ARCN International Journal of Development
ISSN: 2384-5347. Volume 8, Issue 1, (January, 2024) pages 1 – 14
DOI: 2726-4051-0811
arcnjournals@gmail.com
https://arcnjournals.org

Health Risk Assessment of Lead, Cadmium and Zinc Intake Due to Consumption of Carrot (*Daucas carota*) and Lettuce (*Lactusa sativa*) from the Bank of Komadugu River in Gashua, Yobe State, Nigeria

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Abstract: *This study was aimed at investigating the health risk assessment of lead (Pb), cadmium (Cd) and zinc (Zn) intake due to consumption of carrot (Daucas carota) and lettuce (Lactusa sativa) from the bank of Komadugu River in Gashua, Yobe State, Nigeria. Concentrations of the metals were determined by flame atomic absorption spectrophotometry after digestion of the leaves and roots of the vegetable samples. Results obtained were compared with permissible limits from the World Health Organization (WHO), other regulatory bodies and previous works. Values of the pollution load indices (PLIs), estimated daily intake (EDI) and combined total hazard quotient (CTHQ) all indicate no Pb, Cd and Zn pollution in carrot root, lettuce leaf and root. However, there was some degree of pollution of carrot root by these metals.*

Keywords: *Concentration; Health Risk Assessment; Carrot; Lettuce; World Health Organization (WHO); Pollution Load Indices (PLIs); Estimated Daily Intake (EDI); Combined Total Hazard Quotient (CTHQ).*

1 Introduction

Vegetables, especially leafy crops grown in heavy metal contaminated soils, accumulate higher amounts of metals than those grown in uncontaminated soils because of the fact that they absorb these metals through their leaves (Al Jassir *et al.,* 2005). Research has shown that wastewater carries appreciable amount of heavy metals (Pescod, 1992). Household effluents, drainage water, business effluents, atmospheric deposition and traffic related emissions transported with storm water carry a number of pollutants that enrich the urban wastewater with heavy metals (Oliveira *et al.,* 2007). Heavy metal contamination may occur due to irrigation with contaminated water, the addition of fertilizers and metal-based pesticides, industrial emissions, transportation, harvesting process, storage and/or sale. Crops and vegetables grown in soils contaminated with heavy metals have greater accumulation of heavy metals than those grown in uncontaminated soil. Plants take up metals by absorbing them from contaminated soils as well as from deposits on parts of the plants exposed to the air from polluted environment (Khairiah, 2004). In general, food is the main exposure route and human beings are encouraged to consume more fruits, which are a good source of vitamins, minerals, fiber and also beneficial to their health. Unfortunately, these foodstuffs contain both essential and toxic metals over a wide range of concentrations (Radwan and Salama, 2006). It has been reported that nearly half of the mean ingestion of lead, cadmium and mercury through food is due to plant origin (fruit, vegetables and cereals). Moreover, some population groups seem to be exposed, especially vegetarians, since they absorb more frequently 'tolerable daily doses' (Islam, 2007). Dietary intake of heavy metals through contaminated fruits may lead to various chronic diseases. The biotoxic effects of heavy metals depend upon their concentrations and oxidation states, kind of sources and mode of deposition. Lead and cadmium are among the most abundant and toxic heavy metals. The excessive content of these metals in food is associated with etiology of a number of diseases, especially with cardiovascular, kidney, nervous as well as bone diseases (WHO, 1995).

2. Materials and Methods

2.1 The Study Area

The Yobe River, also known as the Komadugu Yobe River is a river in West Africa that flows into Lake Chad through Nigeria and Niger. In Yobe State, it is located on longitud12º52´N and latitude 10º58´E in Gashua, Bade Local Government Area. Its tributaries include River Hadejia , River Jama'are and the Komadugu Gana River. The river forms a small part (150 km) of the international border between Niger and Nigeria and flows a total of 320km (KYBP, 2006). There are concerns about changes in the river flow, economy and ecology due to upstream dams, the largest at present being the Tiga Dam in Kano State, with plans for the Kafin Zaki dam in Bauchi State (NPC, 2006). The River Yobe provides a means of subsistence for hundreds of thousands of people who work in a variety of commercial and agricultural endeavors along its almost 200 km length in the states of the northern region, which spans seven local government areas (LGAs) from Nguru to Yunusari. Notable towns near the river include Gashua, Geidam and Damasak in Nigeria and Diffa in Niger (Wakawa *et al*, 2017).

