



## INTRODUCTION

Adverse impact of spent engine oil (SEO) according to studies has been observed on plants cultivated on SEO polluted soils leading to reduced germination of seeds, yield and increased uptake of toxic components (Akinola *et al.*, 2004). According to Adedokun and Ataga (2007), SEO poses danger to the environment due to its high content in various pollutants both organic and inorganic. The impact of heavy metal contamination on the biological lives has been widely reported especially cadmium and lead which have been found in most crude oil (Raskin and Ensley, 2000; Meagher, 2000). Pollution from SEO has been said to pose environmental problems in Nigeria and such pollution problem has been noted to be more widely spread than crude oil pollution (Odjegba and Sadiq, 2002). Due to the presence of large quantities of hydrocarbons, toxic polycyclic aromatic hydrocarbons and heavy metals, studies have shown that indiscriminate disposal of SEO may adversely affect plants, microbes and aquatic lives (Wang *et al.*, 2000).

Cowpea is a leguminous crop rich in protein, minerals and vitamins (Tharanathan and Mahadevamma, 2003). According to Food and Agriculture Organization (FAO) (2017), the production of cowpea by West Africa sub-region stands at about 81% (4,525,891 metric tonnes) of the global production of cowpea (5,589,216 metric tonnes) in 2014. Globally, Nigeria has been rated as the largest consumer of cowpea (Langyintuo *et al.*, 2003) and this may be connected with its relative affordability and high nutritional value. Due to high quality of protein in cowpea, companies are harnessing its nutritional benefits in industrial food formulation (Hamid *et al.*, 2016). Clinical studies have also documented the relevance of cowpea and other legumes in the reduction of cholesterol and the risks associated with coronary heart diseases (Anderson and Major, 2002; Bouchenak and Lamri-Senhadji, 2013).

The aim of this study was to assess the toxic effects imposed on cowpea grown on SEO contaminated soil and its implication on consumer's health while the specific objectives were to determine the:

- impact of SEO on growth parameters of cowpea
- heavy metal load in cowpea seed and root grown on SEO contaminated soil.
- health risk associated with ingestion of cowpea planted on SEO polluted soil.

The study justification is that in Nigeria, disposing SEO is a serious environmental issue due to inadequacy of waste disposal and recycling facilities. Most generators end up disposing it in water drain systems, rivers, open vacant plots and agricultural lands thus, rendering such lands unsuitable for agricultural practices (Anoliefo and Vwioko, 2001). In cases where such polluted lands are not remediated and farmers plant crops on them, consumers of such crops may stand the risk of ingesting pollutants released into the soil via translocation (Baker *et al.*, 2000). It is against these backdrops that this study is targeted at establishing the dangers associated with consumption of crops grown on such contaminated soils.

We hypothesize that:

- (1) SEO contaminated soil has no significant toxic effect on cowpea planted on it and
- (2) Consumption of cowpea grown on spent engine oil contaminated soil has no significant implication on health.

## MATERIALS AND METHODS

Top soil obtained from agricultural research farm of University of Ibadan was sieved using 2 by 2 mm sieve. SEO from Maintenance Department, University of Ibadan were pooled in a container and thoroughly mixed to give a homogeneous sample. The soil and SEO were assessed for baseline heavy metals. The artificially contaminated soil

was put to a weight of 350g in planting pots for 7 treatments and a control. Each group (treatment and control) was made in 5 replications making a total of 40 pots. Each treatment group was mixed with varying amount of SEO in the order: 0ml (T0), 5ml (T1), 10ml (T2), 15ml (T3), 20ml (T4), 25ml (T5), 50ml (T6) and 75ml (T7) respectively after which soil pH was measured using pH meter.

With the experiment being maintained in a screen house, three cowpea seeds were planted in each pot. Growth parameters such as plant height measured with a meter rule and number of leaves counted visually were monitored weekly for ten weeks. At the end of 10<sup>th</sup> week, the experiment was terminated and each plant was carefully removed from the pot, gently washed under running tap and separated into roots, stem and seed respectively. Harvested samples were immediately oven dried, and milled into powdery form in preparation for digestion and heavy metal analysis. Both root and seed samples for each group were digested separately after which the digested samples were determined for Pb, Cd and Ni on Atomic Absorption Spectrophotometer (AAS). Associated health risk was assessed using hazard quotient (US Environmental Protection Agency (US EPA) 1989). Data generated were analyzed using Statistical Package for Social Sciences (SPSS) version 22.0. Hazard quotient was calculated using the formula:

$$HQ = \frac{(D) \times (C_{\text{metal}})}{Rf D \times BO} \quad (1)$$

Where

D = daily intake of food (Cowpea) (kg/day) taken as 0.1kg/day (Marinangeli *et al*, 2017)

C<sub>metal</sub> = concentration of metal (mg/kg)

Rf D = reference oral dose of metal (Cadmium) (mg/kg of body weight/day), taken as 0.001 (US EPA, 2000)

BO = Average body weight (kg) taken as 55kg (ICMR, 2010)

An index value < 1 is assumed to be safe while above 1 is assumed to be unsafe.

