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ORIGINAL RESEARCH ARTICLE

Comparative Evaluation of Heavy Metals Bioconcentrations in Spinach Grown on Cow Dung-Enhanced and NPK-Enhanced Soils

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ABSTRACT

This study compares heavy metal bioconcentrations in spinach (Spinacia oleracea) cultivated in soils enhanced with cow dung and NPK (15:15:15) fertilizer. Soil samples from agricultural fields around Wukari, Nigeria, were analyzed for physicochemical properties, revealing an alkaline pH, moderate organic carbon, and high nitrogen content, which are conducive to plant growth. Spinach plants were grown under controlled greenhouse conditions with different nutrient treatments: cow dung alone, NPK alone, a combination of both, and no enhancement. After a 37-day growth period, heavy metal concentrations, specifically Pb, Fe, Zn, and Cr, in plant roots and shoots were quantified using atomic absorption spectrophotometry and compared against WHO/FAO permissible limits. The result showed significant difference ($P \leq 0.05$) in the heavy metals accumulation in the plant under study indicating that NPK and cow dung amendments influenced heavy metal uptake, with elevated levels of Pb and Zn observed in tissues, particularly in NPK-enhanced spinach, where Pb in NPK-enhanced shoots (0.617 mg/kg) exceeded WHO limits (0.3 mg/kg). BCF for Pb reached 2.0 in cow dung-NPK enhanced soil, suggesting a potential health risk. Bio-concentration and translocation factors revealed spinach's capacity to accumulate Pb and Zn, highlighting the risk of heavy metal transfer through the food chain. The findings underscore the importance of selecting appropriate soil amendments to mitigate heavy metal uptake, recommending integrated nutrient management practices that minimize health risks while sustaining crop productivity. Overall, the study emphasizes the need for ongoing monitoring and sustainable fertilization strategies in vegetable cultivation to ensure food safety.

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INTRODUCTION

Vegetables such as spinach are good sources of vitamins, minerals, and other components that support antioxidant activity in the diet. They also help manage the side effects of diuretics and hypertension (Gupta and Bains, 2006). However, one of the challenges associated with vegetable products is the elevated levels of heavy metals, which may be traceable to soil factors and other cultural practices along the production chain. For instance, NPK-induced soil acidification enhances Zn mobility (Peng et al, 2015); while organic matter amendment of soil reduces heavy metals bioavailability via complexation (Antonangelo et al., 2021). The presence of heavy metals, even at low concentrations, can affect soil fertility by reducing micro-organisms such as nitrogen-fixing bacteria, which are vital for plant growth, and can bio-accumulate in plant tissues (Aransiola et al., 2019).

When different types of heavy metals are present in soil or irrigation water, plants can absorb them. Iron (Fe), copper (Cu), magnesium (Mg), manganese (Mn), and other metals are categorised as necessary macro-minerals for plants. These metals must be present in appropriate amounts; deficiencies or excesses can have harmful consequences and reduce plant productivity. For example, Mn plays a role in splitting water molecules, which is essential for photosynthesis. Chlorosis in plant leaves can be caused by deficiencies or excesses of metals such as magnesium, which also induces oxidative stress (Bhatla et al., 2018). Zinc (Zn) is an essential micronutrient; however, excessive amounts may harm plants and inhibit growth. Zinc deficiency reduces chlorophyll production, leading to chlorosis in leaves (Peng et al., 2015). Conversely, toxic heavy metals like lead (Pb) negatively impact plant growth by affecting leaf and root development, inhibiting enzymatic activities, and decreasing overall yield (Zulfiqar et al., 2019).

Vegetables grown in areas impacted by heavy metals tend to accumulate higher concentrations of these metals near the root zone. Plants absorb nutrients along with heavy metals, which then bio-accumulate in various plant tissues, especially roots and leaves. Numerous studies have shown

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that leafy vegetables such as Amaranthus spp., spinach, lettuce, and cabbage tend to accumulate higher levels of heavy metals compared to root crops like potatoes, onions, garlic, and carrots (Garba, 2021). Therefore, periodic evaluations of heavy metal levels in vegetables are necessary.