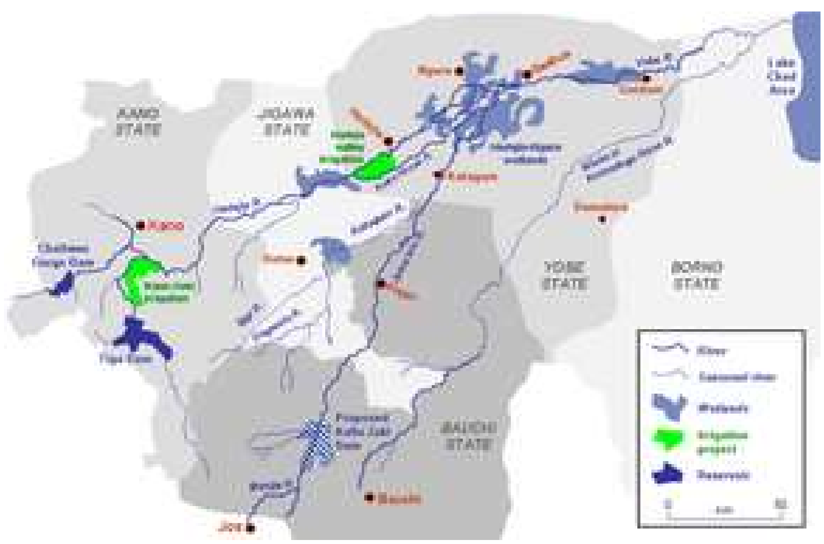


Figure 1: Catchment Area of the Komadugu River

2.2 Instruments, Apparatus and Reagents

All equipment and instruments used in this research were calibrated before conducting the experiments. All glassware used were thoroughly washed with detergent and tap water and then

rinsed with deionized water. Suspected contaminants were cleaned with 10% concentrated nitric acid (HNO_3) and metal surfaces rinsed with deionized water. The digestion tubes were soaked in 1% (w/v) potassium dichromate in 98% (v/v) H_2SO_4.
In preparation of reagents, chemicals of analytical grade purity and distilled water were used. All glassware and plastic containers were washed with detergent.

2.3 Digestion of Carrot and Lettuce Samples

The carrot and lettuce leaves and roots were first separated, air dried and then dried at 100°C in an oven (Model 30GC) for 2 hours. The samples were then cooled and ground to fine powder. A microwave digester (Master 40 serial No: 40G106M) was used in digesting the root and leaf samples in a digestion tube to which 0.1g of sample was added at a time, followed by 6mL of 65% HNO_3 and 2mL of 30% H_2O_2 and allowed to stand for a while. The digestion was carried out at 180°C, 1800W in a time of 30mins.The digestion was followed by cooling at room temperature in the microwave and the sample was diluted with de-ionized water. Potential presence of selected heavy metals in chemicals used in digestion were determined. Blanks were used simultaneously in each batch of the analysis to authenticate the analytical quality (SINEO, 2013).

2.4 Atomic Absorption Spectrometric Analysis

The carrot and lettuce root and leaf extracts were analyzed for lead (283.5nm), cadmium (228.8nm) and zinc (213.9nm) using flame atomic absorption spectrophotometry.Blank determinations were made prior to sample analysis. Heavy metal concentrations in carrot and lettuce root and leaf extracts were obtained in triplicates from calibration curves and expressed as mg/kg. Metals in chemicals used in digestion were determined. Blanks were used simultaneously in each batch of the analysis to authenticate the analytical quality (SINEO, 2013).

2.5 Statistical Analysis

The data were analyzed in triplets and expressed as mean and standard deviation. The mean of all treatments was subjected to a One-way analysis of variance (ANOVA) using IBM SPSS Statistics 23 software and mean differences were performed using the Tukey test. All graphs were plotted using Microsoft Excel 2013.