## RESULTS

### BASELINE HEAVY METAL LOAD IN SOIL AND OIL

Table 1 presents the baseline heavy metal content of soil and SEO respectively. Both SEO and soil were analysed for Pb, Cd and Ni and it was observed that lead and cadmium were present only in the oil at 0.003 ppm and 0.462 ppm respectively while Ni was absent in both soil and SEO.

**Table 1:** Baseline heavy metal load in soil and SEO

Samples	Pb	Ni	Cd
Soil	0.000 ± 0.000 mg/kg	0.000 ± 0.000 mg/kg	0.000 ± 0.000 mg/kg
SEO	0.003 ± 0.0001 mg/L	0.000 ± 0.000 mg/L	0.462 ± 0.0001 mg/L

*Values are means of 3 replicates*

### SOIL pH

The results of soil pH after application of SEO is presented in Table 2 below. The results reveal that SEO contaminated soil samples across all treatments had pH ranging between 4.7 and 5.0

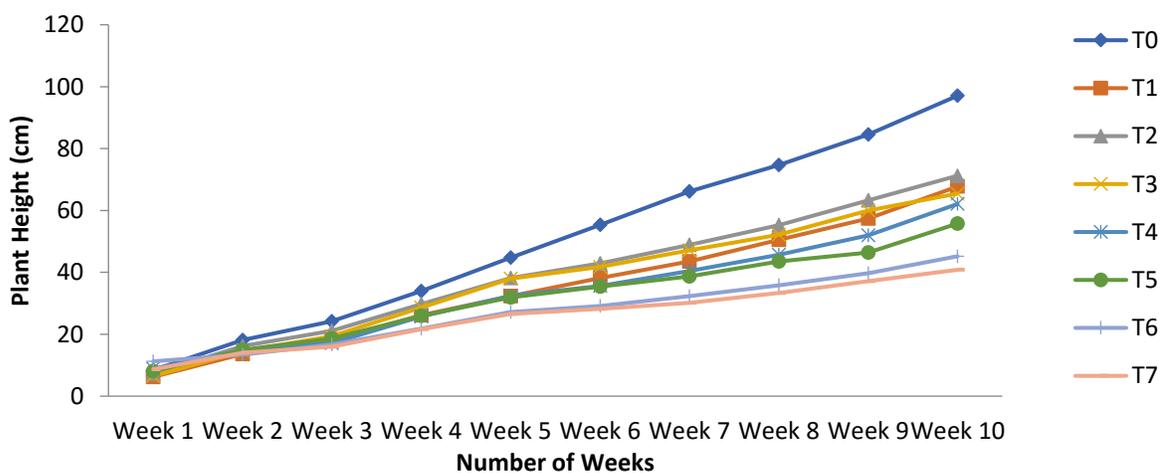
**Table 2:** Average pH values of SEO contaminated soil samples before planting

Treatments	Mean pH $\pm$ SD
T0	4.73 $\pm$ 0.12
T1	4.70 $\pm$ 0.10
T2	4.97 $\pm$ 0.21
T3	4.70 $\pm$ 0.10
T4	4.77 $\pm$ 0.15
T5	5.00 $\pm$ 0.10
T6	4.80 $\pm$ 0.17
T7	4.70 $\pm$ 0.00

