

RESEARCH ARTICLE

Impact of Crude Oil Contamination on Green Leafy Vegetables: Nutritional and Health Risk Assessments

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ABSTRACT

For decades, crude oil spills have been a serious environmental challenge which have led to the establishment of crude oil remediation intervention values (CRIV) by different national agencies to regulate the release of toxic petroleum hydrocarbons via these spills. To ascertain the suitability of these CRIV in crude oil-polluted agricultural sites, the impact of five CRIV on the nutritional composition of two green leafy vegetables (GLV) and associated human health risk was investigated using standard analytical procedures. Briefly, high-performance liquid chromatography was used in determining the composition of the sugars while inductively coupled plasma mass spectrometer and atomic absorption spectrometer were utilized in quantifying the levels of minerals in the harvested samples. This was followed by human health risk assessment based on the evaluation of non-carcinogenic and carcinogenic risks. CRIV at 3000 and 5000 mg/kg total petroleum hydrocarbons (TPH) reduced the fructose and glucose contents of *Lactuca sativa* whereas the sucrose level was enhanced at 10,000 mg/kg TPH. All the tested CRIV had no statistically significant effect ($p > 0.05$) on the Na/K ratio of *Brassica oleracea* as well as the F/G and Ca/P ratios of *L. sativa*. Among the studied nonessential heavy metals, only the samples' Pb contents were below the FAO/WHO Codex maximum level. Multivariate statistical analysis indicated that the 10,000 mg/kg TPH treatment GLV samples' nutrients were the most impacted by the crude oil stress which also primarily had the highest non-carcinogenic and cancer risk levels. The investigation revealed the potential of some crude oil concentrations in agricultural sites to alter the physiology of GLV and their nutritional composition. Consequently, use of CRIV at TPH concentration of ≤ 3000 mg/kg is recommended to protect the quality of GLV from such sites and to safeguard against possible cancer risk.

1 | Introduction

Environmental pollution of agricultural lands with petroleum hydrocarbons (PH), for example, from crude oil, is an environmental challenge, particularly in countries that are directly involved in petroleum exploration and refining operations (Ahmad et al. 2021). The permissible level of these hydrocarbons in polluted soil differs by country (Abdullah et al. 2020) who have developed intervention and/or target values in several national regulations to mitigate PH releases into the environment. These intervention values, for instance, are concentrations at which the presence of PH in the environment would put the general ecosystem at a high degree of risk with levels

above these values indicating contamination warranting remediation (Odukoya et al. 2019a; Pinedo et al. 2014).

Introduction of PH into the soil environment has been noted to alter its physicochemical characteristics, resulting to reduction in oxygen, water, and some essential nutrient levels (Haider et al. 2021). These changes in the soil environment affect plants with recorded negative effects on seed development, growth of the plant, leaf production, and photosynthetic activity (Abdullah et al. 2020; Haider et al. 2021; Khan et al. 2018). PH also have different deleterious effects when they enter the food chain as some of them are recalcitrant/persistent (Abdullah et al. 2020).

Furthermore, Diyaolu et al. (2021) identified crude oil spills as one of the ways in which heavy metals become introduced into the terrestrial environment. Although these toxic metals have different absorption pathways, the food chain has been indicated as a major route of human exposure to them (Aiyesanmi et al. 2020). Previous research works have also revealed that abiotic environmental stress factors, which include contamination from crude oil/PH (Nie et al. 2010; Odukoya et al. 2019b), can affect the quality of vegetables (Odukoya et al. 2019a; Kumar et al. 2014) which through the soil-root pathway may take up heavy metals that are accumulated at elevated concentrations in their edible as well as nonedible parts (Aiyesanmi et al. 2020; Liu et al. 2013; Rehman et al. 2017; Zhou et al. 2016).

As the world's predicted additional population of about 2.3 billion people by 2050 demands the need to boost agricultural production (Ahmad et al. 2021) and ensure foods from crude oil spills-prone regions are also safe for human consumption, this study was aimed at determining the effects of selected crude oil remediation intervention values (CRIV) on the nutrient contents of two green leafy vegetables (GLV) as well as the potential risk their mineral levels pose to human health. This investigation further becomes pertinent as: (a) most studies on the effect of PH on plants have been on growth and yield (Odukoya et al. 2019a), (b) the impact of several countries' established cleanup standards, involving TPH intervention/target values, on crop quality is not clearly understood (Odukoya et al. 2019a), (c) plant adaptation to environmental abiotic stresses may bring about accumulation of compatible solutes (Chen and Murata 2002; Odukoya 2015), and (d) heavy metals contamination has been noted to affect food quality and safety (Rehman et al. 2017; Hu et al. 2017; Chen et al. 2018; Ghasemidehkordi et al. 2018). The three tested research hypotheses are:

- i. Agricultural produce from crude oil-contaminated environment have reduced nutritional value,
- ii. Crops from crude oil-contaminated environment have harmful levels of heavy metals, and
- iii. Consumption of crops from crude oil-contaminated environment will give rise to carcinogenic effects.

2 | Materials and Methods

2.1 | Experimental Set-Up and Samples Preparation

Pot treatments containing the appropriate amount of crude oil to achieve the desired CRIV treatments of 0 (control), 1500, 3000, 5000, or 10000 mg/kg TPH were placed in Cranfield University's research greenhouse facility. The greenhouse experiment was for 60 d with an average temperature and relative humidity of 24°C and 58.21%, respectively. Seeds of kale, *Brassica oleracea* (L.), and lettuce, *Lactuca sativa* (L.), were sown in the pot treatments and, after 10 d of planting, thinning was carried out to reduce the number of plants in the pots to one.

To determine the impact of the crude oil stress, the harvested samples were first thoroughly washed with water followed by snap-freezing with liquid nitrogen. They were then immediately

freeze-dried, ground to powder, and stored at -40°C prior to analyses. Figure 1 shows the growth of the GLV samples in the different pot treatments at the research greenhouse facility.

2.2 | Evaluation of Nonstructural Carbohydrates (Sugars) Content

To estimate the sugar levels in the harvested samples, the modified methods described in Odukoya et al. (2019a) were utilized. Briefly, this involved adding a 3 mL mixture of methanol and water (62:37 v/v) into a 7 mL vial containing 150 mg of the samples to be examined. The vials containing the samples were then agitated using a shaking water bath set at a temperature of 55°C followed by cooling and filtration.

The chromatographic separation involved the use of high-performance liquid chromatography (HPLC) grade water and acetonitrile as the mobile phase. Eventual quantification of the sugars concentration in the sample was then achieved using an Agilent Technologies HPLC system and an evaporative light scattering detector.

2.3 | Evaluation of Crude Protein Content

To determine the crude protein composition of the harvested GLV samples, the total nitrogen level was first assessed using the Dumas method. Thereafter, appropriate steps, as indicated in Mariotti et al. (2008), were followed to express the Dumas nitrogen (DN) content as an estimation of the protein level.