3. Results and Discussion

3.1 Heavy Metal Bioavailability in Plants

Metal bioavailability refers to the fraction of heavy metal in soil that is accessible to the food chain and to the plant (Misra *et al.,* 2009). Plants are known to accumulate heavy metals in their edible portion as a result of cropping contaminated fields. Such accumulation is greatly influenced by the concentration of the pollutant, chemical species of the pollutant in the soil, soil physicochemical properties as well as the plant‘s growth characteristics (Dudka and Chlopecka, 1990). Bioaccumulation takes place when substances are taken in the food chain from food and water. These substances accumulate because they cannot be broken down and used up by the organism or they are taken in faster than they are used up by organisms. Bioaccumulation is not hazardous when the substance accumulated is not harmful. But when the compounds and heavy metals, like Hg that are accumulated are harmful to human health, then bioaccumulation becomes dangerous (Brookes and Grath 1984). When Cd, Zn, Pb, Hg, As, Cu, Cr, Ni and Mg accumulate in the soil over long times, they reduce food quality and quantity. A high heavy metal load in the soil reduces the functioning of soil biota resulting in reduced microbial activity. Heavy metals have specific bioaccumulation characteristic and accumulate differently in the ecosystem. The metal being accumulated is the determining factor of where it bioaccumulates in the ecosystem. Uptake of metals is considered to involve complexation, ion exchange, adsorption, inorganic micro precipitation, oxidation and reduction processes. Metal ions are adsorbed first to the surface of cells by the interactions between the metal ions and functional groups such as carboxyl, phosphate, hydroxyl, amino, sulphide and thiol which is present in the cell (Wang and Chen, 2006). Heavy metals accumulation varies from one plant to another, one heavy metal to another and the plant parts (Linden *et al.,* 1994). Repeated application of poor quality irrigation water can strongly influence speciation of heavy metals that are of anthropogenic origin, reduce the soils sorption capacity for heavy metals and hence the metals eventually leached into soil solution making it more available for plant utilization (Sridhara *et al.,* 2008). Plant uptake of mobile ions present in the soil solution is largely determined by the total quantity of the ions in the soil solution. But in the case of strongly adsorbed ions, absorption seems to depend more upon the amount and surface area of roots. Roots excrete products like high molecular weight organic acids which form complexes and chelates with metal ions thereby modifying heavy metal movement and fixation in soils (Wang *et al.,* 2009).

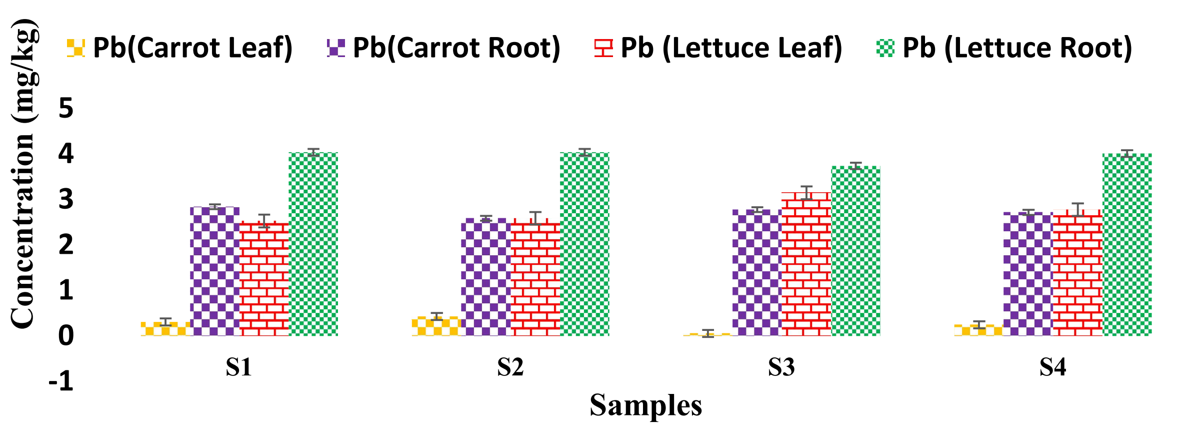
3.2 Abundance of Heavy Metals in Carrot and Lettuce

3.2.1 Lead

Table 1: Minimum, Maximum and Mean Concentrations of Pb in Roots and Leaves of Carrot and Lettuce

	Minimum	Maximum	Mean
Carrot Leaf	0.06	0.43	0.263± 0.15
Carrot Root	2.59	2.84	2.733± 0.11
Lettuce Leaf	2.53	3.15	2.763± 0.28
Lettuce Root	3.74	4.04	3.958± 0.15