Hazardous heavy metals like Cr and Pb pose health risks through oral ingestion, which is a primary exposure pathway for humans (Bansal, 2023). *Spinacia oleracea* (spinach) belongs to the Caryophyllales order and is characterized by a broad surface area, vigorous growth, and a high capacity for heavy metal absorption. Due to these traits, spinach and other Caryophyllales members have been the focus of numerous scientific investigations concerning their growth responses and toxicity levels in contaminated environments (Renu et al., 2021).

Heavy metals in vegetables primarily originate from inorganic and organic fertilizers, with other sources including liming, sewage sludge, and irrigation water applications that can lead to enrichment of metals such as cadmium (Sandeep et al., 2019). Although the levels of heavy metals in agricultural soils are generally low, repeated use of phosphate fertilizers over time can lead to dangerous accumulations.

Since heavy metals are not biodegradable and tend to persist in the environment, they accumulate and pose long-term ecological risks (Ali et al., 2021). They can enter the food chain, and because many are carcinogenic, they may cause neurological and behavioral problems, especially in children. Remediation techniques such as soil flushing, stabilization, vapor extraction, solidification, landfilling, leaching, and soil fixation are vital for removing metal contamination from soils (Liu et al., 2018). Heavy metals can be prevented from entering roots and moving upward via the apoplast and symplast pathways. Xylem loading transports heavy metals from roots to shoots, where they are sequestered in vacuoles. This study aims to assess the extent of heavy metal accumulation in spinach based on two soil nutrient enhancement practices: cow dung and NPK (15:15:15) application.

MATERIALS AND METHODS

Materials and Reagents

Pestle and mortar, funnels, filter paper, sieves, conical flasks, pH meter, volumetric flasks, soil samples, spinach seeds, NPK fertilizer (15:15:15), cow dung, measuring cylinders, weighing balance (PGW 453i), digester (Tecator Digestor 2520 Model), atomic absorption spectrophotometer (Buck Model - Scientific 210VGP). All reagents and chemicals used are of analytical grade and were diluted with deionized water. Reagents employed include hydrochloric acid (HCl) and nitric acid (HNO_3).

Soil Collection

Soil samples were collected from a depth of 0–30 cm from agricultural lands around Federal University Wukari (coordinates: 9.777860°E, 7.871390°N). The soil was homogenized and divided into five experimental pots. Cow dung was obtained from Wukari cattle market, air-dried, and pulverized. NPK (15:15:15), produced by Indorama Eleme Petrochemicals, was purchased from their outlet. Spinach seeds were obtained from the local market in Wukari and identified at the Department of Crop Production and Protection, Federal University Wukari.

Soil Sample Digestion

A 1.0 g soil sample was weighed into a 50 mL beaker, then mixed with 15 mL of HCl and 5 mL of HNO_3 to facilitate digestion. The mixture was transferred to a digester and heated at 150°C for 30 minutes. After cooling, the digest was filtered using Whatman filter paper, and the filtrate was made up to 100 mL with deionized water. The solution was then analyzed using atomic absorption spectrophotometry (Buck Model - Scientific 210VGP) to quantify Pb, Cr, Fe, and Zn concentrations.

Physicochemical Analysis of Soil

The pH was measured by mixing 10 g of air-dried soil (< 2 mm) with 25 mL of distilled water, stirring gently, and letting it stand for 30 minutes. The pH of the supernatant was measured after calibration with buffer solutions at pH 5.5, 7.0, and 8.0.

Organic carbon content was determined after oxidation with potassium dichromate and sulfuric acid, followed by titration. Available phosphorus was measured using a spectrophotometer at 660 nm after extraction with Bray's extractant and coloration with molybdate reagent. Nitrogen was estimated via Kjeldahl digestion and titration.

Exchangeable cations (Ca and Mg) were determined after leaching with ammonium acetate and titration, while Na and K were measured using a flame photometer (Sherwood 410). The soil textural class was determined using the hydrometer method and USDA soil texture triangle (Motsara and Roy, 2008).

