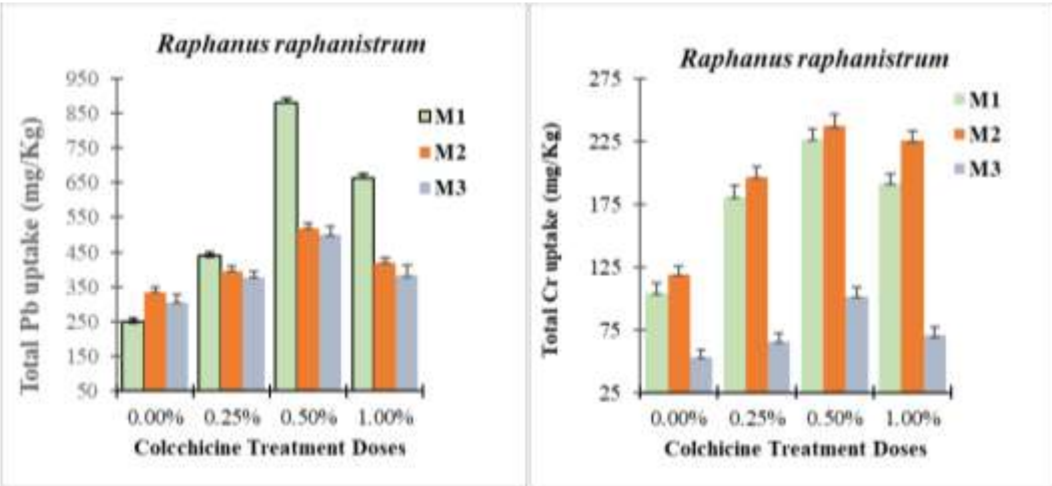

Figure 2: Total PTEs uptakes per enhanced plants *Raphanus raphanistrum*

. 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 agreed with this report on Cr removal efficiency in phytoremediation, including the addition of fungi to enhance plant PTEs uptake, which is reported in several pieces of literature (Hussain *et al.*, 2018).

In the phytoremediation of PTEs from the soil, plant roots played critical roles; comprising of provision of the surface area for the biochemical activities, storage, and channeling of the PTEs from ground to shoot. It forms the soil-plant interface where complex biogeochemical activities occur, including phytoaccumulation, Phytoextraction, and translocation of PTEs to other parts of the plant (Tangahu *et al.*, 2011). Plant's capacity to signal and synthesize various metal chelators including Phytochelatin, metallothioneins, or ferritin, the more that plant can decontaminate PTEs from soils. Synthesis of the chelates enhances plants its PTEs 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. This induces more secretions of chelates, hence hyperaccumulation of PTEs. The plant's response to PTEs, mostly as an antioxidant is the most effective mechanism for PTEs tolerance in plants (Kumar *et al.*, 2019). This complex process is also considerably affected by other processes such as plant species, soil pH, PTEs 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 chelates, EDTA, ammonium nitrate, and nitric acid were applied in the soil. Acidic soil (pH 0.1-pH 6.5) enhances PTEs' bioavailability and then phytoremediation. Soil pH is critical to PTEs' biogeochemistry; it provides the ideal conditions for chemical reactions including redox and potentiometric reactions to take place. Low soil pH increases the solubility of PTEs in soil, consequently increasing PTEs' 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 PTEs using *Bryophyllum laetiveriens* from contaminated garden sludge soil was about 2 to 6 more effective compared to the control, particularly in bioaccumulation of the PTEs in the roots as reported by Li *et al.* (2020). This essentially led to the high uptake of PTEs: Cr and Pb in soil, especially 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 *R. 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 PTEs decontamination (Luo *et al.*, 2016). Similarly, our finding agrees with a study by Pino-Vallejo *et al.* (2021). The phytoremediation potential of chemically induced *R. raphanistrum* was tested using empirical analysis as is presently no single conventionally agreed definition to characterize hyper-accumulating plants, however, various research groups use different criteria (Farooqi *et al.*, 2022). Some criteria have well-defined hyperaccumulation in terms of specific metal elements absorption capacity, for example, Nickel (Ni), Lead, Chromium, Cadmium, or in terms of empirical computation, for example, Bioaccumulation Factor, Bioconcentration Factor, and Translocation Factor in phytoremediation studies (Alaboudi *et al.*, 2018; Deng *et al.*, 2018).

