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The Potential Health Risk Associated with Edible Vegetable Grown on Cr (VI) Simulated Soils

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Abstract

This study reports on the assessment of the growth potential of five edible vegetables which were grown in Cr (VI) spiked soils. The vegetable plants that were used in this study were *Vigna angularis, Cicer arietinum, Spinacia oleracea, Amaranthus dubius Thell and Phaseolus vulgaris.* Dried ground samples from roots, stems and leaves were analysed for various oxidation states of Cr. The daily intake of chromium (DIC), hazard quotient (HQ) and hazard index (HI) methods were employed to assess the potential human health risks posed by these Cr oxidation states through vegetable consumption. The results showed that *Vigna angularis* was the only vegetable that germinated in highly concentrated Cr (VI) in the simulated soil (456 mg/kg). The highest bioaccumulation factor of Cr_T in the roots was found in *Cicer arietinum* at 3.5 ± 0.51 mg/kg DW. The highest translocation factor in stem was that of *Cicer arietinum* and *Vigna angularis* at 1.0 ± 0.00, while *Cicer arietinum* and *Spinacia oleracea* translocated highest Cr to the leaf at 2.1 ± 0.21. A child or an adult consuming such contaminated *Cicer arietinum* were likely to take in between 508 -785 mg/kg/day of total Cr which were above the World Health Organisation guidelines of 220 and 340 mg/kg/day, respectively. The highest HQ was found in *Cicer arietinum* at 8.7 and 13.4 for adults and children, respectively. The same species of plants had also high HI at 17.4 and 27.2 for adults and children respectively. This indicated that consumers of the edible vegetables grown in Cr (VI) rich soil may be exposed to health risks and the children were more likely to be vulnerable to these adverse effects than the adult.

Keywords: Bioaccumulation; edible vegetables; hazard quotient; health index; speciation.

1.0: Introduction

Generally, industrial activities tend to generate a wide range of wastes, which have the potential to impact the ecosystems negatively and also lead to high-costs of treatment when such wastes are discharged to unprotected environments [1]. In developing countries, environmental laws on waste management and waste disposal are either non-existent or ineffective where they do exist [2]. Use of soils from highly contaminated dumpsites as soil improver to grow edible crops, is a common practice in many developing countries. However, such a practice tend to cause bioaccumulation of toxic heavy metals in them. High concentration of these metal pollutants in edible crops is known to be associated with potential health risks to consumers [3, 4]. The contamination of foods is a problem that has the potential to affect populations far away from the place where such crops are grown through trade pacts, or food aid [5].

Tannery effluents and solid wastes generated from tanning process are known to pollute the environment with heavy metals and acids [5]. In developing countries, most of these wastes find their way into the environment through poor open dumping. Open dumping of solid tannery wastes containing chromium has been found to be unsanitary and unaesthetical because they pollute the soils around dumpsites. This is due to the fact that waste dump leachates are transported and distributed to the surroundings by a variety of environmental factors such as rainfall, or spread into the adjacent river system by groundwater flow or chemical reactions such as biodegradation, adsorption, hydrolysis, dissolution, dilution, partitioning, and precipitation [6,7].

Edible vegetable crops grown at contaminated sites, take up and accumulate chromium at concentrations that are potentially toxic to human health [8]. Chromium has been reported to be the second most common heavy-metal contaminant in groundwater, soil and plants [9]. In addition, chromium (III) ions are known to be partially soluble in the soils after complexing with organic matters. At high concentrations, it creates potentially toxic environments for plants growth causing stunted shoots, poor root development, leads to leaves chlorosis, tissue necrosis, decreases enzyme activity, membrane damage, diminished photosynthesis and changing of chloroplasts [10]. The hexavalent form of Cr is the one that is biologically toxic and to date, there are no signs indicating any potential biological role it plays in plants. Its complex electronic chemistry has been a major hurdle in disentangling its toxicity mechanism in plants. Therefore, the hazardous effects of Cr are primarily dependent on the metal speciation, which determines its uptake, translocation and accumulation in roots and edible

portions [11]. The presence of Cr contamination in soil is toxic to edible crops as [12] reported that, the increasing chromium concentrations interfered with the biochemical parameters like, protein, sugars and amino acid contents significantly in the plant as they decreased in all different concentration of Cr treated soil as compared to control.

Indirectly, Cr affects the plant by replacing essential nutrients at cation exchange sites. The high concentration of Cr in soil thus causes several adverse effects on vegetables and in the long run affects the human health. For example, in Hong Kong, Cr contamination in marketed vegetables was found at 0.56% [13].

This study was aimed at evaluating the levels of Cr species that can be accumulated by *Spinacia oleracea, Amaranthus dubius Thell, Phaseolus vulgaris, Cicer arietinum* and *Vigna angularis* as they germinated and grew in Cr (VI) spiked soil. The study also was aimed at assessing which vegetables were consistently able to germinate in the highest concentration, to determine how much they bioaccumulate and translocate in different parts of their tissues (root, stem and leaf) and lastly to estimate human health risk indices through their consumption. The increase in unplanned settlements and urban agriculture around tannery dumpsites, could increase risk of cancer associated with consumption of edible vegetables from contaminated sites. Assessing the health risks could contribute to proactive mitigation.

2.0: Materials

2.1: Chemicals

Anhydrous potassium dichromate (99.0%), nitric acid (55%), hydrogen peroxide (30%), sodium bicarbonate (99.7%), sulphuric acid (98%) and diphenylcarbazide (98%) were purchased from Sigma Aldrich and Merck Millipore (Johannesburg, South Africa). They were of analytical reagent grades and were used without any further purification.

2.2: Equipment

Total chromium concentration in soil and plants was determined using two approaches, including the use of Agilent 700 series Inductively coupled plasma optical emission spectroscopy (ICP-OES) using expert II Varian ICP expert software (Waltham, MA USA). Another method for Cr concentrations measurements employed the use of Lambda 650 Perkin-Elmer UV-visible spectrophotometer (Waltham, MA USA). The soil-water suspension was homogenised by vigorous shaking using a mechanical shaker from Labcon (Florida, USA). The Accsen Multi-parameter probe from XS (Carpi MO, Italy) was used for measurements of pH/EC. A model Defy DMO 350 oven (Zhengzhou, China) was used for drying soil/plant samples. The dried plant materials were ground with a milling machine Tecator Knifetec 1095 sample mill-Technikon from (Johannesburg, South Africa). TOC was measured using spectrophotometer SPEKOL 1500 (Analytik Jena, Germany). Soil hydrometer, ASTM 152H from Gilson (Lewis Center, USA) was used for particle size analysis.