2.4 | Evaluation of Minerals Content

For the trace elements analysis, the World Health Organization (WHO 1996) classification into essential, probably essential, and potentially toxic trace elements was employed. Based on their function in biological systems, Aiyesanmi et al. (2020) noted that arsenic (As), cadmium (Cd), and lead (Pb) also belong to a class of non-essential heavy metals. Five mL of nitric acid and peroxide solution were used for the sample digestion in a fume cupboard before the final digestion. The blank sample and standard reference material were subjected to the same form of treatment.

Thereafter, the levels of all minerals of interest were measured via inductively coupled plasma technology (involving an ICPMS), while potassium (K) and iron (Fe) determinations were made using Perkin-Elmer atomic absorption spectrometer (AAS). Calcium (Ca), phosphorus (P), sodium (Na), and K contents of the harvested GLV samples were used in evaluating the relevant mineral ratios.

2.5 | Health Risk Assessment

Determination of the potential health risk following the ingestion of three potentially toxic trace elements (As, Cd, and Pb) (WHO 1996) in the GLV samples over time was achieved using scientifically established procedures in the literature.



FIGURE 1 | Stages of *Brassica oleracea* and *Lactuca sativa* growth in the greenhouse facility (A = start, B = mid, and C = end of experiment; Y = *Brassica oleracea* and Z = *Lactuca sativa* at the end of experiment). P1–P5 represent CRIV pot treatments at 0 (Control) - 10,000 mg/kg TPH. CRIV, crude oil remediation intervention values; TPH, total petroleum hydrocarbons. [Color figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com)]

2.5.1 | Evaluation of Average Daily Dose (ADD)

The ADD ($\text{mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$), also referred to as chronic daily intake (CDI) by Liu et al. (2013) or daily intake of metals (DIM) by Gupta et al. (2021), was evaluated using the expression of Ametepey et al. (2018) (Equation 1) as well as the input parameters indicated in Odukoya et al. (2021) and Liu et al. (2013) where the ingestion rate was taken as 0.345 kg/day.

$$\text{ADD} = \frac{C \times \text{IR} \times \text{EF} \times \text{ED}}{\text{BW} \times \text{AT}}, \quad (1)$$

where C = Concentration of metal in the harvested sample (mg/kg)

IR = ingestion rate (kg/day)

EF = exposure frequency (day/year)

ED = exposure duration (years)

BW = body weight of consumer (kg)

AT = average time (days).

2.5.2 | Evaluation of Non-carcinogenic Risk (NCR)

The hazard quotient (HQ) values obtained from the expression in Equation (2) was employed in analysing the NCR following the consumption of As, Cd, and Pb in the harvested samples. As pointed out in Ametepey et al. (2018), HQ can be estimated using Equation (2).

$$\text{Hazard quotient (HQ)} = \frac{\text{Average daily dose (ADD)}}{\text{Oral Reference dose (RfD)}}. \quad (2)$$

Table 1 provides details of the oral reference dose (RfD) for the three nonessential heavy metals considered in this study.

According to Lim et al. (2008), $\text{HQ} > 1$ may be indicative of non-carcinogenic effects while the risk becomes acceptable when it is less than unity (Wongsasuluk et al. 2013).

Furthermore, the hazard index (HI) (see Equation 3) is an indication of the potential harm to human health when multiple heavy metals are present (Ametepey et al. 2018). $\text{HI} < 1$ suggests no significant health risk, while $\text{HI} > 1$ and 10.0 reflect

unacceptable non-carcinogenic effects (Wongsasuluk et al. 2013) and serious chronic risks (Li et al. 2018), respectively.

$$HI = HQ_{As} + HQ_{Cd} + HQ_{Pb}. \quad (3)$$

2.5.3 | Evaluation of Carcinogenic Risk (CR)

In line with Lim et al. (2008) and Wongsasuluk et al. (2013), the product of the ADD and the slope factor (SF) (Equation 4) provides an understanding of the carcinogenic/cancer risk, also referred to as Incremental Lifetime Cancer Risk (ILCR) by Sultana et al. (2017).

$$\text{Cancer risk} = \text{ADD}[\text{mg/kg/day}] \times \text{Slope factor} \quad (4)$$

$$[(\text{mg/kg/day})^{-1}].$$

Cancer risk (CR) values $\leq 1 \times 10^{-6}$ signify an acceptable level of risk as these suggest that, with respect to exposure, on average, there is a probability of one person out of one million population to develop cancer (Wongsasuluk et al. 2013; Lim et al. 2008; Alidadi et al. 2019). More specifically, following the US-EPA guidance reported in Qu et al. (2018), CR values $< 10^{-6}$ indicate negligible levels, values within the range of $10^{-6} < CR < 10^{-4}$ have acceptable/tolerable risk levels while CR values $> 10^{-4}$

TABLE 1 | Oral reference dose (RfD) and oral/cancer slope factor (SF) of As, Cd, and Pb.

Potentially toxic trace elements	Oral RfD ^a	Oral/cancer SF ^b
As	0.0003	1.50
Cd	0.001	6.10
Pb	0.0035	ND

Sources: Wongsasuluk et al. (2013), Ametepey et al. (2018), Qu et al. (2018), Odukoya et al. (2021), and Sawut et al. (2018).

Abbreviation: ND, not determined.

^aUnit = mg/kg/day

^bUnit = (mg/kg/day)⁻¹

pinpoint a high cancer risk. With respect to Liu et al. (2013) and Sultana et al. (2017), total/cumulative CR from exposure to multi-carcinogenic heavy metals, in this case from As and Cd (Qu et al. 2018), is expressed as:

$$\text{Total CR} = (\text{ADD}_{As} \times \text{SF}_{As}) + (\text{ADD}_{Cd} \times \text{SF}_{Cd}). \quad (5)$$

2.6 | Statistical Analyses

One-way analysis of variance was used to investigate the impact of the various CRIV on the nutrients content of the GLV and the health risks arising from their heavy metal concentrations. To achieve this, SPSS[®] (version 26) was employed, while JMP Statistical Discovery[™] software (version 15) was used for the multivariate analysis. Where applicable, the results presented are the mean concentration of triplicate determinations.

3 | Results and Discussion

3.1 | Nonstructural Carbohydrates (Sugars) Distribution

For *B. oleracea*, results from the investigation showed that the tested CRIV (1500–10,000 mg/kg TPH) did not affect its total sugar, fructose, and sucrose contents. However, additional increase in the crude oil stress from Treatment 4 (5000 mg/kg TPH) to Treatment 5 (10,000 mg/kg TPH) brought about a reduction in the glucose content (Figure 2A). This decrease in glucose level, in line with Cramer et al. (2011), can be attributed to the impact of abiotic stress factor on sugars. According to these authors, abiotic stress (which in this case was initiated by the crude oil stress) may alter the production, storage, and composition of sugars.