CONCLUSION AND RECOMMENDATION

The physicochemical parameters-soil pH and organic matter concentrations in the study areas were low. Low soil pH enhances soil acidity which reduces organic matter and increases PTEs solubility and bioavailability. Furthermore, it was found that the optimal colchicine treatment for *R. raphanistrum* in PTEs (Cr and Pb) phytoremediation was 0.50 % colchicine in M_1 and M_2. This means that increasing concentrations dose of colchicine from minimum to medium resulted in increased uptake of PTEs by the putative plant, *R. raphanistrum*. The largest amount of absorption was measured at 0.50 % colchicine M_1 and M_2 generations. *R. raphanistrum* showed promising potential for enhanced PTEs phytoremediation, particularly for Cr which removed up to 68.60% and 22.00% of Cr and Pb, respectively. Treatment with colchicine enhanced morphological development in plant heights, root systems, and leaf organs making the putative mutant *R. raphanistrum* has a hyperaccumulation potential.

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REFERENCES

Abello, N., Ruiz, J., Rio, J., & Pascual, P. (2021). In vitro chromosome doubling of tomato var. Improved Pope (Lycopersicon esculentum Mill) via colchicine.
Thai Journal of Agricultural Science, 54(1), 14− 21-14− 21.
Al-Naggar, A., Al-Azab, K. F., Sobieh, S., & Atta, M. (2015). Morphological and SSR assessment of putative drought tolerant M3 and F3 families of wheat
(Triticum aestivum L.). *Biotechnology Journal International*, 174-190.

Alaboudi, K. A., Ahmed, B., & Brodie, G. (2018). Phytoremediation of Pb and Cd contaminated soils by using sunflower (Helianthus annuus) plant. *Annals of agricultural sciences, 63(1), 123-127.*

Ali, H., Khan, E., & Sajad, M. A. (2013). Phytoremediation of heavy metals—concepts and applications. *Chemosphere, 91*(7), 869-881.

Aqafarini, A., Lotfi, M., Norouzi, M., & Karimzadeh, G. (2019). Induction of tetraploidy in garden cress: morphological and cytological changes. *Plant Cell, Tissue and Organ Culture (PCTOC), 137(3), 627-635.*

Arshad, M., Merlina, G., Uzu, G., Sobanska, S., Sarret, G., Dumat, C., . . . Kallerhoff, J. (2016). Phytoavailability of lead altered by two Pelargonium cultivars grown on contrasting lead-spiked soils. *Journal of soils and sediments, 16*(2), 581-591.

Assaad, H. I., Hou, Y., Zhou, L., Carroll, R. J., & Wu, G. (2015). Rapid publication-ready MS-Word tables for two-way ANOVA. *SpringerPlus, 4*(1), 33.

Awa, S. H., & Hadibarata, T. (2020). Removal of heavy metals in contaminated soil by phytoremediation mechanism: a review. *Water, Air, & Soil Pollution, 231(2), 1-15.*

Benjamin, D. J., Berger, J. O., Johannesson, M., Nosek, B. A., Wagenmakers, E.-J., Berk, R., . . . Camerer, C. (2018). Redefine statistical significance. *Nature Human Behaviour, 2*(1), 6.

CHEN, CUI, Q.-z., HUANG, S.-w., WANG, S.-h., LIU, X.-h., LU, X.-y., . . . Yun, T. (2018). An EMS mutant library for cucumber. *Journal of Integrative Agriculture, 17*(7), 1612-1619.

Chen, F., Gao, J., Li, W., Liu, Y., Fang, P., & Peng, Z. (2021). Colchicine-induced tetraploidy influences morphological and cytological characteristics and enhances accumulation of anthocyanins in a red-fleshed radish (Raphanus sativus L.). *Horticulture, Environment, and Biotechnology, 62*(6), 937-948.

Chen, L., Yang, J.-y., & Wang, D. (2020). Phytoremediation of uranium and cadmium contaminated soils by sunflower (Helianthus annuus L.) enhanced with biodegradable chelating agents. *Journal of Cleaner Production, 263*, 121491.

Chen, W., Zhang, Y., Huang, S., Ren, J., & Feng, H. (2022). l-Ascorbic acid sodium salt promotes microspore embryogenesis and chromosome doubling by colchicine in ornamental kale (Brassica oleracea var. acephala). *Plant Cell, Tissue and Organ Culture (PCTOC)*, 1-13.

Das, R., & Jayalekshmy, V. (2015). Mechanism of heavy metal tolerance and improvement of tolerance in crop plants. *J Glob Biosci, 4*(7), 2678-2698.

Deng, T.-H.-B., van der Ent, A., Tang, Y.-T., Sterckeman, T., Echevarria, G., Morel, J.-L., & Qiu, R.-L. (2018). Nickel hyperaccumulation mechanisms: a review on the current state of knowledge. *Plant and Soil, 423*(1), 1-11.