2.3: Soil sampling

The experimental soil with silt loam texture was air dried for 2 days. Containers were filled with 0.5 kg air dried soil. The physicochemical properties of these soils like pH, EC, TOC, moisture, Cr_T and soil texture were determined using appropriate techniques.

2.4: Sampling and preparation of seeds

The seeds of *Amarantha dubius Thell* and *Cicer arietinum* used in this study were collected randomly within Dogbone tannery dumpsite in Kenya. Seeds of *Spinacia oleracea*, *Vigna angularis* and *Phaseoulus vulgaris* were purchased from Agricol, a licenced seeds dealer in South Africa. These seeds were identified and named scientifically at herbarium section of the University of South Africa, Horticulture Department. The seeds of *Cicer arietinum* and *Amarantha dubius Thell* from the field were packed into sterile polythene bags while *Spinacia*

oleracea, *Vigna angularis* and *Phaseoulus vulgaris* seeds came with their sealed packages. The seeds of *Amarantha dubius Thell* and *Cicer arietinum* were sun-dried. Before planting, the seeds were placed in soil packed plastic containers in the greenhouse, the seeds surface were sterilised to prevent fungal and bacteria contamination by first soaking them in 30% bleach for 30 min. The seeds were then rinsed with Millique (MQ) water four times before soaked in MQ water for 30 min. Seed imbibition (hydration) was done with MQ water overnight.

2.5: Preparation of stock solution

A1000 mg/L stock solution of chromium was prepared with appropriate amount of potassium dichromate in deionised water. The stock solution was then serially diluted to get the test solutions of desired chromium concentrations.

2.6: Experimental design in the greenhouse

This study was carried out in greenhouse number 6, Horticulture Department, College of Agriculture and Environment Sciences at the University of South Africa, Science Campus. The site is located at *Latitude*: S 26° 9.501, *Longitude*: E 27° 54.113. The study was conducted between May and July 2018, under controlled conditions.

The experiment was arranged in a complete randomised design with triplicate for each treatment. The air-dried soil artificially spiked with different volume of Cr (VI) solutions i.e. 10, 50, 100 and 200 mL, respectively, along with an untreated control were adapted. The 10 mL and 50 mL solutions were mixed with 100 mL of deionised water to get enough solution before mixing with 0.5 kg soil. Thereafter, chromium solutions were uniformly mixed with air dried soil and kept for one week to stabilise. Three seeds of each of the edible vegetables selected for this study were sowed into the spiked soil and their controls in triplicates. A total of 675 seeds were planted in the 15 containers. The planted seeds were irrigated with 80% deionised water equally three times a week. Before and at the end of the experiments, the soil and plants samples were subjected to various analyses as detailed below.

2.7: Estimation/observation of germination and growth pattern

The seed germination was being monitored after every 24 h until the germination percentage and growth height was constant. For the evaluation of seedling growth, all germinated seedlings of similar type were allowed to grow within concentrations of 23, 114, 228 and 456 mg/kg chromium in the soils. During their growth period, the seeds were monitored for the germination trend, growth rate/pattern, height and physiological changes, especially the stem

and leaves. The plants were harvested carefully after 56 days, washed with distilled water to remove soil particles and analysed for growth attributes such as germination percentage and growth height.

2.8: Germination percentage and growth height

The germination percentage (%G) is the proportion, expressed as percentage of germinated seeds to the total number of viable seeds (675) that were tested by using the formula of [14]. (equation 1). The plant growth height was measured periodically. At the end of the experiment, the mean height was taken for each plant under different concentration i.e. 0, 23, 114, 228 and 456 mg/kg and the stress tolerance index for plant height (TI_{PH}) was calculated using formula of [15], equation (2).

$$\%G = \frac{\text{Number of germinated seeds}}{\text{Total number of planted seeds}} \times 100 \quad (1)$$

$$(TI_{PH}) = \frac{\text{Height of treated plant}}{\text{Height of control plant}} \times 100 \quad (2)$$

2.9: Determination of total chromium in soils and plants

An amount of soil weighing 1 g of the homogenised sample was put into digestion crucibles, then 9 mL of HNO_3 was added to the sample and allowed to react before adding 2 mL of hydrogen peroxide. The solution was given time to stabilise before loading onto the microwave. The vessels were heated at 180^{o}C for 1 h. The digested solution was filtered using Whatman filter paper (0.45µm). Then 1 mL of the filtrate was taken and diluted to 10 mL with deionised water. This solution was analysed for Cr_T.

Plant samples were first cut at root-stem-leaf junctions respectively. The fresh weight of root, stem and leaf samples were measured using an analytical balance and recorded in gram per plant. Then plant parts were dried in an oven at 60˚C for 24 h to get constant dry weight for roots, stems and leaves**.** Plant materials were lyophilised and homogenised by grinding in milling machine before being digested in microwave following the same procedure adapted for soil as modified from method 3052 [16].

2.10: Determination of Cr (VI) in soils and plants

A total of 0.25 g of dried soil samples were put into glass beakers. To that, 25 mL of 0.1 M Na_2CO_3 was added and the mixture boiled on a hot plate for 15 min. The solutions were allowed to cool and then filtered using Whatman filter paper (0.45µm). The filtrate was topped up to

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25 mL. The solution was taken and acidified with 4 mL of 0.2 M sulphuric acid to lower the pH to ~ 2. This was complexed with 1mL of diphenylcarbizide and analysed for Cr (VI) at 540 nm after calibration with prepared standards. Milled plant samples were prepared and analysed for Cr (VI) using the same procedure adapted from [17]. The concentration of Cr (III) was derived from the difference between Cr_T and Cr (VI) concentrations. The occurrence of Cr_T and Cr (VI) in plant samples necessitated the quantification of their risk on human health.

