FUDMA Journal of Sciences (FJS)
ISSN online: 2616-1370
ISSN print: 2645 - 2944
Vol. 7 No. 6, December (Special Issue), 2023, pp 66 -74
DOI: https://doi.org/10.33003/fjs-2023-0706-2173

HEALTH RISK ASSESSMENT FROM THE CONSUMPTION OF *Moringa oleifera* LEAVES CULTIVATED ALONG RIVER GINZO, KATSINA

***[1]Usain Shamsuddeen Alhassan and [2]Jonathan Sylvanus Dajal**

[1]National Environmental Standards and Regulations Enforcement Agency (NESREA), Katsina State Field Office, Nigeria
[2]National Environmental Standards and Regulations Enforcement Agency (NESREA). Port Harcourt Office, Nigeria

*Corresponding authors' email: shamsuusain@gmail.com Phone: +2348036407716

ABSTRACT

Heavy metals have a relatively high density and occur naturally in the environment or introduced by anthropogenic activities. They may be essential or non-essential to living organisms causing toxicity, environmental pollution or contaminate food chain. This research determined the hazard from ingestion of Moringa Oleifera leaves cultivated along River Ginzo, Katsina through the Estimation of Daily Intake of Metals (EDIM), Target Hazard Quotient/Hazard Index (THQ/HI) and Incremental Lifetime Cancer Risk (ILCR) from exposure to Co, Cu Pb and Ni in the leave samples obtained by random sampling. Heavy metals in samples were determined using Atomic Absorption Spectrophotometry (AAS) and analysed using Social Package for Social Science. The Result showed that levels of Co in all samples exceeded the 0.05ppm permissible limit in irrigation water. Values of Pb, Ni, and Cu and Ni in all soil samples exceeded the 0.05ppm, 0.30ppm and 0.05ppm USEPA, 2000 acceptable limits. Concentration of metals in leave samples are within the WHO/FAO 2014 acceptable limits. EDIM in M. oliefera were below tolerable daily intake, implying no health risk. THQ/HI for both essential and non-essential elements in leave samples were >1, implying no health hazard. ILCR showed potential risk of contracting cancer from the consumption of M. oleifera leaves grown in the study areas as Incremental Lifetime Cancer Risk values exceed the acceptable and negligible range of 10^{-4}-10^{-6}particularly of Ni (2.23E-2). As a result, measures should be put in place to prevent disposal of wastes containing heavy metals into River Ginzo used for irrigation purposes.

Keywords: River Ginzo, Heavy Metals, Estimated Daily Intake of Metals, Target Hazard Quotient/Hazard Index, Incremental Lifetime Cancer Risk

INTRODUCTION

Heavy metals are natural elements that are characterized by their rather high atomic mass and their high density. These metals occur naturally in the soil and can also be released into the environment by natural and a number of anthropogenic activities (Osae *et al*., 2023) such as the use of raw sewage for irrigation, discharge of industrial effluent and municipal wastewater into the environment and the excessive use of agrochemicals in agricultural processes (Tongesayi *et al*., 2013).

Heavy metals are categorized as essential and non-essential heavy metals. Essential heavy metals such as Mn, Cu, Zn, Co etc. are essential to the metabolic processes of many living organisms (Sing *et al*., 2016) which may be present in the soil or the medium in which the plant is grown or from heavy metal contaminated water used for irrigation of the crops (Rai *et al*., 2019) which when consumed at higher quantities becomes toxic causing harm to humans (Akoto *et al*., 2008). For example, Zn plays a vital role in immunological function while excessive intake of Cu is associated with liver damage. Unlike essential heavy metals, non-essential elements have no biological function and are toxic to living organisms even in small quantities. Example of these elements include Hg, As, Pb, Cr, Ni etc Researches conducted on the health implications of non-essential heavy metals such as Ni (Mengistu, 2021) reveal gastrointestinal discomfort, rise in red blood cells, decrease in lung function (Zambelli *et al*., 2016). On the other hand, Pb causes elevated arterial pressure and behavioral problems (Vassegghian *et al*., 2020).