Diana, P. (2013). AN OVERVIEW OF STATISTICAL PACKAGES USED IN ENVIRONMENTAL PROTECTION RESEARCHES.

Eng, W.-H., & Ho, W.-S. (2019). Polyploidization using colchicine in horticultural plants: a review. *Scientia horticulturae, 246*, 604-617.

FAO/IAEA. (2018). Manual on Mutation Breeding - Third edition. Spencer-Lopes,
M.M., Forster, B.P. and Jankuloski, L. (eds.). *Food and Agriculture Organization of the United Nations. Rome, Italy*, . 301

Farooqi, Z. U. R., Hussain, M. M., Ayub, M. A., Qadir, A. A., & Ilic, P. (2022). Potentially toxic elements and phytoremediation: Opportunities and challenges. *Phytoremediation*, 19-36.

Fei, X., Lou, Z., Xiao, R., Ren, Z., & Lv, X. (2020). Contamination assessment and source apportionment of heavy metals in agricultural soil through the synthesis of PMF and GeogDetector models. *Science of the Total Environment, 747*, 141293.

Feng, Y., Wang, Q., Meng, Q., Liu, Y., Pan, F., Luo, S., . . . Yang, X. (2019). Chromosome doubling of Sedum alfredii Hance: A novel approach for improving phytoremediation efficiency. *Journal of Environmental Sciences, 86*, 87-96.

George, D., & Mallery, P. (2016). *IBM SPSS statistics 23 step by step: A simple guide and reference*: Routledge.

Gul, I., Manzoor, M., Kallerhoff, J., & Arshad, M. (2020). Enhanced phytoremediation of lead by soil applied organic and inorganic amendments: Pb phytoavailability, accumulation and metal recovery. *Chemosphere, 258*, 127405.

Gworek, B., Dmuchowski, W., Koda, E., Marecka, M., Baczewska, A. H., Brągoszewska, P., . . . Osiński, P. (2016). Impact of the municipal solid waste Łubna Landfill on environmental pollution by heavy metals. *Water, 8*(10), 470.

Helaluddin, A. S. K., Reem; Alaama, Mohamed; Abbas, Syed Atif. (2016). Main Analytical Techniques Used for Elemental Analysis in Various Matrices. *Tropical Journal of Pharmaceutical Research, 15* (2), 427-434. Retrieved from http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v15i2.29. doi:10.4314/tjpr.v15i2.29

Hou, S., Zheng, N., Tang, L., Ji, X., & Li, Y. (2019). Effect of soil pH and organic matter content on heavy metals availability in maize (Zea mays L.) rhizospheric soil of non-ferrous metals smelting area. *Environmental monitoring and assessment, 191*(10), 1-10.

Hussain, A., Hamayun, M., Rahman, H., Iqbal, A., Shah, M., Irshad, M., . . . Islam, B. (2018). Bioremediation of hexavalent chromium by endophytic fungi; safe and improved production of Lactuca sativa L. *Chemosphere, 211*, 653-663.

Iyda, J. H., Fernandes, Â., Ferreira, F. D., Alves, M. J., Pires, T. C., Barros, L., . . . Ferreira, I. C. (2019). Chemical composition and bioactive properties of the wild edible plant Raphanus raphanistrum L. *Food Research International, 121*, 714-722.

Kamunda, C., Mathuthu, M., & Madhuku, M. (2016). Health risk assessment of heavy metals in soils from Witwatersrand gold mining basin, South Africa. *International Journal of Environmental Research and Public Health, 13*(7), 663.

Kara, Z., Sabır, A., Yazar, K., Doğan, O., & Şit, M. M. (2018). Effects of colchicine treatments on some grape rootstock and grape varieties at cotyledon stage. *Selcuk Journal of Agriculture and Food Sciences, 32*(3), 424-429.

Kebaso, L., Frimpong, D., Iqbal, N., Bajwa, A. A., Namubiru, H., Ali, H. H., . . . Chauhan, B. S. (2020). Biology, ecology and management of Raphanus raphanistrum L.: a noxious agricultural and environmental weed. *Environmental Science and Pollution Research, 27*(15), 17692-17705.

Kelepertzis, E. (2014). Accumulation of heavy metals in agricultural soils of Mediterranean: Insights from Argolida basin, Peloponnese, Greece. *Geoderma, 221*, 82-90.