Figure 2: Concentrations of Pb in Roots and Leaves of Carrot and Lettuce



The mean concentrations of lead in the leaves and roots of carrot were 0.263±0.15 and 2.733 ± 0.11 and in the leaves and roots of lettuce were 2.763 ± 0.28 and 3.958 ± 0.15mg/kg respectively. Whereas Pb levels in carrot leaves were in consonance with 0.2mg/kg set by the United States

Environmental Protection Agency (USEPA), the concentrations of Pb in carrot roots, lettuce leaves and roots were above this USEPA value and the permissible limit of 0.3mg/kg set by the World Health Organization (WHO). Concentrations of Pb obtained in this study were much higher than values reported by Bystrická *et al.* (2015):0.05±0.02mg/kg for selected varieties of onion (*Allium cepa L.*) grown in the different locations, Harmanescu *et al.* (2011): 0.13± 0.05mg/kg for vegetables grown in old mining area. The following workers reported slightly higher values of Pb than the mean value reported for carrot leaves in this study; Gupta *et al.* (2022): 0.66±0.60 mg/kg accumulated in vegetables, Leblebici (2020): 0.67±0.16 mg/kg for green vegetables grown in Nevşehir. On the other hand, the following values were higher than the results reported for leaves and roots of carrot and lettuce in this study; Gebrekidan *et al.* (2013): 4.90 -70.79 mg/kg accumulated in vegetables and fruits grown in Ginfel river near Sheba Tannery, Tigray, Northern Ethiopia, Abdullahi *et al.* (2007): 13.199±3.99 mg/kg for tomatoes and onions from irrigated farmlands on the Bank of River Challawa, Kano, Jung (2008): 14.143 + 0.051 mg/kg uptake by plants in the vicinity of a Korean Cu-W Mine.

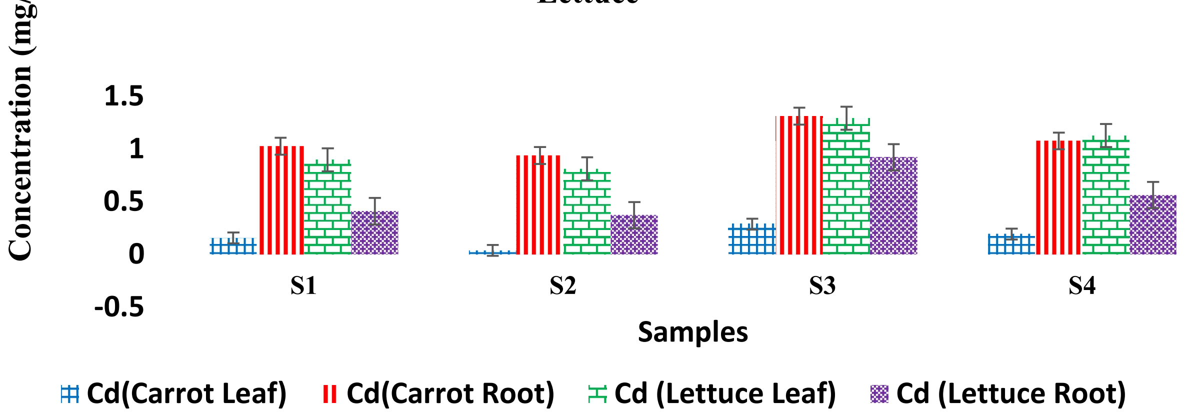
Lead (Pb) is one of the non-essential toxic elements that are most omnipresent in the soil. The plant Pb mainly comes from soil and aerosol (Sharma and Dubey, 2005). Roots are more able to accumulate Pb in plants; however, their subsequent translocation to aerial parts is highly restricted. The availability of lead in soil depends heavily on soil conditions such as soil pH, particle size and capacity for cation exchange. In addition, some other factors such as root surface area, root exudation, mycorrhization and degree of transpiration also affect the availability and uptake of Pb. Plant' root absorbs the Pb through the apoplastic pathway or via Ca^{2+} permeable channels (Pourrut *et al.,* 2011). It accumulates after take-up primarily in root cells due to the blockage inside the endodermis by the Casparian strips. In addition, lead is also trapped on the roots cell wall by the negative charges (Seregin and Ivaniov, 2001).The accumulation of lead in plants has several deleterious effects, either directly or indirectly, on the morphological, physiological and biochemical functions of plants. When Pb enters the cells, toxicity is caused by altering the permeability of the cell membrane, by reacting with active metabolic enzyme groups, by replacing essential ions, and by complex formation with the ADP or ATP phosphate group. Lead toxicity causes inhibition of enzyme activity, disturbed mineral nutrition, water imbalance, hormonal disturbance, inhibition of ATP production, lipid peroxidation, changes in membrane permeability and damage to DNA by overproduction of reactive oxygen species (ROS) (Sharma and Dubey 2005; Pourrut *et al.* 2011; Sethy and Ghosh, 2013).