Meanwhile, for *L. sativa*, the concentration of total sugars in the control samples was found to be higher than those present in samples obtained from pot treatments with crude oil contamination in the range of 3000–5000 mg/kg TPH. Interestingly, the

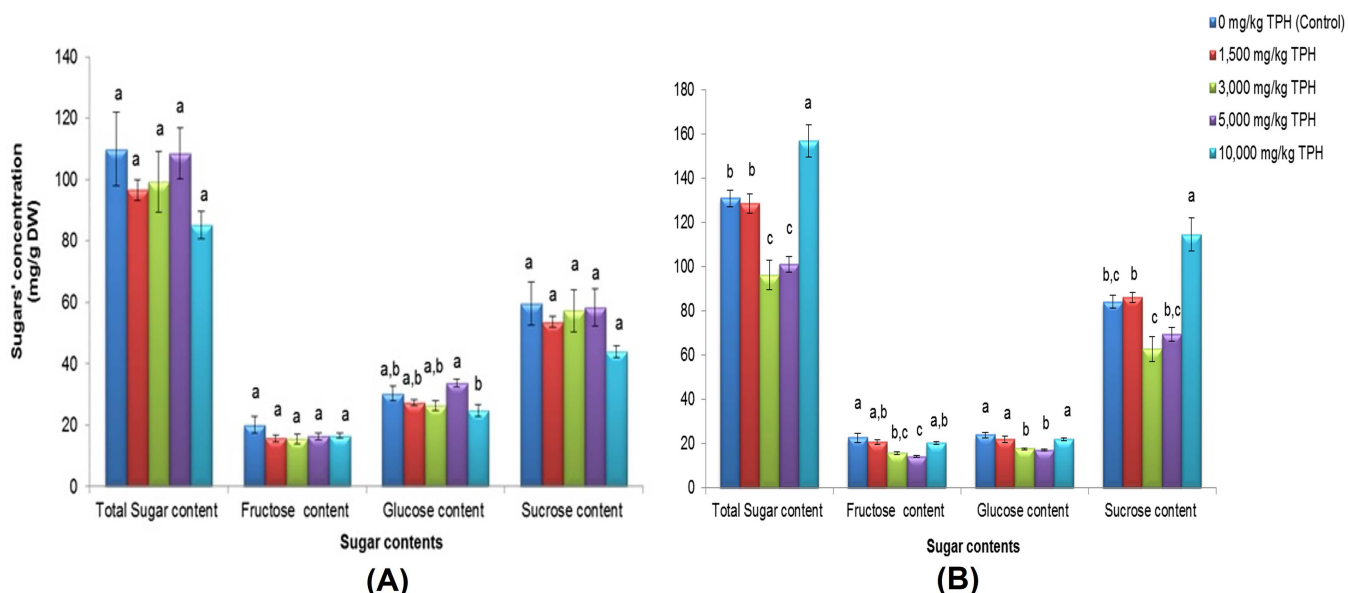


FIGURE 2 | Levels of sugars in the harvested green leafy vegetables (A = *Brassica oleracea*; B = *Lactuca sativa*) obtained at different CRIV. CRIV, crude oil remediation intervention values; TPH, total petroleum hydrocarbons. [Color figure can be viewed at wileyonlinelibrary.com]

highest total sugar level was recorded in samples from treatment with the highest level of crude oil contamination (see Figure 2B). Crude oil stress at 3000–5000 mg/kg TPH led to a reduction in the fructose and sucrose contents of the *L. sativa* samples whereas only the GLV samples from the 10,000 mg/kg TPH treatment had a greater amount of sucrose than the control samples.

As earlier highlighted and in support of Cramer et al. (2011), higher concentration of total sugar, fructose, and glucose recorded in the *L. sativa* control samples than the amount present in samples from pot Treatments 3 and 4, can be attributed to the impact of abiotic stress (Noori et al. 2012), induced by the crude oil contamination, on the synthesis, storage, and composition of these sugars. In support of the view of Wang and Frei (2011), the increase in the sucrose level observed in 10,000 mg/kg TPH treatment samples is an indication of crude oil stress on the plant's physiological status at this CRIV.

Following the report of Muller et al. (2011) and Wingle et al. (2012), the reduced growth of *L. sativa* (reported elsewhere) from treatments containing the highest level of crude oil contamination used in this study (10,000 mg/kg TPH) can be linked to induced soil water deficit which led to carbon accumulation in the harvested samples from this CRIV treatment. This increase in carbon concentration at 10,000 mg/kg TPH according to Muller et al. (2011) may be attributed to organ expansion being affected earlier and more intensively than photosynthesis and metabolism. In addition, the elevated level of sucrose at 10,000 mg/kg TPH suggests that *L. sativa* samples from this treatment experienced water stress as Akinci and Losel (2012) reported that sugars accumulate in plants under water stress condition. This explains the highest total sugar concentration found in *L. sativa* samples harvested from Treatment 5 with the highest level of crude oil contamination in

this study (Figure 2B). Generally, Achuba (2006) considers significant increase in sugar content in plants growing in PH-contaminated soil, with respect to the control samples, as an indication of environmental stress.

3.2 | Crude Protein Contents

The experimental result shown in Figure 3 indicates that the selected GLV samples from Treatment 5 had the highest level of crude protein. There was, however, a statistically significant initial reduction ($p < 0.05$) in crude protein contents of *L. sativa* harvested samples from the 5000 mg/kg TPH treatment before the observed increase at 10,000 mg/kg TPH. This initial decline can be linked to the impact of induced water stress at 5000 mg/kg TPH on the plant metabolism leading to a reduction in protein synthesis (Akinci and Losel 2012), or formation of soluble and insoluble protein-polyphenol complexes arising from the binding affinity of polyphenols for proteins (Papadopoulou and Frazier 2004). Mcmanus et al. (1985) also indicated the ability of polyphenols to associate with proteins.

Furthermore, in line with the report of Achuba (2006), the elevated concentration of crude protein in the two studied GLV at the highest CRIV (10,000 mg/kg TPH) is an indication of induced environmental stress by the crude oil contamination. This view is also supported by the experiment of Al-Hawas et al. (2012) while free proline has been identified by Hare et al. (1998) to accumulate in some plants under environmental stresses, such as water deficit, where it serves as a defense mechanism against osmotic stress by acting as a compatible solute. Rhodes et al. (1999) acknowledged proline accumulation as a common metabolic response of higher plants to water deficit.