Keshavarz, S., Ghasemi-Fasaei, R., Ronaghi, A., & Mousavi, A. A. (2021). Innovative assisted phytoremediation of multi-elements contaminated soil by ryegrass: an electro-bio-chemical approach. *Journal of soils and sediments, 21*(7), 2604-2618.

Khursheed, S., Raina, A., Parveen, K., & Khan, S. (2017). Induced phenotypic diversity in the mutagenized populations of faba bean using physical and chemical mutagenesis. *J Saudi Soc Agric Sci*.

Kim, H. L., Lee, J., & Chae, W. B. (2022). Polyploidization reduces the probability of selecting progenies with high root pithiness and yield potential in radish (Raphanus sativus L.). *Horticulture, Environment, and Biotechnology, 63*(2), 239-247.

Kingston, H., & Walter, P. J. (1998). The art and science of microwave sample preparations for trace and ultratrace elemental analysis. *Inductively coupled plasma mass spectrometry*, 33-81.

Kome, G. K., Enang, R. K., Yerima, B. P. K., & Lontsi, M. G. R. (2018). Models relating soil pH measurements in H2O, KCl and CaCl2 for volcanic ash soils of Cameroon. *Geoderma Regional, 14*, e00185.

Kumar, D., Singh, D., Barman, S., & Kumar, N. (2016). Heavy metal and their regulation in plant system: an overview. *Plant responses to xenobiotics*, 19-38.

Kumar, V., Singh, J., & Kumar, P. (2019). Heavy metals accumulation in crop plants: Sources, response mechanisms, stress tolerance and their effects. *Contaminants in agriculture and environment: health risks and remediation, 1*, 38.

Leung, A. O., Duzgoren-Aydin, N. S., Cheung, K., & Wong, M. H. (2008). Heavy metals concentrations of surface dust from e-waste recycling and its human health implications in southeast China. *Environmental science & technology, 42*(7), 2674-2680.

Li, F., Yang, F., Chen, Y., Jin, H., Leng, Y., & Wang, J. (2020). Chemical reagent-assisted phytoextraction of heavy metals by Bryophyllum laetivirens from garden soil made of sludge. *Chemosphere, 253*, 126574.

Luo, Z.-B., He, J., Polle, A., & Rennenberg, H. (2016). Heavy metal accumulation and signal transduction in herbaceous and woody plants: paving the way for enhancing phytoremediation efficiency. *Biotechnology Advances, 34*(6), 1131-1148.

Magnusson, B., & Örnemark, U. e. (2014). Eurachem Guide: The Fitness for Purpose of Analytical Methods-A Laboratory Guide to Method Validation and Related Topics. *EuraChem*. doi:www.eurachem.org

Manzoor, A., Ahmad, T., Bashir, M. A., Baig, M. M. Q., Quresh, A. A., Shah, M. K. N., & Hafiz, I. A. (2018). Induction and identification of colchicine induced polyploidy in Gladiolus grandiflorus 'White Prosperity'. *Folia Horticulturae, 30*(2), 307-319.

Mwathi, M. W., Gupta, M., Quezada-Martinez, D., Pradhan, A., Batley, J., & Mason, A. S. (2020). Fertile allohexaploid Brassica hybrids obtained from crosses between B. oleracea and B. juncea via ovule rescue and colchicine treatment of cuttings. *Plant Cell, Tissue and Organ Culture (PCTOC), 140*(2), 301-313.

Nedjimi, B. (2021). Phytoremediation: a sustainable environmental technology for heavy metals decontamination. *SN Applied Sciences, 3*(3), 1-19.

Okereafor, U., Makhatha, M., Mekuto, L., Uche-Okereafor, N., Sebola, T., & Mavumengwana, V. (2020). Toxic metal implications on agricultural soils, plants, animals, aquatic life and human health. *International journal of environmental research and public health, 17*(7), 2204.

Oladosu, Y., Rafii, M. Y., Abdullah, N., Hussin, G., Ramli, A., Rahim, H. A., . . . Usman, M. (2016). Principle and application of plant mutagenesis in crop improvement: a review. *Biotechnology & Biotechnological Equipment, 30*(1), 1-16.

Olafisoye, O. B., Adefioye, T., & Osibote, O. A. (2013). Heavy metals contamination of water, soil, and plants around an electronic waste dumpsite. *Pol. J. Environ. Stud, 22*(5), 1431-1439.

Ondrasek, G., Begić, H. B., Zovko, M., Filipović, L., Meriño-Gergichevich, C., Savić, R., & Rengel, Z. (2019). Biogeochemistry of soil organic matter in agroecosystems & environmental implications. *Science of the Total Environment, 658*, 1559-1573.