Greenhouse Experiment

The experiment was conducted in a greenhouse with five pots, each containing approximately 11 liters of soil, while spinach seed (*Spinacia oleracea*) employed was purchased at the new market Wukari, preserved in wood ash and wrapped in perforated cloth to avoid fungal infection and allow for aeration until planting:

Pot 1:

The first pot, cow dung alone, was applied as nutrient enhancement to the soil after 3 days of planting the spinach seeds. At germination, the plant was labeled cow dung amended spinach (CES)

Pot 2:

The second pot was filled with NPK (15:15:15) alone and was applied as nutrient enhancement after 3 days of planting the spinach seeds. At germination, the plant was labeled NPK-amended spinach (NES).

Pot 3:

The third pot was filled with both cow dung and NPK were applied as nutrient enhancement in the ratio of 1:1, after 3 days of planting the spinach seeds. At germination, the plant was labeled cow dung and NPK-enhanced spinach (CNES)

Pot 4:

The forth pot, spinach alone was planted devoid of both cow dung and NPK. At harvest, the plant was labeled devoid of nutrient-enhanced spinach (DES)

Pot 5:

Control, no planting, but irrigation of the soil was maintained.

Spinach was grown for 37 days, with growth length measured at 7-day intervals before harvesting. Watering was performed twice during the growth period.

Spinach Plant Sampling, Preparations and Analysis

After harvest, spinach plant samples were collected separately from each of the experimental pots labelled: spinach enhanced with cow dung (CES), spinach enhanced with NPK (NES), spinach enhanced with both cow dung and NPK (CNES), and spinach without enhanced (DES). The plant samples were washed through running tap water and rinsed with distilled water. The roots were separated from the shoots in each of the experiments and air dried at room. Each respective sample was pulverized by pestle and mortar and was further air dried until a constant weight was achieved.

(a) NPK amended plant

(b) Cow dung amended plant

(c) Cow dung and NPK amended plant

(d) Devoid of Both cow dung and NPK amended plant

Figure 1: Greenhouse Experiment

The root sample of CES air dried and pulverized was digested with the aid of a hot plate (Tecator Digestor 2520 Model) using 10 mL of H_2O_2 and HNO_3 1:1 to 0.5 g of CES in a glass digestion tube, and the sample was heated to 100 °C for about 15 minutes, then allowed to cool. Followed by an additional 5 mL H_2O_2/HNO_3 1:1 and further heating for another 15 minutes before the digest was allowed to cool, then an additional 2 mL of deionized water, 4 mL of 30% H_2O_2 was added and heated to 100 °C until the volume reduced to approximately 5mL. The digest was cooled, filtered by means of Whatman filter paper and diluted to 50 mL with deionized water. The same procedure was carried out to NES, CNES, DES and their respective shoots. Quantification of total Pb, Cr, Fe and Zn was carried out using AAS (Buck Model-Scientific 210VGP), where standard solutions (one-thousand (1000) ppm of each of the analytes) for calibration were bought from Inorganic Ventures (300 Technology Drive, Christiansburg, VA 24,073, USA), explicitly prepared for Atomic Absorption Spectrophotometric Analysis. From each of these standards, a stock solution of one hundred (100) ppm was prepared by dilution factor. A working standard solution was then prepared using the same dilution factor from the stock solution in line with the linear range of the metal under investigation, which was in turn used to prepare the calibration curve. Blank samples were employed to check and correct the reagents and distilled water background effects, as well as to determine the limit of detection of the instrument. The limits of detection of the Atomic Absorption Spectrophotometer ranged from 0.0050 mg/kg (zinc) to 0.080 mg/kg (iron). Standard procedures were followed to ensure quality control. All the laboratory apparatuses were washed several times and rinsed with deionized water before each usage. Accuracy and precision of the procedures were ensured using the reagent blanks and the triplicate samples preparation (Orosun et al., 2023).

Data analysis

One-way ANOVA followed by Turkey's post hoc was used to compare the mean growth profile of spinach enhanced with cow dung, NPK, both cow dung and NPK, and spinach without enhanced, as well as their heavy metal bioaccumulations using the Statistical Package for Social Science (SPSS), version 20, where the mean difference was considered significant at $p \leq 0.05$ (Garba et al., 2023).