Accumulation of proline is also indicated as a response of plants under nutrient deficiency (Delauney and Verma 1993) while

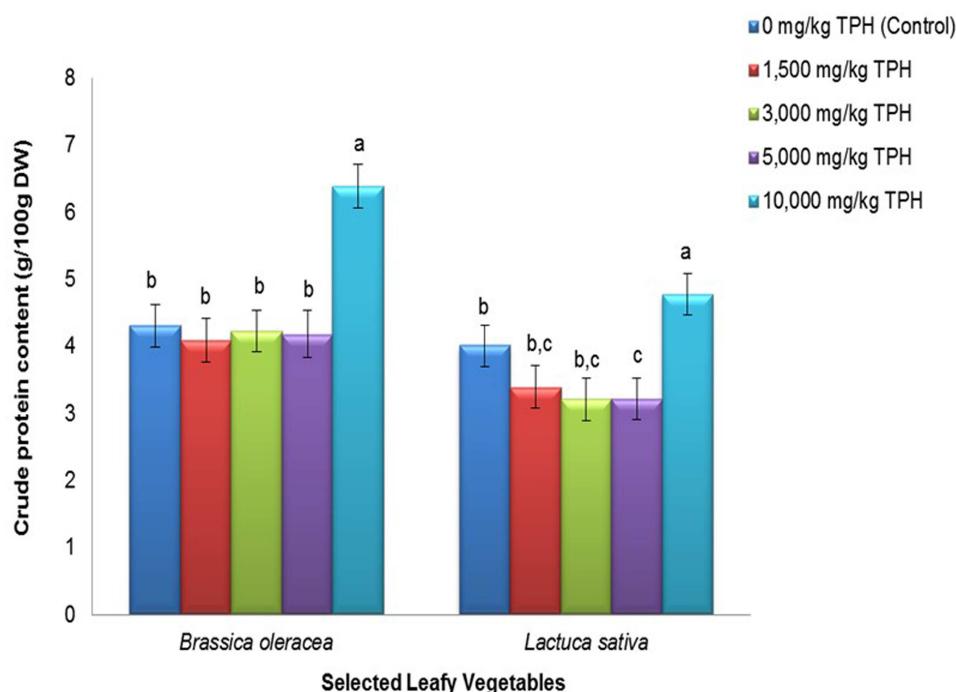


FIGURE 3 | Effect of different CRIV on crude protein content of the harvested green leafy vegetables. CRIV, crude oil remediation intervention values; TPH, total petroleum hydrocarbons. [Color figure can be viewed at wileyonlinelibrary.com]

Smirnoff and Cumbes (1989) as well as Rhodes et al. (1999) noted that proline can be involved in the scavenging of hydroxyl radical in the stressed plants. In view of the anaerobic stress caused by crude oil contamination, Rhodes et al. (1999) as well as Streeter and Thompson (1972) indicated that amino acids, such as alanine and γ -aminobutyric acid (GAB), accumulate in plants under anaerobic conditions. As Pinto et al. (2014) noted the important role of K in protein synthesis, the highest level of K recorded in the two studied GLV at 10,000 mg/kg TPH (see Figure 4A,B), contributed to the observed increased level of crude protein in the GLV samples obtained at this CRIV. This also suggests: (i) the accumulation of some amino acids in these samples following the impact of induced water (osmotic) stress, anaerobic stress, and nutrient deficiency, as well as (ii) more severe effect of crude oil contamination/stress at this CRIV on the dry matter harvest index of the *B. oleracea* and *L. sativa* samples than on their nitrogen harvest index (Wang and Frei 2011; Gooding et al. 2003).

3.3 | Mineral Analysis Results

3.3.1 | Distribution of Major Dietary Minerals

As reported elsewhere in Odukoya et al. (2019a), crude oil stress at the two highest CRIV considered in this study (5000 and 10,000 mg/kg TPH) had a statistically significant reducing effect on the yield of *B. oleracea* compared with that obtained from the control. Hence, the factors responsible for the observed high and significant concentration of Ca and K (Figure 4A) as well as contributing to this response pattern (concentration effect owing to reduced yield) of *B. oleracea* samples at 10,000 mg/kg TPH include: increased transport of these minerals to the aboveground plant part, greater demand for these minerals by the plants, and positive impact on these minerals uptake mechanisms by crude oil contamination at this CRIV (Jarrell and Beverly 1981).

As shown in Figure 4B, crude oil application between 1500–10,000 mg/kg TPH increased the Na contents of the

harvested *L. sativa* samples. The significant reduced yield of samples from CRIV treatment between 3000–10,000 mg/kg TPH reported elsewhere (Odukoya et al. 2019a), led to the observed concentration effect in the treatment samples. This response pattern (*i.e.*, concentration effect), however, was more pronounced in samples from the treatment with the highest level of crude oil contamination, 10,000 mg/kg TPH (with the lowest aboveground plant part fresh weight, see Odukoya et al. [2019a]), as they contain significant higher levels ($p < 0.05$) of Na and K than those found in samples harvested from the control. The enhanced concentration of these minerals at this CRIV is because of the similar factors attributed to the concentration effect in *B. oleracea* samples obtained from the treatment with the highest CRIV (10,000 mg/kg TPH) mentioned earlier. It also supports the report of Martínez-Ballesta et al. (2010) that external conditions (like environmental stress) which alter plant growth to bring about a reduction in growth and biomass at harvest will affect the distribution of mineral nutrient in the crop.

3.3.2 | Distribution of Trace Elements

The effect of CRIV within the experimental range on 13 trace elements (essential: 7, probable essential: 3, and potentially toxic: 3) in the aboveground plant parts of the two GLVs is provided in Table 2. The non-significant effect ($p > 0.05$) of the 5000 mg/kg TPH treatment, despite the reduced aboveground plant part fresh weight of *B. oleracea* at this CRIV, on *B. oleracea* trace elements composition (with the exception of Fe and V contents) in comparison with their amount in the control samples, is a reflection of no direct relationship between crude oil stress at this CRIV and the assessed trace elements (Jarrell and Beverly 1981). The investigation revealed an antagonistic effect (Jarrell and Beverly 1981) of PH within the experimental range used in this study on the Fe and V content of *B. oleracea* as the control samples (with no crude oil contamination) had significantly higher concentrations of these two trace elements. Meanwhile, the factors responsible for the high concentration of