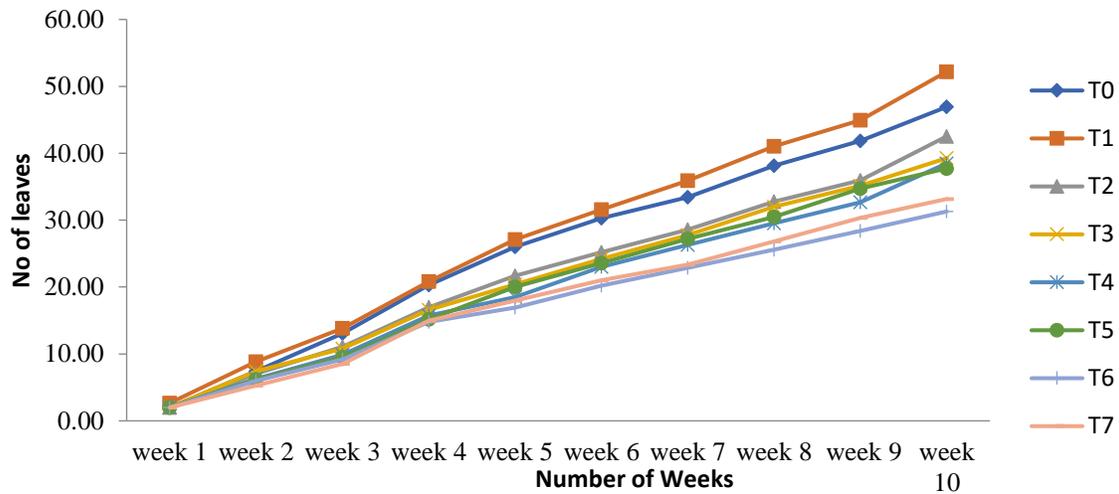
*Values are means of 3 replicates.*

### PLANT GROWTH PARAMETERS

Figures 1 and 2 show the growth pattern of cowpea on SEO contaminated soil during the period of ten weeks. There was a decreasing growth in plant height and number of leaves from T0 to T7 from 1st week to 10th week. For instance, at the 3rd week, average height of T0 (control) was 24.2 cm  $\pm$  2.21, T6 (50 ml) was 16.6 cm  $\pm$  1.39 while T7 (75ml) attained 14.75 cm  $\pm$  2.18. At 10<sup>th</sup> week, T0 reached 97.06cm  $\pm$  5.78; T6 reached 45.2cm  $\pm$  2.28 while T7 reached 39.75 cm  $\pm$  2.57. Similarly, at 3rd and 10th week, average number of leaves on T0 was 13.06  $\pm$  1.34 and 46.96  $\pm$  6.73; T6 was 9.2  $\pm$  1.64 and 31.3  $\pm$  2.64 while T7 was 8  $\pm$  0.87 and 32.75  $\pm$  1.89 respectively.



**Figure 1:** Plant height against number of weeks



**Figure 2:** Number of leaves against number of weeks

### HEAVY METAL LOAD IN COWPEA SEED AND ROOT

There was a significant difference ( $P < 0.001$ ) between Cd load both in seed and root across T0 and other treatment groups (Table 3). For instance, in the root, average Cd load for T0 was 0.000 mg/kg; T3 was 0.0787 mg/kg while T7 was 0.2633 mg/kg. Similarly, in the seed, Cd load for T0 was 0.000 mg/kg, T3 was 0.0557 mg/kg and T7 was 0.1193 mg/kg. Pb was found to be absent both in seed and root across all treatment groups.

**Table 3:** Heavy metal load in cowpea seed and root

Treatment	Cadmium in root (mg/kg)	P-value	Cadmium in seed (mg/kg)	P-value	Lead in root (mg/kg)	Lead in seed (mg/kg)
T0	0.000 ± 0.000	Ref	0.000 ± 0.000	Ref	N.d	N.d
T1	0.057 ± 0.009	<0.001	0.046 ± 0.015	<0.001	N.d	N.d
T2	0.070 ± 0.015	<0.001	0.057 ± 0.011	<0.001	N.d	N.d
T3	0.079 ± 0.003	<0.001	0.056 ± 0.007	<0.001	N.d	N.d
T4	0.091 ± 0.005	<0.001	0.077 ± 0.008	<0.001	N.d	N.d
T5	0.113 ± 0.030	<0.001	0.081 ± 0.003	<0.001	N.d	N.d
T6	0.163 ± 0.013	<0.001	0.102 ± 0.014	<0.001	N.d	N.d
T7	0.263 ± 0.016	<0.001	0.119 ± 0.026	<0.001	N.d	N.d

Values are means of 3 replicates.