RESULTS AND DISCUSSION

Physicochemical parameters of soil

The degree to which heavy metals affect the biological and biochemical characteristics of the soil is significantly influenced by the soil's organic matter, clay concentration, and pH, as shown in Table 1 (Olaniran et al., 2013).

The pH of the soil collected from farms near Agbungshu Villa Wukari was 6.10. According to USDA categorization, the pH of the soils surrounding Agbungshu Villa was moderately alkaline (7.9 to 8.4) (Motsara and Roy, 2008). The high pH values of the soil indicate that the soil samples have low levels of heavy metals and plant nutrients, including phosphate, iron, zinc, copper, and manganese, that are available for plant uptake. Weathering of parent material rich in calcium carbonate or irrigation with alkaline water can cause high soil pH (Agbaji et al., 2015).

According to USDA classification, the percentage of organic carbon in soil is as follows: less than 0.4% (very low), 0.4–1.0% (low), 1.0–1.5% (moderate), and greater than 1.5% (high). As a result, 1.215% of the soil under study fell within the moderate range of 1.0–1.5%. According to Table 1, the soils' percentage organic matter concentration was 5.36%. According to Yerima et al. (2018), there is a positive correlation between the soils' organic carbon content and their organic matter content.

Soils with a nitrogen level of 1.26% are categorized as high by the U.S. Department of Agriculture (USDA) (> 0.20). The use of nitrogenous fertilizer throughout the growing season is the cause of the high nitrogen concentration in farmland. Similarly, the study of particle size showed that the textural class was primarily sandy loam. Texture affects the soil's ability to retain water and air as well as its fertility (Al-Saedi et al., 2016; Yerima et al., 2023).

The soil under study has a low effective cation exchange capacity (ECEC) of 4.98 Meq/100g, falling within the range of 1.01 to 5.00. This suggests that the soil lacks a sufficient number of negatively charged sites to adhere cations to its surface (Yerima et al., 2023).

Table 1: Physicochemical parameters of soil

S /no	Parameter	Test soil	USDA/NRCS value	Remark
1	pH	8.10	6.5 – 8.5	Alkaline
2	Organic matter (%)	5.36	-	-
3	Organic carbon (%)	3.11	> 1.5%	High
4	Available phosphorus	4.32	<5 = Low; >30 = High	Low
5	Total nitrogen (%)	1.26	> 0.2%	High
6	ECEC (Meq/100g)	4.98	< 25	Low
7	Texture	Loamy sand		

Growth Profile of Spinach Plant

The growth profile of spinach planted by: cow dung enhanced spinach (CES), NPK enhanced spinach (NES), cow dung and NPK enhanced spinach (CNES) and devoid of enhancement spinach (DES) is presented in Figure 2.

The experiment was set up on November 10th, 2024, NES grew up to 4 cm after the interval of one week, while CES grew by 5.5 cm approximately 6 cm in height after the interval of one week.

Afterwards, a significant growth was observed within week 2 to week four, which was more pronounced among CES and NES, respectively, followed by CNES, then DES. This trend is traceable to additional vitality derived from soil enhancement in terms of cow dung and NPK.

Beyond week 4, CES was observed to have grown up to 27 cm long, NES grew up to 30 cm, CNES grew up to 19 cm long, and DES grew up to 16 cm height, respectively, by the fifth week, which was harvested.

Cumulatively, the result obtained revealed a significant difference ($P \leq 0.05$) in the growth profile of CNES and

DES, implying that cow dung and NPK soil enhancement have a significant effect on soil enrichment and consequently spinach growth (Machado et al., 2020).