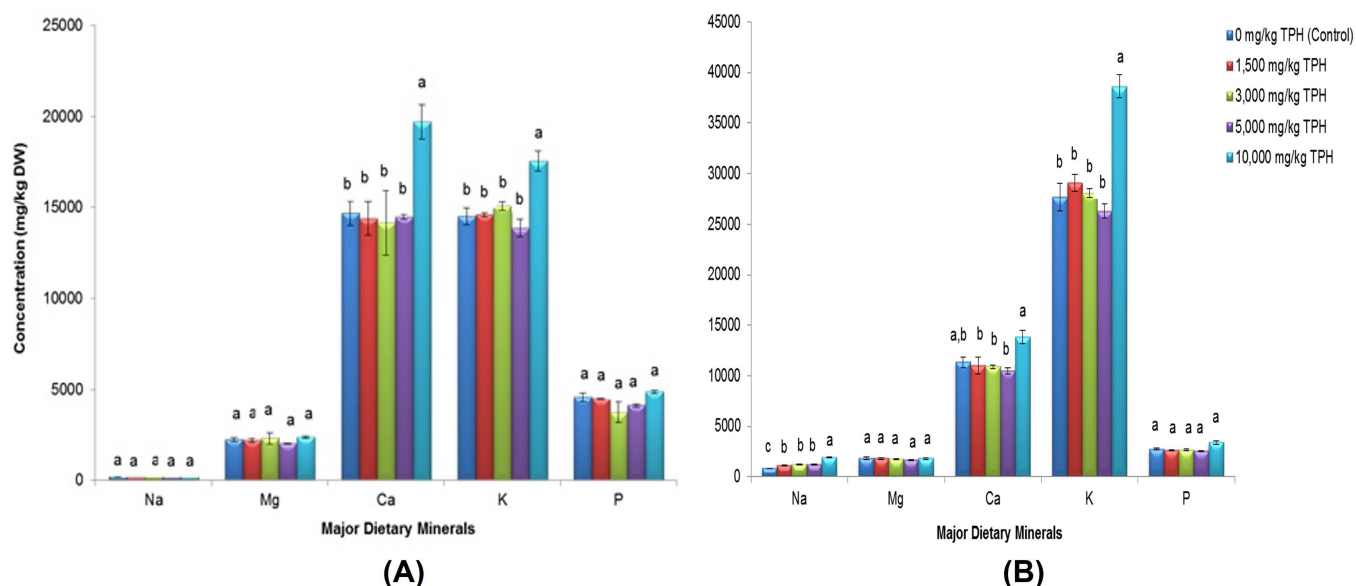


FIGURE 4 | Effect of different CRIV on the major dietary minerals in (A) - *Brassica oleracea*, and (B) - *Lactuca sativa* harvested aboveground plant parts. CRIV, crude oil remediation intervention values; TPH, total petroleum hydrocarbons. [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 2 | Distribution of trace elements (mg/kg DW).

Treatments	Essential					Probable essential			Potentially toxic				
	Cr	Co	Cu	Zn	Mo	Fe	Se	Mn	V	Ni	As	Cd	Pb
<i>Brassica oleracea</i>													
0 mg/kg TPH, Control	1.23 ^a ± 0.36	0.32 ^a ± 0.08	1.79 ^a ± 0.14	19.86 ^a ± 2.69	1.14 ^{ab} ± 0.13	48.10 ^a ± 3.75	0.28 ^a ± 0.02	86.9 ^b ± 12.43	0.06 ± 0.01	< LOD	0.16 ^a ± 0.00	0.18 ^a ± 0.02	< LOD
1500 mg/kg TPH	1.12 ^a ± 0.39	0.24 ^a ± 0.01	1.77 ^a ± 0.15	16.79 ^a ± 0.37	0.91 ^b ± 0.45	27.73 ^b ± 0.96	0.27 ^a ± 0.03	80.73 ^b ± 8.48	< LOD	< LOD	0.16 ^a ± 0.04	0.18 ^a ± 0.01	< LOD
3000 mg/kg TPH	0.71 ^a ± 0.06	0.30 ^a ± 0.10	1.79 ^a ± 0.34	17.55 ^a ± 0.33	1.19 ^{ab} ± 0.04	27.60 ^b ± 0.95	0.32 ^a ± 0.02	92.13 ^b ± 5.67	< LOD	< LOD	0.19 ^a ± 0.06	0.18 ^a ± 0.01	< LOD
5000 mg/kg TPH	0.74 ^a ± 0.07	0.31 ^a ± 0.02	1.86 ^a ± 0.08	17.79 ^a ± 0.72	1.23 ^{ab} ± 0.05	34.70 ^b ± 2.59	0.33 ^a ± 0.06	98.19 ^b ± 9.65	< LOD	< LOD	0.19 ^a ± 0.02	0.18 ^a ± 0.00	< LOD
10,000 mg/kg TPH	0.97 ^a ± 0.08	0.52 ^a ± 0.04	2.13 ^a ± 0.33	21.31 ^a ± 2.51	2.12 ^a ± 0.12	36.87 ^b ± 1.38	0.37 ^a ± 0.02	152.26 ^a ± 7.74	< LOD	1.75 ± 0.36	0.23 ^a ± 0.04	0.19 ^a ± 0.01	< LOD
<i>Lactuca sativa</i>													
0 mg/kg TPH, Control	0.84 ^b ± 0.00	0.09 ^b ± 0.00	0.90 ^a ± 0.14	17.81 ^a ± 0.81	0.03 ^a ± 0.01	94.87 ^b ± 1.62	0.47 ^b ± 0.06	77.33 ^b ± 5.07	0.15 ^b ± 0.03	< LOD	0.09 ^a ± 0.01	0.19 ^{a,b} ± 0.01	0.06 ^a ± 0.01
1500 mg/kg TPH	0.71 ^c ± 0.04	0.10 ^b ± 0.01	0.79 ^a ± 0.12	18.76 ^a ± 1.15	0.01 ^a ± 0.00	72.60 ^b ± 7.78	0.63 ^b ± 0.04	71.39 ^b ± 6.04	0.17 ^b ± 0.02	< LOD	0.13 ^{a,b} ± 0.01	0.21 ^{a,b} ± 0.01	0.04 ^a ± 0.00
3000 mg/kg TPH	0.84 ^b ± 0.04	0.10 ^b ± 0.00	1.03 ^a ± 0.23	18.11 ^a ± 0.55	0.03 ^a ± 0.00	83.80 ^b ± 10.08	0.61 ^b ± 0.03	85.79 ^b ± 4.37	0.19 ^b ± 0.02	< LOD	0.13 ^{a,b} ± 0.01	0.22 ^b ± 0.01	0.05 ^a ± 0.01
5000 mg/kg TPH	1.01 ^b ± 0.03	0.12 ^b ± 0.02	1.29 ^a ± 0.04	17.29 ^a ± 0.38	0.04 ^a ± 0.01	85.07 ^b ± 14.05	0.63 ^b ± 0.04	96.99 ^b ± 9.45	0.19 ^b ± 0.02	< LOD	0.16 ^b ± 0.00	0.22 ^b ± 0.01	0.09 ^a ± 0.01
10,000 mg/kg TPH	1.46 ^a ± 0.09	0.22 ^a ± 0.01	1.67 ^a ± 0.69	17.27 ^a ± 0.58	0.07 ^a ± 0.04	259.13 ^a ± 12.61	1.47 ^a ± 0.06	172.53 ^a ± 3.03	0.49 ^a ± 0.02	< LOD	0.34 ^c ± 0.02	0.17 ^a ± 0.02	0.14 ^a ± 0.02
WHO ^a	5	50	40	60		450		500					
FAO/WHO ^{b,c,d}		50	40	60		450	N/A			68	0.2	0.2	0.3

Abbreviations: FAO, Food and Agriculture Organization; LOD, limit of detection; N/A, not available; TPH, total petroleum hydrocarbons; WHO, World Health Organization.