N.d = Not detected

### RELATIONSHIP BETWEEN GROWTH PARAMETERS OF COWPEA AND HEAVY METAL LOAD

Table 4 shows a significant positive linear correlation between plant height and number of leaves ( $r=0.886$ ,  $P < 0.001$ ), Cd in root and Cd in seed ( $r=0.915$ ,  $P < 0.001$ ), seed weight and number of leaves ( $r=0.507$ ,  $P < 0.001$ ) while an inverse

relationship was observed in Cd in seed and seed weight ( $r = -0.420$ ,  $P < 0.001$ ) and Cd in seed and number of leaves ( $r = -0.411$ ,  $P < 0.001$ )

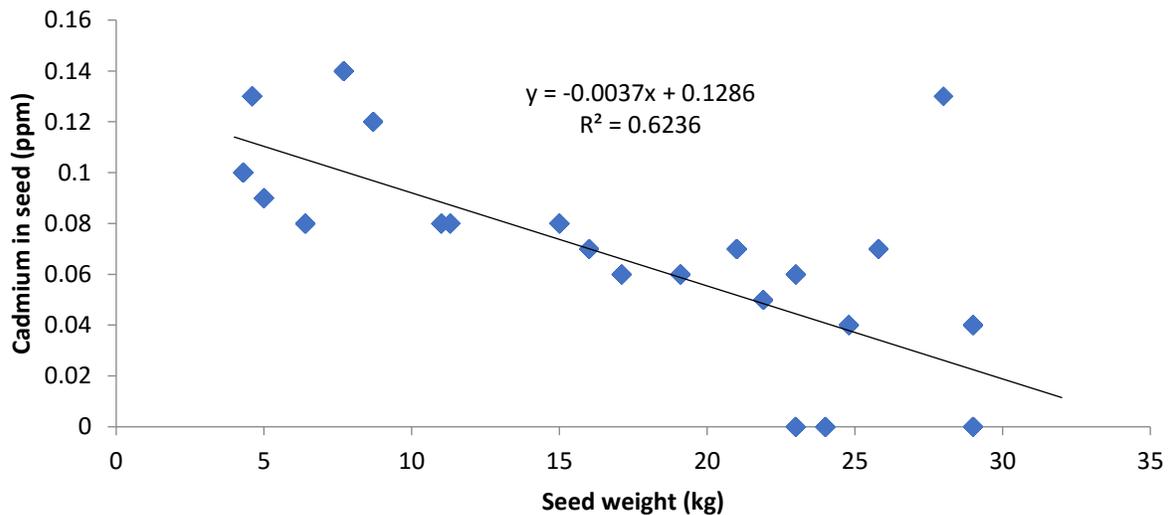
**Table 4:** Relationship between growth parameters and uptake of heavy metals

	Plant Height	No of leaves	Seed Weight	Cadmium in Seed	Cadmium in root
Plant Height	1				
No of leaves	0.886 **	1			
Plant Weight	0.102		1		
Seed Weight	0.080	0.507 **		1	
Cadmium in Seed	-0.225	-0.411 **	-0.420 **		1
Cadmium in root	-0.116	-0.048	-0.305 **	0.915 **	

\*\* Correlation is significant at  $P < 0.005$ .

### RELATIONSHIP BETWEEN CADMIUM IN SEED AND SEED WEIGHT

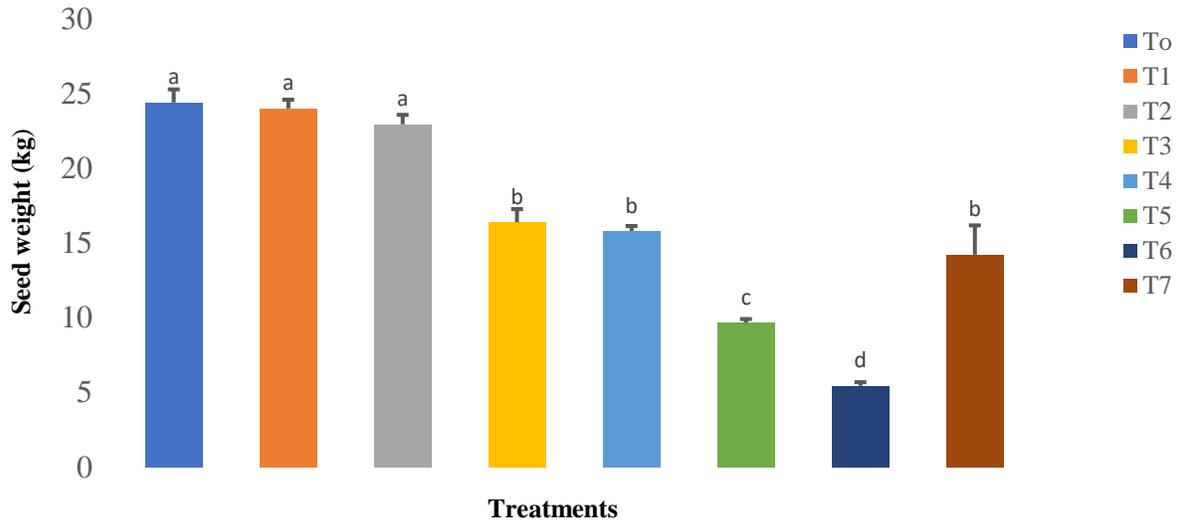
A scatter plot of Cd in seed against seed weight is illustrated in figure 3 with correlation coefficient ( $R^2$ ) of 0.6236. The relationship depicts an average negative linear relationship between the concentration of Cd in seed and seed weight.