[18,19, 20, 21, 22] proposed that the quantification of risk potential due to Cr ingestion like other heavy metals on human health may be assessed or estimated using bioaccumulation/ bioconcentration factor (BF/BCF), translocation factor (TF), daily intake of chromium (DIC), hazard quotient (HQ) and Hazard Index (HI) which were calculated using equations (3) to (7)**.** Statistical analysis was done using STATA version 14.2. It was used to to generate the means and standard deviation for the data sets, compare the mean values of the tested parameters for all the different sampled parts of edible vegetable at 0.05 level of significance using Tukey's HSD as the post hoc test. Principal component analysis was used to test for the relationship between the edible vegetable species and their bioaccumulation and translocation factor of Cr_T, Cr (VI) and Cr (III).

Bioaccumulation/Bioconcentration factor (BF) is computed according to the following formula (3) adapted from [23,21,24].

$$\mathrm{BF/BCF} = \frac{[C1]}{[C2]} \tag{3}$$

where C_1 and C_2 are average concentrations of metal in plant and soil, respectively.

Translocation factor (TF) was calculated by equation (4) modified from [20, 21].

$$\mathrm{TF} = \frac{\text{Cr content in the leaf (mg/kg)}}{\text{Cr content in the roots (mg/kg)}} \tag{4}$$

Daily intake of chromium (DIC) was estimated by the modified equation (5) of [22]

$$\mathrm{DIC} = \mathrm{DIV} \times Cr\ content\ of\ vegetable \tag{5}$$

Where DIV - daily intake of vegetable; Cr - Cr_T / Cr (VI) content of vegetable.

Hazard quotient (HQ) an estimate of potential hazard was determined by equation (6) modified from [19].

$$\mathrm{HQ} = \frac{Div \times Cmetal}{RfD \times BO} \tag{6}$$

Where- Div is the daily intake of vegetable leaves (mg/kg/day), (C_{metal})- is the concentration of Cr_T/Cr (VI) in the vegetable (mg/kg), RfD- is the oral reference dose (RfD value for Cr is 1.5 mg/kg of body weight/day) and BO- is the human body weight (60 kg for adults and 25 kg for children).

Hazard index (HI) is calculated as an arithmetic sum of the hazard quotient for each pollutant as shown in the following modified equation (7) of [22].

$$HI = \sum_{i=0}^{n} HQ \quad (7)$$

3.0: RESULTS AND DISCUSSION

3.1: General properties

Various physico-chemical properties of the homogenised soil used in this study were measured and are depicted in Table 1. The textural particle size of experimental soil had 25% sand, 15% silt and 60% clay suggesting the soil to be predominantly silt loam. Silty loam soils are the most balanced and support the greatest diversity of plant life. The types of plants that grow well in silty loam soil include grasses, bamboo, wetland and aquatic plants, vegetables and fruit trees [26].

Table 1*:* Physico-chemical properties of experimental soil.

Soil Properties	Average values
pH	6.2 ± 0.05
EC (µS/cm)	42.8 ± 0.01
Total organic carbon (%)	0.98 ± 0.01
Moisture (%)	10.9 ± 0.15
Sand (%)	25
Silt (%)	15
Clay (%)	60
Texture Class	Silt Loam
Cr_T in soil (mg/kg)	1.2 ± 0.03

3.2: Effect of chromium concentration on seed germination and growth

The observed results of the present study show that higher chromium concentration adversely influence the germination process of various vegetable seeds (Figure 1). Chromium treatment in soils at the level of 23 to 456 mg/kg had different effects. It was observed that 24% germination took place in the control soils but decrease to 16% was recorded in soils with concentrations of 23 mg/kg, 10% germination corresponded to soils with 114 mg/kg of chromium concentration, 7% germinated in 228 mg/kg Cr levels and 1% managed to germinate in 456 mg/kg Cr levels. Significant variations in Cr tolerance and sensitivity in terms of seed germination have been recorded in literature according to [18]. This study has established that the germination of different plant seeds is affected differently with different Cr levels (Figure 1).

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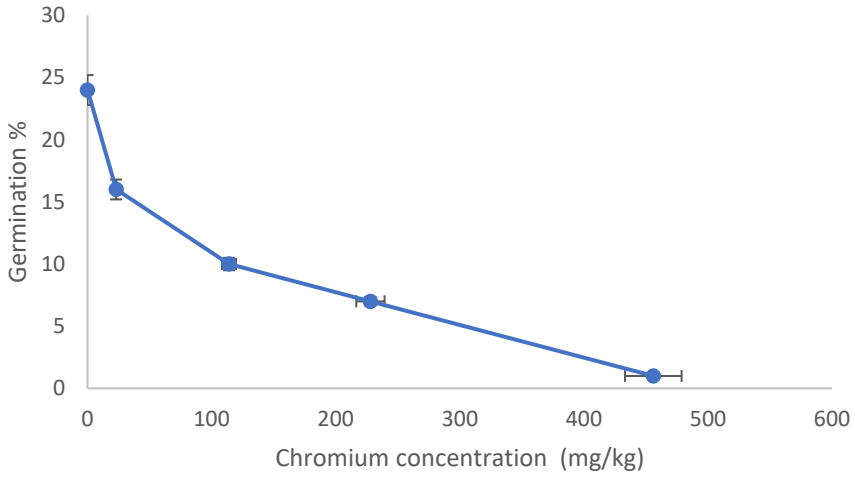

Figure 1: Germination percentage of plants in different concentrations of Cr (VI) (n = 3, SD).

The germination time in this study was prolonged to (8 ± 1 days) while the control germination was observed after (6 ± 1 days). The prolonged germination period was observed as the levels of chromium increased from 23 mg/kg to 456 mg/kg. This suggests that the seeds may have undergone secondary induced dormancy in Cr conditions before germination and this lengthened their germination period. According to, [27] at treatment level of 400 mg/kg of chromium in soil, a prolonged germination time (9 ± 1 days) was observed unlike the control (4 ± 1 days) and this implied that germination time increased with increase in chromium dosage.