3.2.2 Cadmium

Table 2: Minimum, Maximum and Mean Concentrations of Cd in Roots and Leaves of Carrot and Lettuce

	Minimum	Maximum	Mean
Carrot Leaf	0.040	0.289	0.171± 0.10
Carrot Root	0.940	1.313	1.090± 0.16
Lettuce Leaf	0.813	1.293	1.034± 0.22
Lettuce Root	0.374	0.922	0.568± 0.25

Figure 3: Concentrations of Cd in Roots and Leaves of Carrot and Lettuce



The mean concentrations of cadmium in the leaves and roots of carrot were 0.171±0.10 and 1.090±0.16 and in the leaves and roots of lettuce were 1.034±0.22 and 0.568±0.25mg/kg respectively. Whereas Cd levels in carrot leaves were in consonance with 0.1mg/kg set by the World Health Organization (WHO), the concentrations of Cd in carrot roots and in lettuce leaves and roots were higher than this WHO value and the permissible limit of 0.2mg/kg set by USEPA. Results reported by the following workers were lower than Cd concentrations in carrot roots, lettuce leaves and roots; Harmanescu *et al.* (2011) reported a value of 0.01±0.00 in vegetables, Hellen *et al.* (2014) reported a value of 0.21±0.19 in tomatoes and selected vegetables from Lushoto District-Tanzania, Gebrekidan *et al.* (2013) reported a value of 0.207±0.01 mg/kg in vegetables and fruits, Bvenura *et al.* (2012) reported a value of 0.21±0.19 mg/kg in vegetables cultivated in home gardens in the Eastern Cape, Jung *et al.* (2008) reported a value of 0.291 to 0.347mg/kg in plants in the vicinity of a Korean Cu-W Mine.

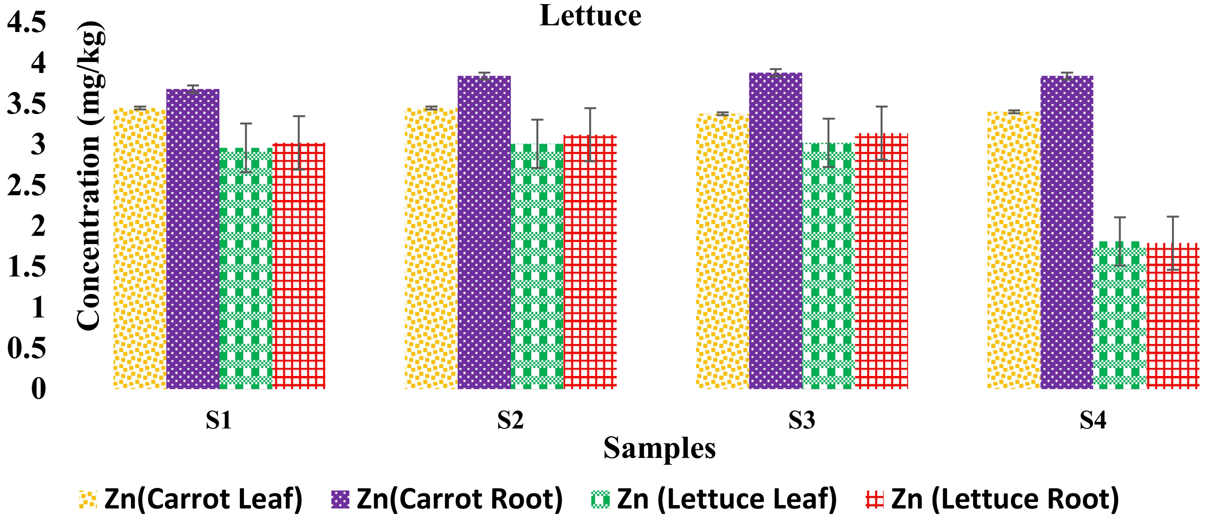
A natural metallic element, cadmium (Cd) is generally present in the earth's crust at low levels. However, cadmium is present at elevated levels in some soils, rocks, metal ores, fossil fuels (especially coal), human and animal waste, and as an impurity in phosphate rock. Fertilizer contains cadmium because phosphate rock is used to produce phosphorus fertilizers. Cadmium concentrations vary significantly by geography. Plants readily take up cadmium into their leaves, stems, roots and tubers, and to a lesser extent their seeds, grains, and fruits. Cadmium concentrations are typically higher in leaves than in other parts of the plant, but translocation varies by species and environment. As Cd becomes more into focus and buyers include metals in risk management programs. Vegetable packers and processors may be asked to monitor for Cd and limit Cd uptake in vegetable crops. More recently cadmium (Cd) has been identified as a potential human concern and vegetables are a significant source of Cd in human diets. Chronic exposure to Cd (low level over an extended period of time) can result in kidney, bone and lung diseases. Cd is also thought to be a potential carcinogen. Cadmium exposure due to the accumulation of Cd in living systems and food chains affects the susceptibility to infection and the detailed mechanism has not been investigated (Johnson, 2019).