^{a,b}Odukoya et al. (2021).

^cSihlahla et al. (2019).

^dRahmdel et al. (2018).

Ca and K as discussed in Section 3.3.1 also explains the significant high level of Mn and detectable amount of Ni (Table 2) in *B. oleracea* samples harvested from the treatment having the highest CRIV (10,000 mg/kg TPH).

For the potentially toxic trace elements distribution in the *B. oleracea* samples, the experimental results revealed that there was no statistically significant effect of the tested CRIV on the As and Cd levels (Table 2). Interestingly, they all have acceptable levels of Cd (Alimentarius 2019) and Pb (below the level of detection [LOD]). Among the studied potentially toxic trace elements, only the level of As in samples from treatment with the highest CRIV (10,000 mg/kg TPH) was higher than the EU and WHO/FAO maximum limit of 0.20 mg/kg (Sihlahla et al. 2019).

Practically, the general variations in the *B. oleracea* samples mineral distribution are associated with the reduction in total biomass accumulation of the plant than some nutrients' uptake and/or changes in nutrients assimilation pattern brought about by the influence of crude oil contamination/stress on the plant's carbon assimilation (Piikki et al. 2007). The observed variability in mineral composition within this same vegetable type is also a reflection of the difference in soil and environmental conditions (Szefer and Nriagu 2007; Martinez-Ballesta et al. 2010).

Concentration effect, as earlier discussed, also brought about an increased level of Cr, Co, Fe, Se, Mn, V, and As in *L. sativa* samples obtained from the treatment containing the highest amount (10,000 mg/kg TPH) of crude oil (Table 2). Although the level of Ni in all the harvested *L. sativa* samples was below the LOD, samples from this treatment also had the highest As content which exceeds the EU and WHO/FAO safe limit of 0.20 mg/kg (Sihlahla et al. 2019). Pb contents of all the *L. sativa* samples and Cd content of those from the control and 10,000 mg/kg TPH treatments are below the respective FAO/WHO maximum levels (Alimentarius 2019). As indicated in Samara et al. (1992), higher levels of this latter potentially toxic trace element (Cd) noted in the harvested *L. sativa* samples from the other crude oil containing treatments are still below the maximum permissible concentration (1 mg/kg DW) for vegetables in the human diet.

Generally, for *L. sativa*, in comparison with the control samples, the observed reduced yield at 3000 mg/kg TPH treatment only favored the concentration of Na in GLV samples harvested at this CRIV while the reduced yield at 5000 mg/kg TPH treatment increased the As content. Hence, with the exception of Na and As, and despite the reduced yield recorded, there is no direct correlation (Jarrell and Beverly 1981) between crude oil contamination at CRIV of 3000–5000 mg/kg TPH and most of the analyzed minerals in *L. sativa* harvested samples from these treatments.

3.4 | Relevant Ratios

3.4.1 | Fructose/Glucose (F/G) Ratio

As fructose consumption can induce functional gut symptoms (Barrett and Gibson 2007), Food Intolerance Diagnostics (F.I. Diagnostics, Fructose 2023) suggested that the fructose/glucose (F/G) ratio should be below one because glucose presence in food aids fructose tolerance. Table 3 provides the result of the

TABLE 3 | Result of the assessed ratios.

Treatments	F/G ratio		Ca/P ratio		Na/K ratio	
	<i>B. oleracea</i>	<i>L. sativa</i>	<i>B. oleracea</i>	<i>L. sativa</i>	<i>B. oleracea</i>	<i>L. sativa</i>
0 mg/kg TPH, Control	0.66 ^a ± 0.04	0.95 ^a ± 0.04	3.20 ^b ± 0.09	4.13 ^a ± 0.13	0.02 ^a ± 0.00	0.03 ^a ± 0.00
1500 mg/kg TPH	0.58 ^{a,b} ± 0.04	0.94 ^a ± 0.04	3.21 ^b ± 0.19	4.19 ^a ± 0.32	0.01 ^a ± 0.00	0.04 ^{a,b} ± 0.00
3000 mg/kg TPH	0.59 ^{a,b} ± 0.02	0.89 ^a ± 0.02	3.78 ^{a,b} ± 0.12	4.11 ^a ± 0.12	0.02 ^a ± 0.00	0.04 ^{b,c} ± 0.00
5000 mg/kg TPH	0.48 ^b ± 0.01	0.84 ^a ± 0.01	3.52 ^{a,b} ± 0.07	4.05 ^a ± 0.08	0.02 ^a ± 0.00	0.05 ^{b,c} ± 0.00
10,000 mg/kg TPH	0.66 ^a ± 0.02	0.92 ^a ± 0.03	4.05 ^a ± 0.17	4.07 ^a ± 0.28	0.01 ^a ± 0.00	0.05 ^c ± 0.00

Abbreviations: *B. oleracea*, *Brassica oleracea*; Ca/P, calcium/phosphorus; F/G, fructose/glucose; *L. sativa*, *Lactuca sativa*; Na/K, sodium/potassium; TPH, total petroleum hydrocarbons.

TABLE 4 | Average daily dose (ADD) values results.

Treatment samples	ADD (adult)			ADD (children)		
	As	Cd	Pb	As	Cd	Pb
<i>Brassica oleracea</i>						
0 mg/kg TPH ^a	0.0008	0.0009	0.0000	0.0035	0.0039	0.0000
1500 mg/kg TPH	0.0008	0.0009	0.0000	0.0035	0.0039	0.0000
3000 mg/kg TPH	0.0009	0.0009	0.0000	0.0041	0.0039	0.0000
5000 mg/kg TPH	0.0009	0.0009	0.0000	0.0041	0.0039	0.0000
10,000 mg/kg TPH	0.0011	0.0009	0.0000	0.0050	0.0041	0.0000
<i>Lactuca sativa</i>						
0 mg/kg TPH ^a	0.0004	0.0009	0.0003	0.0019	0.0041	0.0013
1500 mg/kg TPH	0.0006	0.0010	0.0002	0.0028	0.0045	0.0009
3000 mg/kg TPH	0.0006	0.0011	0.0002	0.0028	0.0048	0.0011
5000 mg/kg TPH	0.0008	0.0011	0.0004	0.0035	0.0048	0.0019
10,000 mg/kg TPH	0.0017	0.0008	0.0007	0.0073	0.0037	0.0030

Abbreviation: TPH, total petroleum hydrocarbons.

^aControl.

evaluated F/G ratios of the two GLV (*B. oleracea* and *L. sativa*) samples.

Although all the harvested *B. oleracea* samples have F/G ratios < 1, consumption of samples from the treatment with CRIV of 5000 mg/kg TPH would have the highest tolerance level of fructose as crude oil contamination at this CRIV had a significant reduction ($p < 0.05$) on the F/G ratio of the treatment samples (0.48) compared with that found in the control samples. For the *L. sativa* samples, all the investigated CRIV (1500–10,000 mg/kg TPH) had no significant impact on their F/G ratio (< 1) and, consequently, no effect on their tolerance level of fructose.