Heavy Metal Concentration in the Root and Shoot of Spinach Plant

The heavy metal levels of the studied soil, root and shoots of the spinach plant alongside the WHO/FAO maximum permissible limit are shown in Table 3. The result showed a significant difference ($P \leq 0.05$) in the heavy metal accumulation in the plant under study. Due to uptake from the studied soil enhancement, the Pb content in the roots of spinach plants labelled: CES, NES, CNES, and DES was 0.309±0.001, 0.370±0.001, 0.494±0.002 and 0.124±0.001, indicating NES and CNES levels to be slightly greater than the 0.30 mg/kg permissible limit of WHO/FAO. Likewise, the shoot concentrations of 0.741±0.002, 0.617±0.002, 0.309±0.001 and 0.062±0.001, respectively, indicate an appreciable level of Pb beyond the permissible limit with the exception of DES. Implying that the application of soil nutrient enhancement ends up increasing the Pb content, in contrast to Antonangelo et al. (2021), who suggested a lowering effect on the addition of organic fertilizer due to organic matter complexation reducing Pb bioavailability. The observable increase in Pb is likely due to NPK-induced soil acidification, enhancing the mobility (Peng et al., 2015).

Concentration of Fe in the roots of spinach plants: CES, NES, CNES and DES as 0.400±0.001, 0.400±0.001, 0.200±0.001 and 0.200±0.002 mg/kg as well as in the shoot: 0.500±0.001, 5.00±0.002, 0.400±0.001 and 1.00±0.001 mg/kg respectively were generally less than 425 mg/kg maximum permissible limit of the EHO/FAO, suggesting less accumulation of the element in the plant tissues.

Zinc content of CES (0.800±0.002 mg/kg), NES (0.800±0.002 mg/kg), CNES (0.400±0.001mg/kg) and DES (0.200±0.001mg/kg) in the root tissues as well as CES (1.500±0.002 mg/kg), NES (0.500±0.002 mg/kg), CNES (1.300±0.002mg/kg) and DES (2.400±0.002mg/kg) in the shoot were generally less than the 60 mg/kg threshold for zinc to exhibit toxicity in vegetable (WHO/FAO, 2007). As well as the 23.39 mg/kg zinc content recorded for the spinach control sample in Hunkuyi local government of Kaduna, Nigeria (Mijinyawa et al., 2022).

Accumulation of Cr from soil into the roots of spinach plants labeled: CES, NES, CNES and DES were 0.007±0.001, 0.007±0.002, 0.004±0.002, and 0.008 ± 0.002 mg/kg, while the shoots were 0.005 ± 0.002, 0.003 ± 0.001, 0.001 ± 0.001 and 0.009±0.002 mg/kg. This indicates the Cr content to be below the threshold limit to exhibit toxicity in vegetables (WHO/FAO, 2007).