3.2.3 Zinc

Table 3: Minimum, Maximum and Mean Concentrations of Zn in Roots and Leaves of Carrot and Lettuce

	Minimum	Maximum	Mean
Carrot Leaf	3.38	3.45	3.420± 0.04
Carrot Root	3.68	3.88	3.810± 0.09
Lettuce Leaf	1.81	3.02	2.700± 0.59
Lettuce Root	1.79	3.14	2.768± 0.65

Figure 4: Concentrations of Zn in Roots and Leaves of Carrot and Lettuce



The mean concentrations of zinc in the leaves and roots of carrot were 3.420±0.04 and 3.810±0.09 and in the leaves and roots of lettuce were 2.700±0.59 and 2.768±0.65mg/kg respectively. Though, there is no specific limit for Zn in vegetables set by World Health Organization WHO, the United States Environmental Protection Agency (USEPA) set a value of 200 mg/kg. The Zn results reported in this study were higher than the results of Ikechukwu *et al.* (2019), who reported a value of 2.447±0.010 mg/kg in selected fruits in Umuahia market. However. Harmanescu *et al.* (2011) reported a value of 4.65±2.20 mg/kg in vegetables,

Gebrekidan *et al* (2013) reported a value 70.10 mg/kg in vegetables and fruits. Zinc is an essential trace element that poses great importance in human dietary nutrition and health (Hambidge 2000; Gammoh and Rink 2017). Therefore, it is known to be the second most abundant trace metal in human body after iron (Rink and Gabriel 2000). It consists of 2-4 g within a human body mass with plasma concentration of 12-16 µM (Rink and Gabriel 2000). The role of zinc on human health was originally observed and reported by (Prasad *et al.,* (1963). Since there is no specialized Zn storage system in human body, daily intake of Zn is necessary to maintain a steady state (Rink and Gabriel 2000). One of the roles of zinc in human being is immunity (Wessels *et al.,* 2017; Maywald *et al.,* 2017). Zinc deficiency can result in immunodeficiency (Rink and Gabriel 200). Zinc ions are crucial in the regulation of intracellular signaling pathway in innate and adaptive immune cells (Wessels *et al.,* 2017). Immune system is known to be susceptible to alteration in Zn levels and every immunological response by human body is related to Zn in varying extent (Gammoh and Rink 2017). There are two immunological mechanisms in human physiology; innate and adaptive immunity. Innate immunity is the first line of human biological defense countering various forms of pathogens. Innate immunity of human consists of polymorphonuclear cells (PMNs), macrophages, and natural killer cell (NK). Zinc deficiency is associated to reduced PMN chemotaxis and phagocytosis. Deficiency as well as excess of Zn could also inhibit the activity of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, which functions to destroy pathogens after it was phagocyted (De Coursey *et al.,* 2003; Hasegawa *et al.,* 2000). Chelation-free Zn was also observed to abolish the formation of neutrophile extracellular traps (NETs) in vitro. This is a matrix of DNA, chromatin and granule proteins that capture extracellular pathogenic protein (Brinkmann *et al.,* 2004).