3.4.2 | Mineral Ratios

Although the tested CRIV had no statistically significant impact on the Ca/P ratio and Na/K ratio of the *L. sativa* and *B. oleracea* harvested samples, respectively (Table 3), crude oil contamination at the highest CRIV (10,000 mg/kg TPH) elevated the Ca/P ratio of *B. oleracea*. In line with Nieman et al. (1992), this revealed that *B. oleracea* samples from this treatment, compared with those obtained from the control and 1500 mg/kg TPH treatment, will present lower Ca-binding effect of oxalic acid.

On the other hand, increase in Na/K ratio was recorded in *L. sativa* samples subjected to crude oil stress between 3000 and 10,000 mg/kg TPH. Despite this significant rise in Na/K ratio, the experimental results revealed that consumption of any of *B. oleracea* and *L. sativa* samples would not give rise to hypertension (Odukoya et al. 2021; Nieman et al. 1992) as they all exhibited Na/K ratios < 0.6.

3.5 | Health Risk Assessment

The ADD obtained from the application of Equation (1) are shown in Table 4. These values were used in evaluating the HQ and HI for adults and children (Table 5) following the consumption of the two GLV samples from the different crude oil treatments as well as the control. Although the control and Treatment 1 (1500 mg/kg TPH) *B. oleracea* samples had the least HQ values for As, all the HQ values following the consumption of As in the *B. oleracea* samples by adults are greater than one which may be indicative of non-carcinogenic effects (Lim et al. 2008). Similarly, consumption of As and Cd in the harvested *B. oleracea* samples by children with HQ > 1 could also be a sign of non-carcinogenic consequences (Lim et al. 2008). Meanwhile, the Cd and Pb health risk via the consumption of *B. oleracea* samples by adults may be acceptable as

TABLE 5 | Health risk assessment results.

Treatment samples	Risk of non-carcinogenic effect						Cancer risk					
	HQ (Adult)			HQ (Children)			CR (Adult)			CR (Child)		
	As	Cd	Pb	As	Cd	Pb	As	Cd	Pb	As	Cd	Pb
	HI (Adult)	HI (Child)	HI (Adult)	HI (Child)	HI (Adult)	HI (Child)	As	Cd	Pb	As	Cd	Pb
<i>Brassica oleracea</i>												
0 mg/kg TPH ^a	2.629	0.887	0.000	11.520	3.888	0.000	3.517	15.408	0.001	0.005	0.024	0.029
1500 mg/kg TPH	2.629	0.887	0.000	11.520	3.888	0.000	3.517	15.408	0.001	0.005	0.024	0.029
3000 mg/kg TPH	3.122	0.887	0.000	13.680	3.888	0.000	4.010	17.568	0.001	0.006	0.024	0.030
5000 mg/kg TPH	3.122	0.887	0.000	13.680	3.888	0.000	4.010	17.568	0.001	0.006	0.024	0.030
10,000 mg/kg TPH	3.780	0.937	0.000	16.560	4.104	0.000	4.716	20.664	0.002	0.007	0.025	0.032
<i>Lactuca sativa</i>												
0 mg/kg TPH ^a	1.479	0.937	0.085	6.480	4.104	0.370	2.500	10.954	0.001	0.003	0.025	0.028
1500 mg/kg TPH	2.136	1.035	0.056	9.360	4.536	0.247	3.228	14.143	0.001	0.004	0.028	0.032
3000 mg/kg TPH	2.136	1.085	0.070	9.360	4.752	0.309	3.291	14.421	0.001	0.004	0.029	0.033
5000 mg/kg TPH	2.629	1.085	0.127	11.520	4.752	0.555	3.841	16.827	0.001	0.005	0.029	0.034
10,000 mg/kg TPH	5.587	0.838	0.197	24.480	3.672	0.864	6.623	29.016	0.003	0.011	0.022	0.033

Abbreviations: CR, carcinogenic risk; HI, hazard index; HQ, hazard quotient; TCR, total cancer risk; TPH, total petroleum hydrocarbons.

^aControl.

their HQ values are less than unity (Wongsasuluk et al. 2013). This is also the case for the consumption of Pb in the *B. oleracea* samples by children (HQ < 1). For *L. sativa*, all the HQ values following the consumption of As and Cd in the vegetable samples by adults and children, except for the consumption of Cd by adults in the control and Treatment 5 samples, suggest non-carcinogenic effects (HQ > 1) (Lim et al. 2008). Nonetheless, based on the HQ values being less than unity, the risk of the consumption of Pb in all the harvested *L. sativa* samples by adults and children may also be acceptable (Wongsasuluk et al. 2013).

Surprisingly, evaluation of the HI revealed that all the HI values for the GLV for adults were > 1 pinpointing an unacceptable non-carcinogenic effect on health (Ametepey et al. 2018; Wongsasuluk et al. 2013) while those for children being > 10 (with the highest values at 10,000 mg/kg TPH), according to Li et al. (2018), signals serious chronic risks. In addition, all the GLV samples had their total CR values > 10⁻⁴ for adults and children (Table 5) which revealed a high CR (Qu et al. 2018) and the need for intervention (Alidadi et al. 2019). The health risk assessment results further illustrated that GLV samples from treatments containing crude oil, particularly those of CRIV ≥ 3000 mg/kg TPH, had the highest CR. The high HI values (HI > 1 for adults and HI > 10 for children) as well as total CR > 10⁻⁴ (for adults and children) associated with the consumption of the *B. oleracea* and *L. sativa* control samples also suggest the need to analyse soil samples for the presence of these potentially toxic trace elements/nonessential heavy metals (As, Cd, and Pb) when selecting farmlands for growing these vegetables.

3.6 | Multivariate Analysis Results

For the multivariate statistical analysis, exploratory methods—principal components (PCA) and hierarchical cluster analyses (HCA) (Odukoya et al. 2021; Granato et al. 2018)—were used.

3.6.1 | PCA

For *B. oleracea*, two principal components (PCs), PC1 and PC2, describing 54.8% variation in the data were generated (Figure 5A). PC1 revealed 39% of variance while PC2 explained an additional 15.8%. The PCA biplot provided in Figure 5A shows separation of clusters of the control (0 mg/kg TPH) sample composition in Quadrant 1 and those from the treatment containing the highest level of crude oil contamination (10,000 mg/kg TPH) in Quadrant 4. Meanwhile, a close relationship (near the center position) is observed in the clusters of 1500, 3000, and 5000 mg/kg TPH treatment samples' composition. This separation of clusters of the control samples' composition, with no crude oil stress, from others clearly showed the impact of crude oil contamination on the essential nutrients and minerals composition of *B. oleracea* in which composition of samples from the highest CRIV treatment considered in this study was the most affected. The biplot also revealed that the crude protein, Ca, K, Mo, Mn, and Ni contents of *B. oleracea* samples from this treatment (10,000 mg/kg TPH) contributed principally to the observed separation of this treatment sample composition clusters from others.