The Cr transported to the aerial parts or retained at the roots might have affected the physiological processes of plants growth as it contributed to their various reduction in height as shown in Figure 2. The mean values of shoot length were affected as concentration increased from 23 mg/kg to 456 mg/kg. In the control, maximum height was that of *Cicer arietinum* (21 cm) followed by *Phaseoulus vulgaris* (17 cm) then *Vigna angularis* (12 cm), second last was *Amarantha dubius Thell* (10 cm) and the last was *Spinacia oleracea* (8 cm). In the control soil which was free of Cr (VI), thus maximum height observed. In 23 mg/kg Cr level in soil, the plant height follows the trend of *Cicer arietinum* being the highest (18 cm) followed by *Phaseoulus vulgar* (15 cm), then *Vigna angularis* (8 cm) while *Amarantha dubius Thell* was (7cm) and *Spinacia oleracea* was (5 cm). *Vigna angularis, Spinacia oleracea* and *Amarantha dubius Thell* had an observable reduction in height in this concentration. This was a significant decrease of height and could be attributed to Cr (VI) concentration. In the 114 mg/kg Cr levels, the highest height was that of *Cicer arietinum* (20 cm) and *Phaseoulus vulgaris* was (11 cm),

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then *Vigna angularis* (4 cm) as lowest was *Spinacia oleracea* and *Amarantha dubius Thell* at (1 cm). The *Cicer arietinum* species had an increase in height, while the other species registered decrease in height in the same concentration. The most reduced height recorded was for *Spinacia oleracea* and *Amarantha dubius Thell*. This may have implied that *Spinacia oleracea* and *Amarantha dubius Thell* seeds growth were very sensitive to high increase in Cr (VI) concentrations at 114 mg/kg in comparison to other plants. At the same concentration, *Cicer arietinum* species had an increase in height suggesting that its growth hormones may have been stimulated by higher Cr (VI) concentration. [14] concurs by stating that, the increasing Cr (VI) concentration and number of days, resulted into increase of the shoot and root length of chickpea (*Cicer arietinum*) cultivars. This may have been attributed to the presence of suitable cations and anions that had influence on the effect of the nutrient uptake, which helped in plants physiological metabolic activity that allowed the plant to grow easily in such environment.

In 228 mg/kg levels the maximum height was that of *Phaseoulus* vulgaris (7 cm) followed by *Cicer arietinum* (3 cm) and lastly *Vigna angularis* (2 cm). The *Spinacia oleracea* and *Amarantha dubius Thell* seeds never germinated in that concentration. *Vigna angularis* was the only plant that managed to germinate and grow up-to 1 cm in the highest Cr (VI) level of 456 mg/kg. According to [28,14] the concentrations of 500 mg/kg Cr (VI) in soil affected shoot growth of wheat and oat. It led to decrease in plant height as there was reduced root growth and consequent decreased nutrients and water transport to the higher parts of the plant. Thus Cr transported to the aerial part of the plant directly impacted cellular metabolism of shoots contributing to the reduction in plant height as seen in Figure 2 of this study.

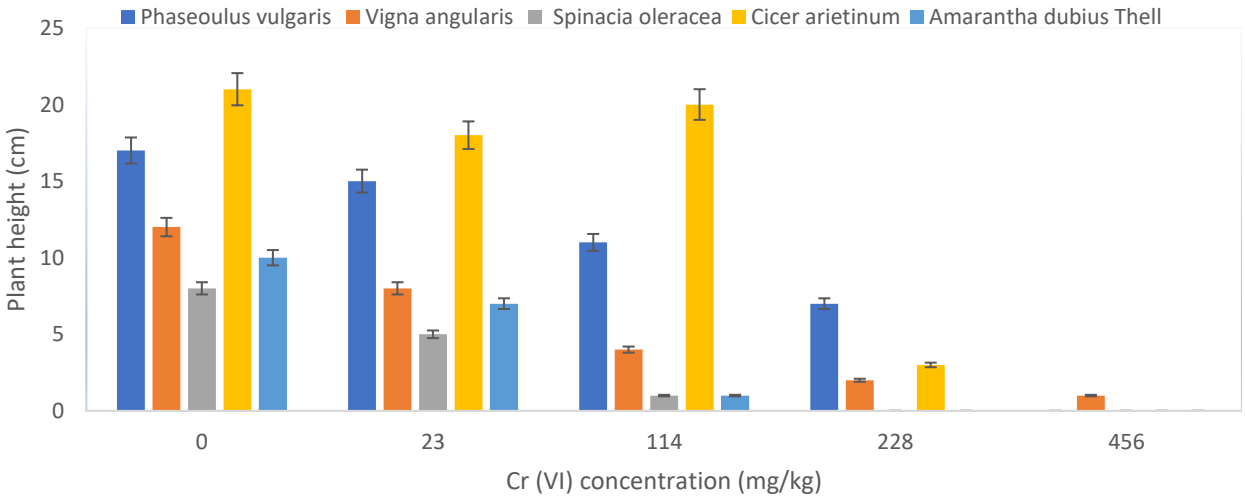

Figure 2: Effect of Cr (VI) concentration on different plant heights in a simulated soil. (n=3, SD)

Simulated studies using Cr (VI) as the spiking agent to investigate different crops reactions have been reported by [29, 30, 31]. Cr is considered strongly toxic because Cr (III) compounds in the soil are more or less insoluble and their ions are tightly bound to humus and clay particles while Cr (VI) is very soluble and easily pass through the plant cells into vacuoles where they combine with cations and form stable compounds which either accelerate or retard plants growth [32]. The current study observed a significant variation in the germination of edible vegetable seeds in polluted soil. *Amarantha dubuis Thell* and *Spinacia oleracea* germinated in low concentration only while *Cicer arietinum, Phaseoulus vulgaris and Vigna angularis* germinated in both low and high concentration of Cr (VI). These findings are in agreement with those reported by [33] who reported that higher concentrations of heavy metal significantly reduced the strength of germination as compared to the lower concentrations which have least harmful effect on germination. [34] also found that there was no germination of spinach with applied Cr level at 320 mg/kg in the soil. Therefore, it may be possible to conclude that seed coats of different plants impermeability and embryos selectivity can affect the tolerance of chromium impacts. Those events naturally could have helped in the selection of high and low tolerant species or chromium varieties during the germination, early seedling stages and growth height in this study.