Globally, environmental pollution by heavy metals and contamination of food items has become a concern. The United State Environmental Protection Agency (USEPA) proposed the integral approach that is globally used to assess the risks posed to people from the exposure to particular environmental substances (Osae *et al*., 2023.). The risk assessment tool is used to determine the potential risk of exposure to heavy metals using the variety of effect that they have on human health (Fallahzadeh, *et al*., 2019). The present study is aimed at determining the heavy the heavy metals in River Ginzo used for the irrigation of vegetables cultivated in the study area, the agricultural soil, levels of the of the metalloids in leaves of *M. oleifera,* the EDIM, THQ/HI and the cancer risk to the people of the research area.

Study Area

Katsina metropolis lies on Latitude 12°59'26''N and longitude 7° 36'6.37''E with a total land area of about 3,370km^2 (Danjuma, 2012) and elevation of about 500 meters above sea level (Mohammed & Hamisu, 2015). Katsina is the capital city of Katsina State and covers a total land area of about 24,192km^2. According to Wikipedia, the population of Katsina State as at 2019 was 9,300,382 while the city population was put at 681,662 (population.gov.ng) which must have now increased due to natural population increase and immigration. The State is bordered by Republic of Niger to the North. by the East, South and West, the State is bordered with Kano and Jigawa, Kaduna and Zamfara respectively.

Sampling Stations

Sampling sites for this study are Kofar Marusa (Abdullahi Sarki Muktar Road, Katsina), Kofar Durbi (Nagogo Road, Katsina) and Kofar Sauri (Muhammadu Dikko Road) irrigation farming sites all located along the major wastewater channel called River Ginzo (Figure 1). The river is located in a densely populated area of Katsina metropolis, Katsina State. The study sites were selected purposely based on the productivity and regular consumption of *M. oleifera* and *L. sativum* by residents of the research area.

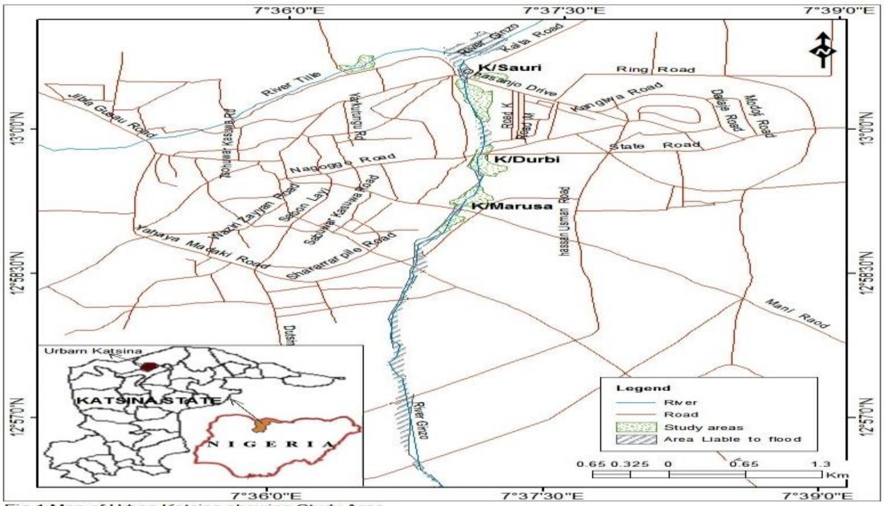

Fig.1:Map of Urban Katsina showing Study Area
Source:- National Aeronoutic and Space Administration Spot Image 2020

Figure 1: Chart of Katsina Metropolis Showing Research Areas

MATERIALS AND METHODS

Sample Collection

Water Sample

Samples were collected by adopting the standard method of the American Public Health Association (APHA) (1998) for the period of March, April and May, 2020. Before sample collection, sampling bottles were pre-cleaned and appropriately coded. Collected samples were kept in ice chest and then taken to the laboratory for analysis.

Soil Sample

Soil samples were collected at different depths of 0-20cm, 20-40cm and 40-60cm and thoroughly mixed using meter rule, auger, masking tape and labeled polythene bags as described by Mohammed and Suresha (2015). Before the analysis, samples were allowed to dry, crumpled and then sieved to obtained fine particles with the use of 2mm sieve. The prepared sample were then stored in labeled polythene bottles for analysis.