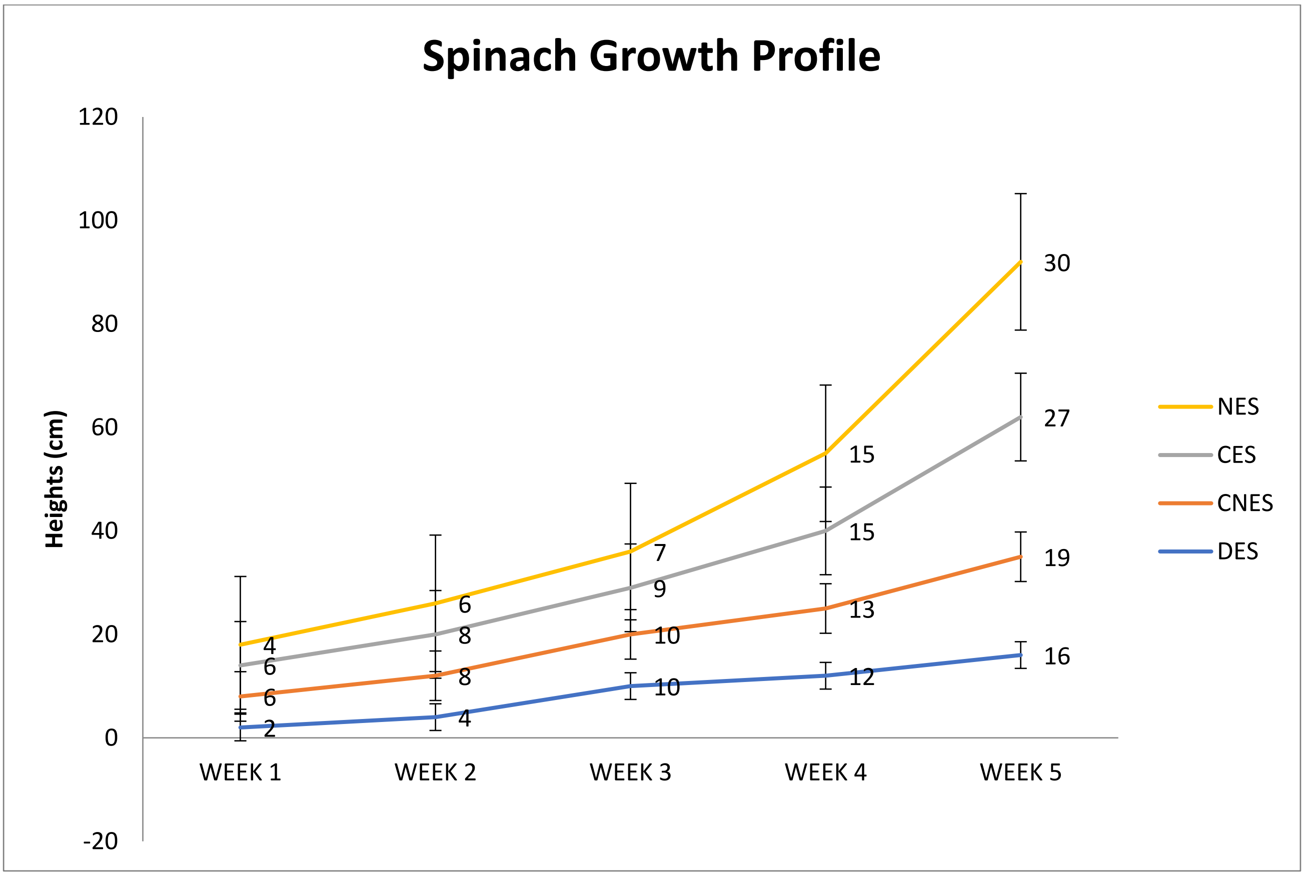

Figure 2: Growth profile of spinach with respect to time

Table 3: Heavy metals content (mg/kg) in soil and spinach tissue

		Heavy metal			
		Pb	Fe	Zn	Cr
DES	Shoot	0.062±0.001	1.000±0.001	2.400±0.002	0.009±0.002
DES	Root	0.124±0.001	0.200±0.002	0.200±0.001	0.008±0.002
CNES	Shoot	0.309±0.001	0.400±0.001	1.300±0.002	0.001±0.001
CNES	Root	0.494±0.002	0.200±0.001	0.400±0.001	0.004±0.002
NES	Shoot	0.617±0.002	5.000±0.002	0.500±0.002	0.003±0.001
NES	Root	0.370±0.001	0.400±0.001	0.800±0.002	0.007±0.002
CES	Shoot	0.741± 0.002	0.500±0.001	1.500±0.002	0.005±0.002
CES	Root	0.309±0.001	0.400±0.001	0.800±0.002	0.007± 0.001
Soil		0.247±0.001	3.100±0.001	0.600±0.002	0.024±0.002
WHO Standard	Vegetable	0.3	425	60	1.3

WHO = WHO/FAO (2007) Standard adopted from Oketayo et al. (2022)

Bio Concentration Factor of Heavy Metals in Spinach

The bio-concentration factor (BCF), which is the ratio of the metal content in the plant's root or shoot to that in the soil, can be used to measure a plant's capacity to accumulate metals from soils (Pedron et al., 2017; Yerima et al., 2022), where BCF ≥ 0.2 and TF > 1 are conditions for a plant to be considered an accumulator (Wahsha et al., 2012). As presented in Table 4, the soil-root bio concentration factor of Pb, Zn, Fe and Cr based on the CES, NES, CNES and DES cultural practice of soil nutrient enhancement revealed spinach as an accumulator of Pb and Zn having BCF ≥ 0.2 (Wahsha et al., 2012; Mijinyawa et al., 2022). However, the accumulation factor of Pb by spinach plant was much less than that recorded by lily pad (217) and algae (853), which are considered hyperaccumulators (Yerima et al., 2024).