Opaluwa, O. D., Aremu, M. O., Ogbo, L. O., Abiola, K. A., Odiba, I. E., Abubakar, M. M., & Nweze, N. O. (2012). Heavy metal concentrations in soils, plant leaves and crops grown around dump sites in Lafia Metropolis, Nasarawa State, Nigeria. *Advances in Applied Science Research, 3*(2), 780-784.

Pino-Vallejo, M., Latorre, A. R., Robayo, M. H., Orozco, A. A., & Cahueñas, N. P. TREATMENT OF LEAD CONTAMINATED WASTE SLUDGE USING THE WILD RADISH VEGETABLE SPECIES Raphanus raphanistrum.

Poursattari, R., & Hadi, H. (2022). Lead Phytoremediation, Distribution, and Toxicity in Rapeseed (Brassica napus L.): the Role of Single and Combined Use of Plant Growth Regulators and Chelators. *Journal of Soil Science and Plant Nutrition*, 1-18.

Rahman, Z., & Singh, V. P. (2019). The relative impact of toxic heavy metals (THMs)(arsenic (As), cadmium (Cd), chromium (Cr)(VI), mercury (Hg), and lead (Pb)) on the total environment: an overview. *Environmental monitoring and assessment, 191*(7), 1-21.

Rezvani, M., & Zaefarian, F. (2011). Bioaccumulation and translocation factors of cadmium and lead in'Aeluropus littoralis'. *Australian Journal of Agricultural Engineering, 2*(4), 114-119.

Rodiansah, A., & Puspita, M. I. (2020). *In vitro polyploidy induction of foxtail millet (Setaria italica (L) beauv) cv. buru hotong using colchicine treatment.* Paper presented at the IOP Conference Series: Earth and Environmental Science.

Solis, C., Andrade, E., Mireles, A., Reyes-Solis, I., Garcia-Calderon, N., Lagunas-Solar, M., . . . Flocchini, R. (2005). Distribution of heavy metals in plants cultivated with wastewater irrigated soils during different periods of time. *Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms, 241*(1-4), 351-355.

Tabinda, A. B., Irfan, R., Yasar, A., Iqbal, A., & Mahmood, A. (2018). Phytoremediation potential of Pistia stratiotes and Eichhornia crassipes to remove chromium and copper. *Environmental technology*.

Tangahu, B. V., Sheikh Abdullah, S. R., Basri, H., Idris, M., Anuar, N., & Mukhlisin, M. (2011). A review on heavy metals (As, Pb, and Hg) uptake by plants through phytoremediation. *International Journal of Chemical Engineering, 2011*.

Tricault, Y., Matejicek, A., & Darmency, H. (2018). Variation of seed dormancy and longevity in Raphanus raphanistrum L. *Seed Science Research, 28*(1), 34-40.

Vandenhove, H., Duquène, L., Tack, F., Baeten, J., & Wannijn, J. (2009). Testing the potential of enhanced phytoextraction to clean up NORM and heavy metal contaminated soils. *Radioprotection, 44*(5), 503-508. doi:10.1051/radiopro/20095093

Walkley, A., & Black, I. A. (1934). An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. *Soil Science*, *37*(1), 29-38.

Warwick, S. I. (2011). Brassicaceae in agriculture. *Genetics and Genomics of the Brassicaceae*, 33-65.

Xie, Y., Fan, J., Zhu, W., Amombo, E., Lou, Y., Chen, L., & Fu, J. (2016). Effect of heavy metals pollution on soil microbial diversity and bermudagrass genetic variation. *Frontiers in plant science, 7*, 755.

Yamagishi, H. (2017). Speciation and diversification of radish. In *The radish genome* (pp. 11-30): Springer.

Yan, A., Wang, Y., Tan, S. N., Mohd Yusof, M. L., Ghosh, S., & Chen, Z. (2020). Phytoremediation: a promising approach for revegetation of heavy metal-polluted land. *Frontiers in plant science, 11*, 359.

Yuan, L., Guo, P., Guo, S., Wang, J., & Huang, Y. (2021). Influence of electrical fields enhanced phytoremediation of multi-metal contaminated soil on soil parameters and plants uptake in different soil sections. *Environmental Research*, *198*, 111290.

Zahedi, A., Hosseini, B., & Fattahi, M. (2018). Effect of different concentration of colchicine on some morphological and phytochemical characteristics of Dracocephalum kotschyi Boiss. *Journal of Plant Productions (Agronomy, Breeding and Horticulture), 40*(4), 31-40.