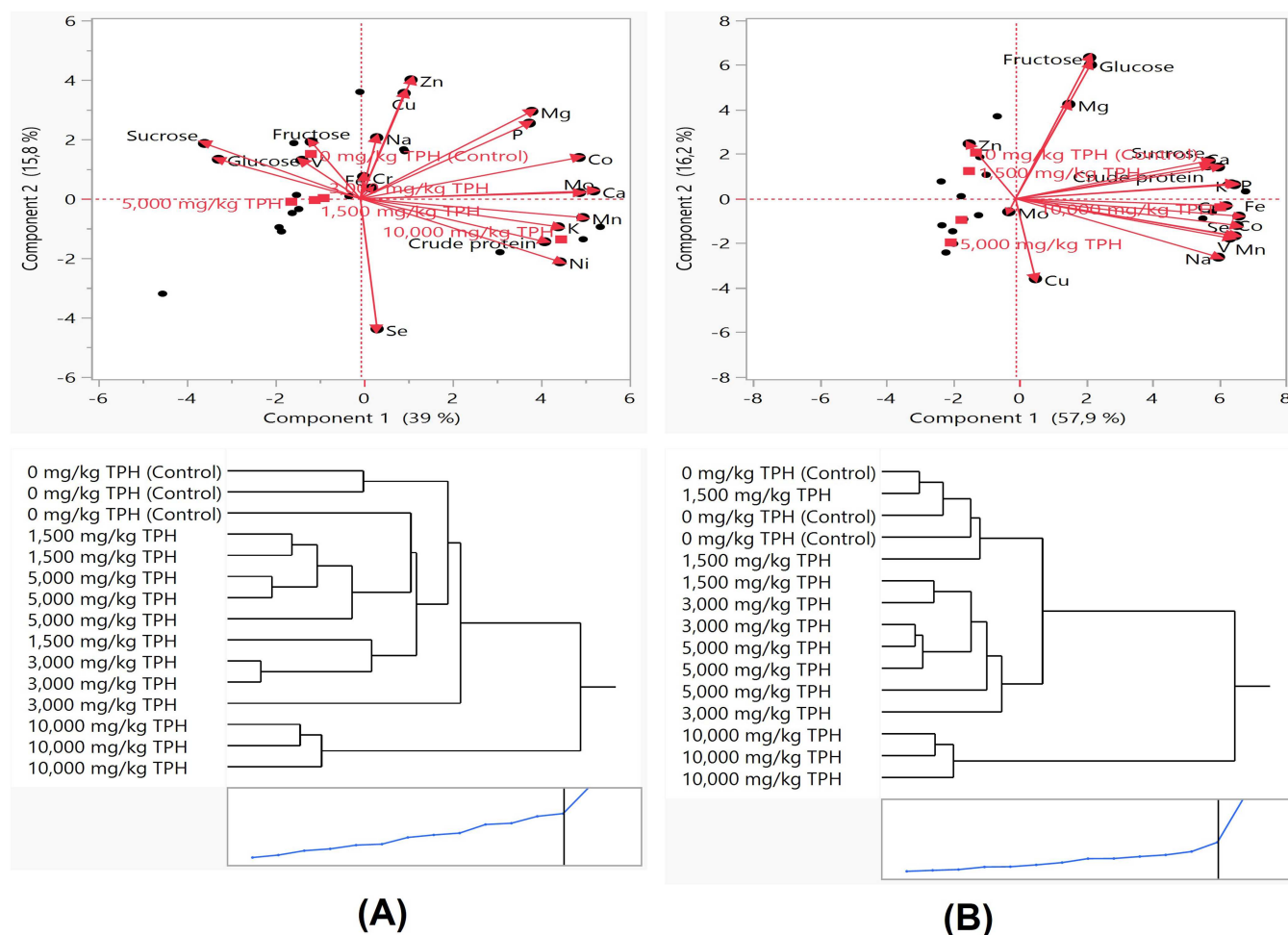


FIGURE 5 | Result of the multivariate analysis of the *Brassica oleracea* (A) and *Lactuca sativa* (B) aboveground plant parts from the different treatments [Top: Biplots of the PCA; Bottom: Dendrograms from the HCA]. HCA, hierarchical cluster analysis; PCA, principal components analysis. [Color figure can be viewed at wileyonlinelibrary.com]

The PCA biplot for *L. sativa*, presented in Figure 4B, shows PC1 and PC2 accounting for 74.1% of the variation. A somewhat similar pattern to *B. oleracea* samples' composition clustering is recorded with clusters of the control (0 mg/kg TPH) and 1500 mg/kg TPH treatment samples composition separated in the first quadrant. As explained earlier, drift of clusters of 10,000 mg/kg TPH treatment samples' composition to the right reflects the influence of crude oil contamination at this CRIV on the essential nutrients and minerals composition of the *L. sativa* samples. As seen in Figure 5B, the sucrose, crude protein, Na, Ca, K, P, Cr, Co, Fe, Se, Mn, and V contents of *L. sativa* samples from the experimental treatment with the highest level of crude oil contamination (10,000 mg/kg TPH) chiefly brought about this drift.

3.6.2 | HCA

HCA of the essential nutrients and minerals composition of the *B. oleracea* and *L. sativa* samples in Figure 5 revealed a similar form of distribution with two main clusters (Cluster-I and II) identified. The composition of samples from the 0 to 5000 mg/kg TPH treatments was grouped in Cluster 1 while that from the highest CRIV (10,000 mg/kg TPH) is separated into Cluster II. To a large extent, this ascertained the significant impact of crude oil

stress at 10,000 mg/kg TPH on the studied essential nutrients and minerals composition of *B. oleracea* and *L. sativa*.

4 | Conclusion

The current study provides an expanded understanding of the impact of different CRIV, as recommended in some national environmental regulations, on the nutritional composition and health risks of two selected GLVs. The investigation revealed that, at some of these CRIV, the level of essential nutrients in agricultural produce may become affected by the crude oil stress. It further shows that there is generally an increase in CR associated with the consumption of GLVs from agricultural sites as the level of crude oil contamination increases.

Hence, governments of regions of the world prone to crude oil spills should be mindful of the impact of the presence of PH in agricultural sites, even at $\leq 10,000$ mg/kg TPH, on agrifood production. Additionally, the research findings indicated the need for careful selection of CRIV and GLV/crops to be grown in crude oil contaminated or remediated farmlands. Levels of bioaccumulated polycyclic aromatic hydrocarbons in crops harvested from crude oil contaminated sites, within the range considered in this study, also warrant further investigation.

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Conflicts of Interest

The authors declare no conflicts of interest.

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