The consistent germination and significant growth of *Phaseoulus vulgaris*, *Cicer arietinum* and *Vigna angularis* in chromium contaminated soil observed in this study seems to suggested that these plants might be tolerant or have some mechanism that allow them to germinate and grow in Cr toxic environment. It could be observed that at the roots of *Vigna angularis* at highest concentration was modified with less hair roots as compared to those from low concentrations and control. This could be the part of mechanism this plant applied in the roots to exclude Cr (VI) to its aerial parts which made it possible for it to grow in such high concentration.[30] stated that toxic properties of Cr (VI) could be reduced by oxidising agents as well as from the formation of free radicals during the reduction of Cr (VI) to Cr (III) inside the root cell.

[35] reported that, plants exhibit diverse strategies to high concentrations of Cr such as indicators, excluders and accumulators. In order to understand practically how these plants may have applied these strategies, the sampled plants were divided into root, stem and leaf. Then measurement involving the assessment of different levels of Cr oxidation states; Cr_T, Cr (VI) and Cr (III) in the vegetables grown in the spiked soil was undertaken and their occurrence given in Table 2. Different vegetable cultivars differ in their ability to take up Cr oxidation states as the occurrence varied in this study as depicted in Table 2. In the table, *Cicer arietinum*

had the highest Cr_T in the root at (3.5 ± 0.51 mg/kg DW) while *Spinacia oleracea* had the lowest level in the root at (1.1 ± 0.03 mg/kg DW). *Vigna angularis* had high Cr (VI) in the root at 0.9 ± 0.04 mg/kg DW and *Spinacia oleracea* registered the lowest Cr (VI) in the root at 0 .1 ± 0.03 mg/kg DW. The root of *Cicer arietinum* had Cr (III) at the highest level of 2.9 ± 0.05 mg/kg DW while the lowest was in *Amarantha dubuis Thell* at the range of 0.9 ± 0.03 mg/kg DW. The level of total Cr translocated to the stem was high in *Cicer arietinum* and *Vigna angularis* (1.0 ± 0.00 mg/kg DW) and least in *Phaseoulus vulgaris* (0.1 ± 0.07 mg/kg DW). *Vigna angularis* accumulated high Cr (VI) in the stem as *Spinacia oleracea* registered nil in the stem. *Phaseoulus vulgaris* had high concentration of Cr (III) in stem at 1.2 ± 0.08 mg/kg DW as lowest level were recorded in *Amarantha dubuis Thell* (1.0 ± 0.03 mg/kg DW). The level of Cr_T translocated to the leaf parts was highest in *Cicer arietinum* (2.1 ± 0.21 mg/kg) and least in *Phaseoulus vulgaris* at 1.0 ± 0.00 mg/kg. *Cicer arietinum* accumulated maximum Cr (VI) in the leaf (0.2 ± 0.12 mg/kg) as lowest concentration was observed in *Phaseoulus vulgaris* at 0.1 ± 0.03 mg/kg. *Spinacia oleracea* had high levels of Cr (III) in the leaf (2.1 ± 0.00 mg/kg DW) while least concentration was found in *Amarantha dubuis Thell* at 1.0 ± 0.02 mg/kg DW.

The chromium oxidation states in the sampled parts of the plants were found to be significantly different to their control plants parts ($p < 0.05$). This could be attributed to the Cr (VI) spiked in soils which could have been accumulated by these plants through their tolerance mechanisms. Residual chromium concentrations (Cr_T, Cr (VI) and Cr (III)) were still detected in simulated soil at the end of the experiment, despite the effects of soil natural matrices and uptake by plants. This indicated Cr is persistent in soil environments and this is a source of concern.[1] stated that, the risk that could be associated with Cr (VI) amendment in soils may involve the chance of Cr persistence in soil and accumulating in plants which may eventually enter into the food chain. From the concentrations derived were used to assess the transfer of Cr pollutant from soil to plants portion using pollution indices such as BF and TF as monitoring tools for pollution effect on edible vegetables grown in Cr contaminated sites.

Table 2: The occurrence of Cr_T, Cr(VI) and Cr(III) in root, stem and leaf of different plant species in the simulated soil.

Name of Plant	Portion of plant	Chromium oxidation states (mg/kg)						P>|t| (tukey effect)
		Cr_T	control	Cr (VI)	control	Cr(III)	Control	
Phaseoulus vulgaris	root	2.8 ± 0.31	0.2 ± 0.00	0.7 ± 0.03	ND	2.1±0.34	0.2 ± 0.00	0.000
	stem	0.1 ± 0.07	0.2 ± 0.01	0 .01 ±0.00	ND	1.2±0.08	0.2 ± 0.01	
	leaf	1.0 ± 0.00	0.2 ± 0.00	0.1 ± 0.03	ND	1.0±0.03	0.2 ±0.00	
Vigna angularis	root	3.4 ± 0.61	0.2 ±0.00	0.9 ± 0.04	ND	2.4±0.57	0.2 ± 0.00	0.000
	stem	1.0 ± 0.00	0.2 ± 0.00	0 .1 ± 0.02	ND	1.1±0.02	0.2 ± 0.00	
	leaf	1.8 ± 0.33	0.2 ± 0.00	0 .2 ± 0.03	ND	1.6±0.30	0.2 ± 0.00	
Spinacia oleracea	root	1.1 ± 0.03	0.1 ± 0.00	0 .1 ± 0.03	ND	1.0±0.03	0.1 ± 0.00	0.000
	stem	0.01±0.06	ND	ND	ND	1.1±0.06	ND	
	leaf	2.1 ± 0.00	ND	0 .01± 0.00	ND	2.1±0.00	ND	
Cicer arietinum	root	3.5 ± 0.51	0.3 ± 0.01	0.8 ± 0.00	ND	2.9±0.05	0.3 ± 0.01	0.000
	stem	1.0 ± 0.00	0.2 ± 0.03	0.01 ± 0.00	ND	1.1±0.01	0.2 ± 0.03	
	leaf	2.1 ± 0.21	0.3 ± 0.03	0 .2 ± 0.12	ND	2.0±0.01	0.3 ± 0.03	
Amarantha dubuis Thell	root	1.2 ± 0.01	ND	0.3 ± 0.03	ND	0.9±0.03	ND	0.000
	stem	0.11±0.02	ND	0.001±0.01	ND	1.0±0.03	ND	
	leaf	1.2 ± 0.03	0.09±0.06	0.2 ± 0.01	ND	1.0±0.02	0.1±0.07	
Chrome simulated soil	Soil	4.9 ± 0.00	1.2 ± 0.03	1.8 ± 0.07	ND	3.0±0.13	1.2±0.03	0.000