3.3 Pollution Index Parameters

- The most common pollution index parameters include pollution load index (PLI), estimated daily intake (EDI) and target hazard quotient (THQ).
- LPI is a preliminary parameter, which gives only the sign of pollution by a metal in a sample.
- A PLI > 1 indicates that heavy metal pollution may exist subject to the values of EDI and THQ.
- A PLI < 1 shows out rightly that no pollution exists in a sample (Tomlinson *et al.,* 1980).
- EDI and THQ are confirmatory parameters.
  - If EDI > RfD (the oral reference dose of a metal) and HTQ > 1, pollution by a metal exists in a sample.
  - If HTQ < 1, no pollution by a metal exists in a sample.
- The three parameters are calculated from the following equations;
  - $LPI = \left(CF_1 \times CF_2 \times CF_3 \times CF_4 \times \ldots\ldots\ldots\ldots \times CF_n\right)^{\frac{1}{n}}$, where CF_n is the concentration of the nth element under investigation.
  - $EDI = \dfrac{D_{intake} \times C_F \times C_m}{B_{weight}}$, where

- D_{intake} is the average daily intake of food= 0.585kg of vegetable/person/day
- C_F is the conversion factor =0.08045
- Cm is the metal concentration in the vegetable (mg/kg dry weight basis)
- B_{weight} is the average body weight for adults (70 kg) Yaqub *etal*., (2021)

- $THQ = \dfrac{EDI}{RfD}$, Where, EDI = Estimated Daily Intake, RfD = Oral Reference Dose.

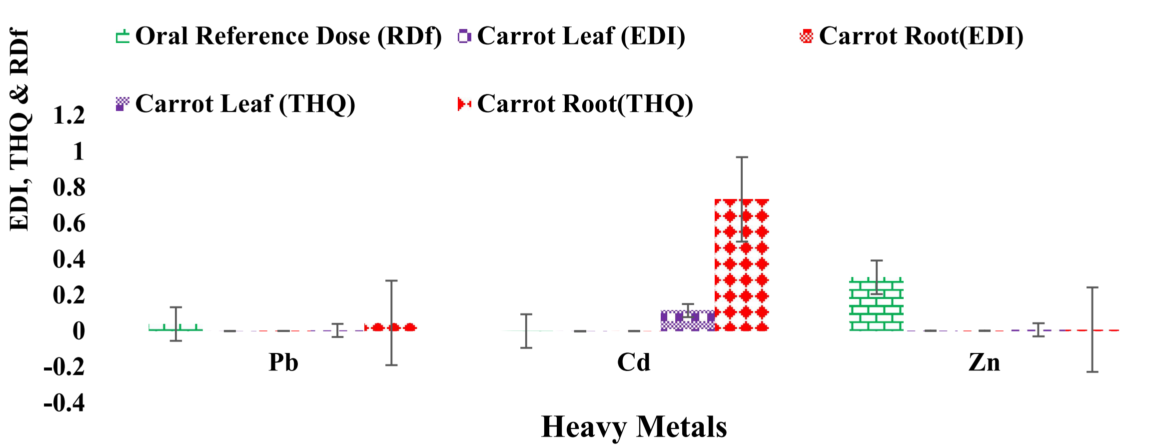
3.4 Human Health Risk Assessment Due to Carrot and Lettuce Intake

3.4.1 Carrot

The load pollution index (LPI) of Pb, Cd and Zn in carrot leaf and carrot root were 0.5358 and 2.2473 respectively, suggesting no pollution of these metals in the leaf, but some degree of pollution in the root might exist, subject to the values of the estimated daily intake (EDI) and target hazard quotient (THQ).

Figure 5 shows the estimated daily intake (EDI), target hazard quotient (THQ) and oral reference dose of Pb, Cd and Zn in carrot leaf and root.