The soil-to-root BCFs of Pb in spinach planted in cow dung-enhanced soil (CES), NPK-enhanced soil (NES), cow dung and NPK-enhanced spinach (CNES) and devoid of cow dung and NPK-enhanced spinach (DES) were 1.25, 1.49, 2 and 0.50, respectively. This indicates that the BCFs of Pb are higher in cow dung enhanced spinach and NPK enhanced spinach (CNES) with a value of (2) than NES, CES and DES with the following concentration values 1.49, 1,25 and 0.50, respectively.

The soil-to-root BCFs of Zn in spinach planted in CES, NES, CNES and DES were 1.33, 1.33, 0.66 and 0.33, respectively, indicating that CES and NES have the highest concentration value of Zn at 1.33, than CNES (0.66) and DES (0.33), respectively.

The soil-to-root BCFs of Fe and Cr in spinach plants were all less than 0.2, implying that the plant is not an accumulator of these heavy metals and will have no toxicity effect on the consumers (Wahsha et al., 2012).

Translocation Factor of Heavy Metals in Spinach Plant

Like bio-concentration factor (BCF), translocation factor (TF), which is defined as the ratio of metal content in the plant’s shoot to that in the root (Pedron et al., 2017; Yerima et al., 2022), where TF > 1 is also a condition for a plant to be considered an accumulator (Wahsha et al., 2012).

As displayed in Table 5, the TF of Pb in CES, NES, CNES and DAS were 2.39, 1.66, 0.62 and 0.50, respectively. The TF of Pb is higher in CES with a value of (2.39) than in NES, CES and DES with 1.66, 0.62 and 0.50, respectively.

The TFs of Zinc in CES, NES, CNES and DAS were 1.87, 0.62, 3.25 and 1.2, respectively, indicating significant root to shoot transfer of Zn in all except NES. However, only CES spinach has both BCF and TF values greater than 1, implying significant soil-to-root uptake of Zn as well as significant translocation of Zn from root to shoot.

The TFs of Fe from root to shoot of spinach in CES, NES, CNES, and DES were 1.25, 12.5, 2 and 5, respectively. Indicating the highest translocation factor of Fe in the NES-spinach with a value of (12.5), however, there was no significant transfer of Fe from the soil to the root. The TF of Cr from root to shoot of spinach were all less than 1, except for DES-spinach (1.12); however, there was no significant transfer of Cr from the soil to the root.

Table 4: Soil to root bioconcentration factor of heavy metals

Spinach Sample	Lead	Zinc	Iron	Chromium
CES	1.25*	1.33*	0.12	0.029
NES	1.49*	1.33*	0.12	0.029
CNES	2*	0.66	0.06	0.016
DES	0.50	0.33	0.06	0.033

*= effective transfer from soil to root

Table 5: Root-shoot translocation factor of heavy metals in spinach plant

Spinach sample	TF (Pb)	TF (Zn)	TF (Fe)	TF (Cr)
CES	2.39*+	1.87+	1.25	0.71
NES	1.66+	0.62	12.5*	0.42
CNES	0.62	3.25*	2	0.25
DES	0.50	1.2	5	1.12*

* = maximum translocation factor

\+ = effective transfer from soil to root and root to shoot

Health Risk Assessment

The hazard quotient (HQ) and hazard index (HI) model were used to assessed the risk of cow dung enhanced spinach (CES), NPK enhanced spinach (NES), cow dung and NPK enhanced spinach (CNES) and devoid of enhancement spinach (DES), as presented in equation I, the hazard quotient is the ratio of the Estimated Daily Intake (EDI) of a specific metal in a substance to its oral reference dose (RfD) or acceptable daily intake (mg/kg of body weight per day). It is an estimate of an oral exposure per day of the human population which does not cause deleterious effects during a lifetime in non-cancer health assessments (Olawale et al., 2023).

$$HQ = \frac{EDI}{RfD} \qquad (I)$$

The values of RfD for Pb, Zn, Cr and Fe were 0.035, 0.3, 1.5 and 0.7 ($mg.kg^{-1}.day^{-1}$) respectively (USEPA IRIS, 2006; Adedokun et al., 2016; Ekere et al., 2020).