3.3: Bioaccumulation/Bioconcentration Factor

BF/BCF is an index that measures the ability of the plant to accumulate a particular metal with respect to its concentration in the soil and the root of the plant [36]. Under normal conditions, concentration of Cr in plants is supposed to be less than 0.001 mg/kg DW [37]. But in this study, Cr oxidation states levels revealed significant variations in the five studied vegetables as shown in Table 3. In this study, *Vigna angularis* and *Cicer arietinum* were found to have high BF of Cr_T, Cr (VI) and Cr (III) while *Spinacia oleracea* and *Amarantha dubuis Thell* had the lowest BF of the same Cr oxidation states. According to [38] categorisation system, *Cicer arietinum* could be grouped as Cr moderate accumulator plants while *Vigna angularis, Spinacia oleracea, Phaseoulus vulgaris* and *Amarantha dubuis Thell* were low Cr accumulator plants. There was no non-accumulator and hyper accumulator plants in this study, instead these plants could be grouped as phytoextractants or phytostabilisers. Since they are edible crops, this disqualifies them for phytoremediation of Cr contaminated sites. Given that they have shown bioaccumulation potential for Cr in edible parts, they are of major health risk concern to human population in Kenya and South Africa.

The high values of BF/BCF confirmed that the roots are the main bio-accumulators of Cr in all its oxidation states. The highest Cr accumulation in the roots occurred due to their direct contact with Cr oxidation states in the soil solution. This Cr accumulation in the roots could be apportioned to that fraction of Cr ions physically adsorbed to the root cell walls and another fraction absorbed by the cells that were immobilised in the root vacuoles. Since there was an increase in the shoot biomass, these mechanisms were suspected to have played a key role in the BF/ BCF of the plants under toxic conditions in the soil. [39] explained that, enhanced accumulation of chromium in the roots of *angularis* and *arietinum* species may have been due to the presence of organic acids (carboxylic acid and amino acids) in the root exudates which form complexes with chromium, thereby making them available for the uptake by roots.[20] reiterated that, the concentration of Cr is always higher in the roots than in the shoots. [37] reported that *Amaranthus* and *Spinacia* vegetables have limited potential for the bioaccumulation of higher Cr concentration in their roots thus giving the possible reasons why they were unable to germinate and grow in 228 and 456 mg/kg levels in this study. This difference in Cr accumulation in different parts of the plant suggested that different cellular mechanisms of bioaccumulation of Cr took place and this influenced Cr bioaccumulation and partitioning in these plants, thus the need to find out the quantity that was translocated from the root to the shoot.

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Table 3: The BF and TF of Cr_T, Cr(VI) and Cr (III) in the different parts of vegetables plants at the harvesting stage.

Name of Plant	Treatment	BF		TF
		Root	Stem	Leaf
Phaseoulus vulgaris	Cr_T	0.8	0.05	0.3
	Cr (VI)	0.4	0.01	0.2
	Cr (III)	0.4	0.04	0.1
Vigna angularis	Cr_T	1.0	0.3	0.7
	Cr (VI)	0.5	0.2	0.3
	Cr (III)	0.5	0.1	0.4
Spinacia oleracea	Cr_T	0.3	0.02	0.2
	Cr (VI)	0.1	-	0.01
	Cr (III)	0.2	0.01	0.2
Cicer arietinum	Cr_T	1.0	0.3	0.7
	Cr (VI)	0.4	0.01	0.4
	Cr (III)	0.6	0.02	0.3
Amarantha dubuis Thell	Cr_T	0.3	0.04	0.4
	Cr (VI)	0.1	0.02	0.3
	Cr (III)	0.2	0.02	0.1

3.4: Translocation factors

Though Cr is absorbed by roots from nutrient solution as Cr (III) or Cr (VI), and translocated to aerial portions and roots, it is largely retained in the roots [25]. In this study, the translocation to the stem was highest for Cr_T in the *Cicer arietinum* and *Vigna angularis* at 0.30 (Table 3). *Vigna angularis* translocated high Cr (VI) at 0.2 and Cr (III) at 0.1 in the stem respectively. In the leaves, *Cicer arietinum* and *Vigna angularis* translocated the highest amount of Cr_T at 0.7. *Cicer arietinum* had high Cr (VI) in the leaf at 0.4. Cr (III) was translocated more in *Vigna angularis* at 0.3.

Figure 3 gives the principal component analysis (PCA) to illustrate the relationship between plant species BF/BCF and TF of Cr oxidation states. Cr_T and Cr (III) showed negative relationship to Cr (VI). Cicer *arietinum* and *Vigna angularis* had higher positive relationship with Cr (VI), while *Spinacia oleracea* had lower positive relationship with Cr_T and Cr (III). *Amarantha dubuis Thell* had lower negative relationship with Cr_T and Cr (III). *Phaseoulus vulgaris* had higher negative relationship with Cr (VI). Exposure to *Cicer arietinum* and *Vigna*

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angularis implied higher risk to Cr (VI) while exposure to *Spinacia oleracea* could lead to risk of Cr_T and Cr (III).

Although the tendency to retain more Cr in the roots was seen to be common in most of the plant species studied, there was also translocation quantitatively among the plant species. According to [40] the maximum amount of Cr is accumulated in the roots followed by leaves and then fruits which was close to the result in this study; where Cr > root > leaf > the stem. This may mean that plants with high levels of Cr in their leaves may be trying to phytovolarise the metal into the atmosphere through their leaves as seen yellowing from the edge towards the petal and eventually droping off the plant after possibly translocating it and starting a new leaf growth. According to [20] dicotyledon species, such as *Vigna angularis* uptake and transport more Cr to shoots than monocotyledons plants such as maize due to differences in the rooting patterns, transpiration rates and metabolism between these two groups of plants while [24] found that *Amaranthus dubius* tolerated high Cr (VI) concentrations by accumulating and transferring them to aerial parts. The outcome of this study also compares to the reported findings by [41] which found that increase in Cr concentration in the leaves may be related to higher soil Cr concentration from the spiked soil and therefore, the metal was bioaccumulated from the roots to the leaves. Lower accumulation of Cr in leaves than in roots can as well be related to conservation of photosynthesis processes from toxic levels of trace elements according to [42]. The levels of Cr (VI) transferred and detected in *Cicer arietinum, Phaseoulus vulgaris, Amarantha dubuis Thell, and Vigna angularis* in the leaf portions of the plants signify high health risk to consumers.