Figure 5: Estimated Daily Intake (EDI), Target Hazard Quotient (THQ) and Oral Reference Dose (RDf) of Pb, Cd and Zn in Carrot Leaf and Root



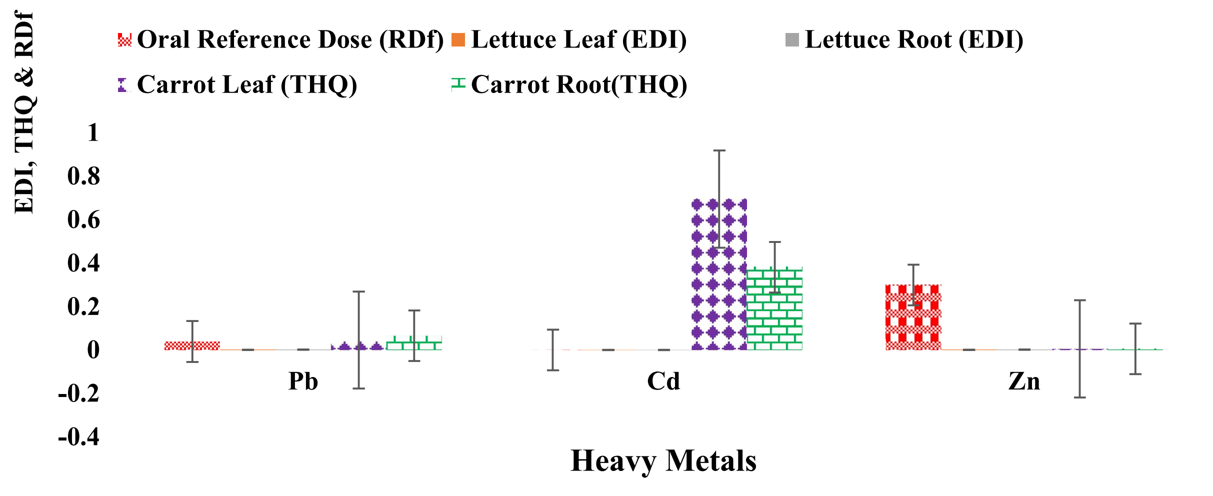
The estimated daily intakes (EDIs) of Pb, Cd and Zn in carrot leaf were 0.00018, 0.00012 and 0.00230 respectively. There was no pollution of the carrot leaf by these metals, since their oral reference doses; Pb (0.04), Cd (0.001) and Zn (0.3) are greater than the EDIs. Furthermore, the target hazard quotients (THQs) of the metals in carrot leaf; Pb (0.00442), Cd (0.11504) and Zn (0.00767) were all less than 1. Similarly, there was no pollution of the carrot root by these metals.

3.4.2 Lettuce

The load pollution index (LPI) of Pb, Cd and Zn in lettuce leaf and lettuce root were 1.9759 and 1.8393 respectively, suggesting some degrees of pollution of the leaf and root, subject to the values of the estimated daily intake (EDI) and target hazard quotient (THQ).

Figure 6 shows the estimated daily intake (EDI), target hazard quotient (THQ) and oral reference dose of Pb, Cd and Zn in lettuce leaf and root.

Figure 6: Estimasted Daily Intake (EDI), Target Hazard Quotient (THQ) and Oral Reference Dose (RDf) of Pb, Cd and Zn in Lettuce Leaf and Root



The estimated daily intakes (EDIs) of Pb, Cd and Zn in carrot leaf were 0.00186, 0.00070 and 0.00182 respectively. There was no pollution of the carrot leaf by these metals, since their oral reference doses; Pb (0.04), Cd (0.001) and Zn (0.3) and are greater than the EDIs. Furthermore, the target hazard quotients (THQs) of the metals in lettuce leaf; Pb (0.04647), Cd (0.69562) and Zn (0.00605) were all less than 1. Similarly, there was no pollution of the lettuce root by these metals.

4. Conclusion

Concentrations of Pb, Cd and Zn in carrot (*Daucas carota*) and lettuce (*Lactuca sativa*) obtained in this study were compared with permissible limits set by the World Health Organization (WHO), United States Environmental Protection Agency (USEPA) and results reported by previous workers. The pollution load index (PLI), estimated daily index (EDI) and target hazard quotient (THQ) were used to assess pollution potentials of; Pb, Cd and Zn in carrot (*Daucas carota*) and lettuce (*Lactuca sativa*) leaves and roots. There was no Pb, Cd and Zn pollution in carrot root, lettuce leaf and root. However, there was some degree of pollution of carrot root by these metals.

Acknowledgement
The authors are grateful to Mal. Idris Baba of Chemistry Research Laboratory, Yobe State University, Damaturu for his invaluable contribution in the sample analysis.

Authors' Contributions
Dagari M.S.: Conceptualization, design and supervision of the research work; Editing of the write-up
Musa S.: Undertaking the research work, write-up and data analysis.

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