**Figure 3:** Relationship between Cd in seed and seed weight

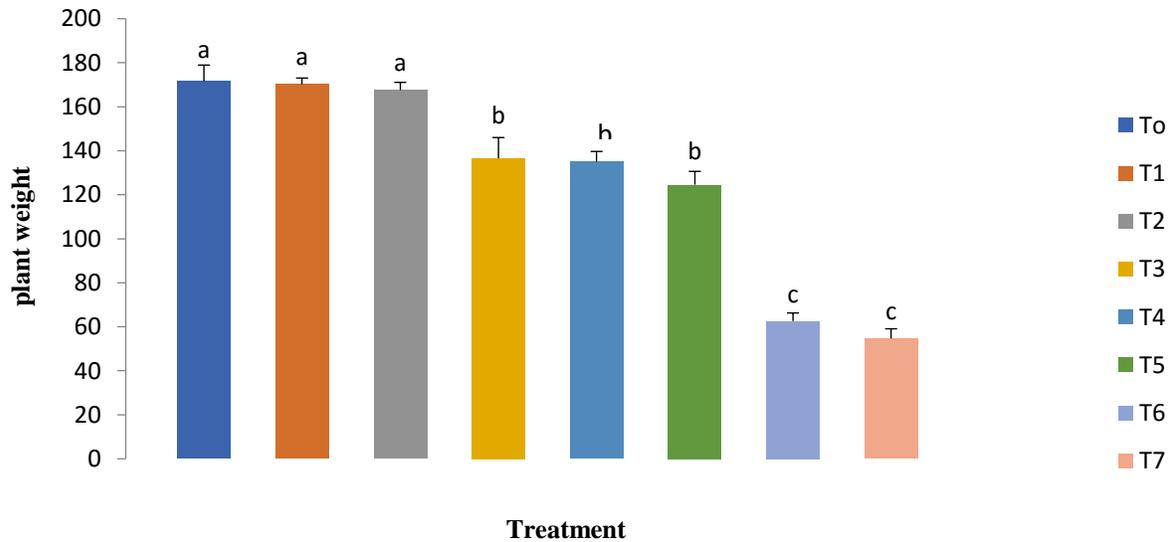
## SEED WEIGHT AND TOTAL PLANT WEIGHT

Weights of seed and total plant across all treatment groups are presented in Figures 4 and 5. The seed weights for T3 ( $16.38 \pm 6.27$  kg), T5 ( $9.66 \pm 1.76$  kg), T6 ( $5.44 \pm 1.7$  kg) and T7 ( $14.21 \pm 6.74$  kg) were observed to be significantly lower compared to controls ( $24.40 \pm 4.21$  kg),  $p < 0.001$ . Similarly, the plant weights for T3 ( $136.60 \pm 66.41$  kg), T5 ( $124.58 \pm 42.80$  kg), T6 ( $62.60 \pm 15.89$  kg) and T7 ( $54.67 \pm 14.20$  kg) were significantly lower compared to control ( $171.60 \pm 51.78$  kg),  $p < 0.001$ .



**Figure 4:** Seed weight across Treatment groups

*\*Means with the same letters are not significantly different from each other*



**Figure 5:** Plant weight across Treatment groups

*\*Means with the same letters are not significantly different from each other*

## HEALTH RISK ASSESSMENT

Using daily intake of cowpea as 0.1 kg/day, reference oral dose as 0.001 mg/kg/day, average body weight as 55 kg for various concentrations of Cd obtained in the study, hazard quotient increased with increasing concentrations of Cd albeit, they all existed far below the danger level (Table 5).