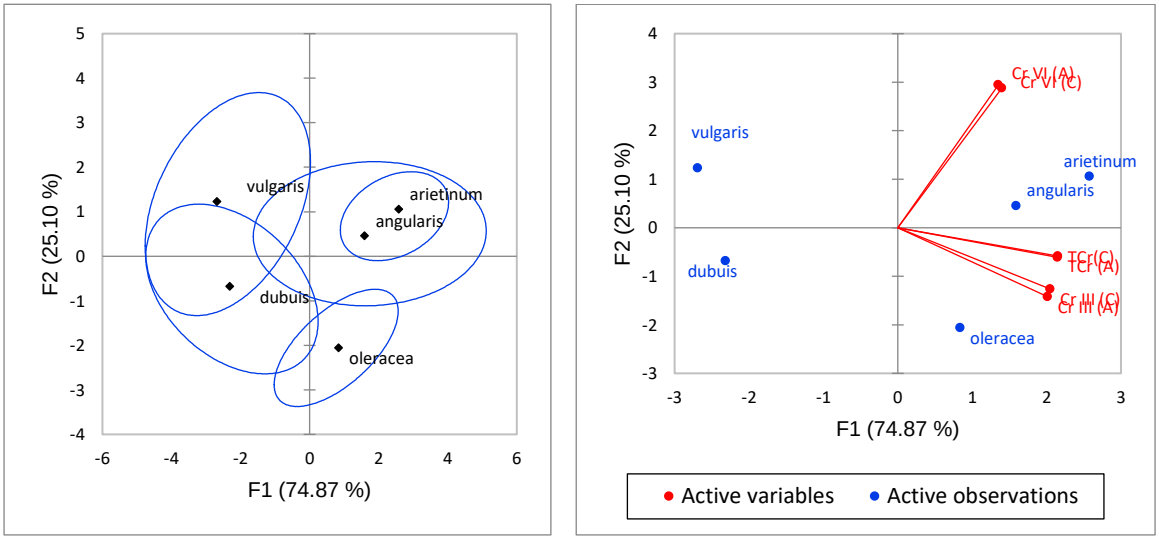

Figure 3: Principal component analysis of the vegetable species in terms of BF/BCF and TF in different portions of the plants.

3.5: Daily intake of chromium (Cr_T, Cr (VI), Cr (III)) through edible vegetables grown on Cr (VI) spiked soils

This study focused on the cultivation of vegetables on Cr (VI) simulated soils and then estimated the average daily intake of Cr by adult and child consumers of these vegetables. A child and an adult consuming *Cicer arietinum* were likely to take in between 508 - 785 mg/kg/day of high Cr_T. The least quantity of the Cr_T they could have consumed was in the range of 220 – 340 mg/kg/day for child and adult from *Phaseoulus vulgaris* species in the same environment. When a child and an adult consumed *Cicer arietinum* they were likely to get exposed to Cr (VI) in the order of 70 -109 mg/kg/day as the highest amounts of the contaminant. The least amount of Cr (VI) they could get exposed to was from 0.4 to 0.7 mg/kg/day after eating leaves of *Amaranthus dubius Thell*. An adult or a child who consumed *Spinacia oleracea* from this simulated site was going to be exposed to between 460 - 711 mg/kg/day as the maximum levels of Cr (III) in the leaves of this plant while the minimum a child and an adult could take in from the same Cr (III) ranged from 191 - 296 mg/kg/day of *Phaseoulus vulgaris.* However, the required amount of vegetables in people's daily diet is supposed to be between 300 -350 g per person's (adult) and 200 - 230 g per child as suggested by guidelines of [43].

Figure 4 depicts the possible daily intake of high Cr_T, Cr (VI) and Cr (III) by human beings from the edible vegetables grown in spiked soil in this study. The intake values were calculated by taking the average value of Cr oxidation states to assess the level exposure in all the five varieties of the vegetables (Table 3) and taking into account that each adult and child in Kenya and South Africa (assuming 60 and 25 kg of body weight for adult and child respectively) consumes approximately 340 and 220 g respectively of vegetables per day according to [43]. However, the amounts in Figure 4 give a picture showing that daily intake of vegetable species were far above recommended intake levels. This is so because vegetables, in this study may have accumulated Cr_T, Cr (VI) and Cr (III) in concentrations greater than the maximum permissible limits of [44] for Plant (0.10 mg/kg DW) in the spiked soil.

In the consumption habits of local residents of Kenya and South Africa, *Spinacia oleracea, Cicer arietinum, Phaseoulus vulgaris, Amarantha dubuis Thell, and Vigna angularis* are consumed as leafy vegetables which accounts for 90% of total consumption of vegetables within the region with the remaining percentage being taken as seeds or pods [46, 4]. This means that a very large population from these two countries are potentially at health risk due to exposure to these Cr_T, Cr (VI) and Cr (III). Therefore daily intake of chromium by human

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consumers of these vegetables are likely to expose them to these types of clinical disorders i.e respiratory, carcinogenic, renal, hepatic, gastrointestinal, cardiovascular, haematological, reproductive and developmental, genotoxic and mutagenic effects [46].

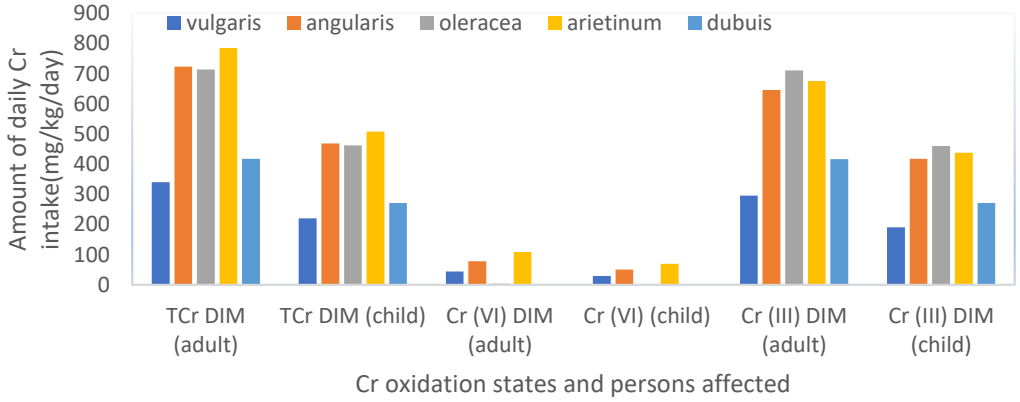

Figure 4: Daily intake of Cr_T, Cr (VI) and Cr (III) from the leaves of different vegetables from soil spiked with Cr (VI).