The daily intake of metals (DIM) presented in Table 6 was calculated to estimate the daily intake (EDI) of metal into the body system of a specified body weight of a consumer, as well as reveals the relative phyto-availability of metal based on the formula displayed in equation II.

$$EDI = \frac{C \times C_F \times DIM}{BW} \qquad (II)$$

Where, C is the heavy metal content in vegetables (mg/kg), C_F is the conversion factor equivalent to 0.085 to convert fresh vegetable weight to dry weight, daily vegetable intake of 65 g/day (Adedokun et al., 2016), while the average body weight used was 60 kg for this study (Olawale et al., 2023).

In this study, the HQ of Fe, Zn and Cr were far less than 1 in CES, NES, CNES and DES spinach; therefore, it does not pose health risk concerns except for Pb in CES (1.95) and NES (1.62). However, the hazard index (HI), which is the summation of all the individual HQ of Fe, Zn and Cr in spinach, was CES (2.48), NES (2.43), CNES (1.00) and DES (1.00), respectively. A HQ and HI less than or equal to one indicates negligible hazard, while a hazard quotient greater than one indicates hazard possibility (Adedokun et al., 2016; Olawale et al., 2023).

Table 6: Hazard quotient and estimated daily dose of heavy metals in spinach

Heavy metals	Sample	C (mg/kg)	CF	DIM	BW (kg)	EDI	RfD ($mg/kg.day^{-1}$)	HQ
Pb	CES	0.74	0.085	65	60	0.068	0.035	1.95
	NES	0.62	0.085	65	60	0.057	0.035	1.62
	CNES	0.31	0.085	65	60	0.028	0.035	0.81
	DES	0.06	0.085	65	60	0.006	0.035	0.16
Fe	CES	0.50	0.085	65	60	0.046	0.7	0.07
	NES	5.00	0.085	65	60	0.460	0.7	0.66
	CNES	0.40	0.085	65	60	0.037	0.7	0.05
	DES	1.00	0.085	65	60	0.092	0.7	0.13
Zn	CES	1.50	0.085	65	60	0.138	0.3	0.46
	NES	0.50	0.085	65	60	0.046	0.3	0.15
	CNES	1.30	0.085	65	60	0.119	0.3	0.39
	DES	2.40	0.085	65	60	0.221	0.3	0.74
Cr	CES	0.005	0.085	65	60	0.00046	1.5	0.0003
	NES	0.003	0.085	65	60	0.00028	1.5	0.0002
	CNES	0.001	0.085	65	60	9.21E-05	1.5	6.14E-05
	DES	0.009	0.085	65	60	0.00083	1.5	0.0006

C = heavy metal content in spinach, C_F = conversion factor, HQ = hazard quotient, BW = Body weight

CONCLUSION

In conclusion, the soil quality parameters showed the studied soil was alkaline with relatively high organic matter/carbon, which will aid stabilized heavy metal mobility, relatively rich in nitrogen and phosphorus content to enable plant grow even on little or no soil enhancement. The growth profile of spinach was in the order NES > CES > CNES > DES, while the potentially toxic heavy metal content of the soil was within acceptable limits. However, of all the studied heavy metals only Pb and Zn were both bio-accumulated in the root as well as efficiently distributed on the leaves of solely NPK enhanced spinach and solely cow dung amended soil with health risk thereby presenting cow dung/NPK blend enhancement and devoid of nutrient enhance soil a more preferred cultural practice for heavy metal controlled spinach production. Based on the findings, it is advisable to promote the use of enhancements that minimize heavy metal uptake while maintaining soil fertility, such as cowdung/NPK blended enhanced soil.

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