**Table 5:** Hazard quotient values

Treatments	Hazard quotient	Remark
T0	0.000	
T1	0.084	
T2	0.104	
T3	0.102	Hazard quotient is less than 1 in all treatments.
T4	0.140	
T5	0.147	
T6	0.185	
T7	0.216	

## DISCUSSION

The absence of heavy metals from the natural soil may not be unconnected with the fact that the location where the soil samples were taken has not been involved in prior industrial or vehicular activities. However, the presence of Cd in the SEO may indicate the possibility of the heavy metals being picked from the circulation of oil round the engines (Whisman *et al.*, 1974; Wang *et al.*, 2000). This is in line with Ahamad *et al.* (2015) who reported presence of lead oxide, detergents and trace metals in SEO. Cheng (2003) reported that soil pH is the major factor affecting the mobility and bioavailability of heavy metals to plants. Thus, in the present study, soil used in the experiment presented a favourable pH condition for the movement of heavy metals from the soil to cowpea plant as indicated by the pH values of all treatments in acidic region.

A decreasing growth pattern in plant height and number of leaves from T0 to T7 in this study is an indication that plant growth may be significantly impaired by SEO. In a related study, Olayinka and Arinde (2012) reported a significant reduction in plant height and number of leaves of groundnut in order of increasing SEO. Similarly, Kayode *et al.* (2009) reported that both *Zea mays* and *Vigna unguiculata* grew better in non-polluted soil while Vwioko and Fashem (2005) reported growth retardation in *Vigna* seedlings planted on SEO.

The presence of Cd in both seed and root of cowpea plant in this study is an indication of possible translocation of soil contaminants into various plant parts. In this study, Cd increased in both seed and root with increasing concentration of SEO. The inability of Pb to be detected in the seed and root may be due to very small quantity (0.003 mg/kg) initially present in the soil. In related studies, Adekunle *et al.* (2018) reported bioaccumulation of Pb and Cd in *Amaranthus Cruentus* L., grown on heavy metal polluted soil, while Nwite and Alu (2015) reported increased uptake of Pb by maize grains cultivated on SEO and Agbogidi *et al.* (2007) reported increasing buildup of

heavy metals in *Zea mays* with increasing crude oil contamination. Mohammed and Asem (2018) also demonstrated translocation potential of heavy metals from contaminated soil to various plant areas in the order: roots > shoots > fruits which is consistent with results recorded in this present study with the order: roots > seed.

The positive correlation between plant height and number of leaves in this study shows the dependency between the two while positive correlation between Cd in seed and root may reflect translocation to seed based on the quantity in the root. Conversely, an inverse relationship between Cd in seed and seed weight may reflect the impact of heavy metal on yield. A significant difference between seed and plant weights across treatments and overall decline in seed and total plant weight recorded in this study may not be unconnected with the unfavorable conditions imposed on plant environment by the SEO. Okonokhua *et al.* (2007) noted a significant decrease ( $p < 0.05$ ) in grain yield planted on polluted soil in order consistent with the level of contamination. Such yield decline was also reported by Wang *et al.* (2002) who recorded a dry matter yield decrease to about 68%.

### **IMPLICATIONS ON HEALTH**

Ingestion of heavy metals through consumption of contaminated crops may constitute health risk to consumers of such crops particularly if accumulated to elevated concentration (Gupta and Gupta, 1998). According to Food and Agriculture Organization (2017), the maximum intake limits of 0.3 and 0.2 mg/kg for Pb and Cd were recommended in green leafy vegetables by Committee of food additives and contaminants. The concentrations of cadmium recorded in the seeds of cowpea used in this study were below the limit set by the committee. Similarly, hazard quotients obtained across all treatments were less than 1 thus, adverse effects are unlikely via ingestion of cowpea used in this study. However, planting on heavily polluted soil may pose serious danger of higher accumulation as exemplified in the increasing hazard quotient with increasing concentration of SEO. Continuous intake of crops grown on polluted soils even with low metal concentrations may biomagnify in human systems (Pinto *et al.*, 2003; Patra *et al.*, 2011; Carneiro *et al.*, 2018) and this may cause serious health problems.

### **CONCLUSION**

SEO affected the growth of cowpea cultivated on contaminated soil by causing a significant reduction in plant growth and yield as well as contaminating the plants with heavy metals. Hazard quotient increased with increased contamination hence, the need for integrated health risk assessment for inhabitants of areas polluted with such contaminants.

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