3.6: Hazard quotient

The potential non-cancer risk for Cr_T, Cr (VI) and Cr (III) is expressed as hazard quotient (HQ). If HQ >1, it implies that there may be a concern for potential non-cancer effects when the chronic daily intake exceeds threshold [36]. The results of HQ for Cr oxidation states in this study are shown in Table 4. For different exposure population, HQ of Cr_T and Cr (III) oxidation states were all above 1, which means that the daily intake of Cr oxidation states through the consumption of *Spinacia oleracea, Cicer arietinum, Phaseoulus vulgaris, Amarantha dubuis Thell, and Vigna angularis* will likely cause adverse health effects for residents of South Africa and Kenya. Even though the levels of Cr (VI) were less than 1 implying that consumers were safe when they got exposed to vegetables containing it. However, according to [44] guidance for Plant, the maximum permissible limits of (0.10 mg/kg DW) was exceeded in most of the vegetables except for *Spinacia oleracea* and *Amarantha dubuis Thell* for both adult and child which were found below permissible levels. It can also be shown from Table 4 that HQ of Cr_T, Cr (VI) and Cr (III) for children is higher than that for adults in all the plants analysed, which agrees with previous studies of [19].

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Table 4: Hazard quotients of Cr_T, Cr (VI) and Cr (III) to consumers of vegetables grown on soil spiked with Cr (VI).

Plant name	Total Cr		Cr(VI)		Cr(III)	
	Adult	Child	Adult	Child	Adult	Child
P. vulgaris	3.8	5.8	0.5	0.8	3.3	59
V.angularis	8.0	12.3	0.9	1.3	7.1	11
S. oleracea	7.9	12.2	0.04	0.06	7.9	12.1
C. arietinum	8.7	13.4	1.2	1.8	7.5	12
A. dubuis (T)	4.6	7.1	0.007	0.01	4.6	7.1

3.7: Hazard Index

The estimation of hazard index (HI), which takes care of the chemical mixtures, is very important in assessing multiple effects of the heavy metals like Cr. In nature, chronic low-level intake of toxic metal elements can have negative effect on human health and the detrimental impact only becomes known after several years of exposure [48]. The HI method was used to assess the total of all potential health risks of Cr_T, Cr (VI) and Cr (III) accumulation through leafy vegetable consumption for adults and children. The risk is considered unacceptable at HI >1. The results of the five leaf vegetables were all found to be above 1, which may present risk to adults and children in terms of Cr_T, Cr (VI) and Cr (III) exposure. HI values were observed in this decreasing order for adults and children as *Cicer arietinum* > *Vigna angularis* > *Spinacia oleracea* > *Amarantha dubuis Thell* > *Phaseoulus vulgaris* as shown in Table 5. This suggested that the potential HI of Cr oxidation states through vegetable consumption were higher in *Cicer arietinum* 17.2 and 27.2 mg/kg/day for both adults and children respectively. These results implied that the potential health risk of Cr oxidation states through consumption of leafy vegetables were higher for all vegetable types.

The HI values of Cr oxidation states through vegetable consumption for children were higher than the values for adults in all vegetables. Therefore, Cr oxidation states are likely to contribute to the potential health risks of vegetable consumption for residents living and accessing sites contaminated with tannery Cr wastes. The HI findings in this study were lower than that of [22] who found HI for Cr in children toys at 91.9 mg/kg/day. [36] stated in their work that Cr speciation was of concern. This was because, according to them health risk from Cr exposure may be overestimated if Cr (III) co-exists with Cr (VI). Cr (III) is considered essential in the metabolism of carbohydrates in animals but high levels are equally risky under HI assessment as seen in this study. Therefore, this study considered Cr speciation in vegetables

from polluted sites and found out that their HI is potentially risk to both adults and children consuming them.

Table 5: Hazard index of Cr_T, Cr (VI) and Cr (III) in vegetables grown in soil spiked with Cr (VI) for adult and child.

HI	*Phaseoulus vulgaris*	*Vigna angularis*	*Spinacia oleracea*	*Cicer arietinum*	*Amarantha dubuis*
Adult	7.6	16	15.8	17.4	9.2
Child	11.6	24.6	24.4	27.2	14

3.8: Conclusion

The present study used 5 species of vegetables on Cr (VI) contaminated soil in a greenhouse. *Vigna angularis* was the only vegetable which managed to germinate at a high Cr concentration of 456 mg/kg. *Phaseoulus vulgaris* and *Cicer arietinum* germinated at a Cr concentration of up to 228 mg/kg while *Spinacia oleracea* and *Amarantha dubuis* germinated and grew at Cr concentration of up to 114 mg/kg. Bioaccumulation/bioconcentrations factor of *Vigna angularis* and *Cicer arietinum* were higher than those of *Phaseoulus vulgaris, Spinacia oleracea* and *Amarantha dubuis.* These plants can be grouped as moderate and low accumulators but cannot be used for phytoremediation because they are edible vegetables. An adult and child who could get exposed to these vegetables, especially *Cicer arietinum* and *Spinacia oleracea* through daily intake of Cr_T, Cr (VI) and Cr (III), were likely to consume above recommended values of WHO (340 and 220 g), respectively. The HQ and HI of Cr_T and Cr (III) oxidation states were all above 1 while that of Cr (VI) was below 1. This portends carcinogenic risks to the consumers with children being more vulnerable to such risks. Therefore remediation of sites polluted with tannery based Cr wastes is recommended.

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5.0 Conflict of interest

No conflict of interest in this work.

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