Plant Samples

A procedure described by Maleki *et al.,* (2013) was adopted. The crops were collected randomly at distances of 10, 30, 50, 70, 120 and 140m away from the first sub-samples. After collection, the samples were thoroughly mixed to give a composite representative fraction. After mixing the samples, exactly 500g of the leaves were collected from the composite sample, labeled and then placed in black polythene bags, and then taken to the laboratory for the determination of heavy metals (Gwana *et al.,* 2016).

Preparation of Samples

Plant samples

Samples were washed under tap water to remove the dirt and then washed with deionised water and allowed to dry. After drying, the samples were subjected to 75ºC for 24h in oven to remove moisture. The dried sample were crumpled into powder and then sieved at room temperature using motar and pestle and then stored in a labeled, air tight and sealed plastic bottles until required for analysis (Doherty *et al.,* 2012).

Soil samples

The procedure described by Doherty *et al.,* (2012) was used adopted. Before drying, all non-soil particles were removed from each sample including gravels. The samples were dried using oven at 95ºC for 48h and then grounded using pestle and motar into powdery form, sieved, labeled and stored in a polythene bag ready for analysis.

Heavy Metals Determination in Samples

Water samples

To 100ml of water sample in a beaker, 2ml of HNO_3 and 5ml of concentrated HCl were added to the sample. The sample was heated using a hot plate at a heat range of 90 to 95ºC until the volume changed to 15-20ml and allowed to cool. The residue was filtered with 0.45-μmfilter paper into a graduated transparent polythene bottle. The mixture was then increased to 100ml using deionized water and then refrigerated at 4ºC prior to analysis for the presence of heavy metals in the sample (Rohrbogh *et al.,* 1986).

Soil samples

A modified method of Zheljazkov and Nelson (1996) was implored for soil digestion. During the process, one gram (1grm) of the sample was placed in a 250ml beaker and 10ml of concentrated HNO_3 was added to the sample. The sample was heated for 45min at 90ºC using hot plate and then increased to 150ºC and the mixture was allowed to boil for 8 hours until a clear solution was obtained. During this process, at least 5ml of concentrated HNO_3 was occasionally added three times until the volume was reduced to about 1ml. The interior wall of the beaker was rinsed with deionized water and then swirled throughout the digestion process to keep the wall of the beaker clean and to prevent loss of sample. After cooling, 5ml of 1% HNO_3 was then added to the solution and then filtered into a transparent polythene container using a Whatman No. 42 filter paper into a transparent graduated polythene bottle and the filtrate was kept in a refrigerator at 4ºC for the presence of heavy metals.

Plant samples

Triplicate samples (0.5g) of leaves were measured in a digestion flask and 5ml of HNO_3 of concentrated HNO_3 was added to the flask. As demonstrated by Sahito *et al.,* (2002), a blank sample was prepared by adding 5ml of HNO_3 into an empty digestion flask and heated for 2 hours at 80-90^oC and later raised to 150^oC and allowed to boil. Occasionally, 3-5ml of 30% H_2O_2 and HNO_3 were added to the mixture and the digestion process was continued until a clear solution was obtained which was allowed to cool and then filtered with into a 50ml polythene bottles. The filtrates were stored in a refrigerator at 4^oC for the determination of metalloids using Buck Scientific VGP210 Atomic Absorption Spectrophotometer machine.

Estimation of Daily Intake of Metals (EDIM)

This was determined using the equation below as adopted from Onyedikachi *et al.,* (2018). Calculations were made with reference to the standard assumption for an integrated United State Environmental Protection Agency (2002) risk analysis as follows:

$$\text{EDIM} = \frac{\text{CHM x Cf x DFI}}{\text{ABW}} \quad (1)$$

Where EDIM – Estimation of Daily Intake of Metal

CHM - Concentration of Heavy Metals (ppm)

Cf - Conversion factor (weight of fresh samples was converted to dry weight using 0.085).

DFI - Daily Food Intake (0.345 kg person^{-1} day^{-1} and 0.173 kg person^{-1} day^{-1} for Nigerian adults and children respectively).

ABW - Average Body Weight (60 kg and 32.7 kg for Nigerian adults and children respectively).

Determination of Human Health Risk

Estimation Hazard Quotient (HQ)

Target Hazard Quotient (THQ) from ingestion of *M. oleifera* leaves were determined using the equation below adopted from Onyedikachi *et al*., (2018).

$$\text{THQ} = \frac{\text{EDIM}}{\text{R}f\text{D}} \quad (2)$$

HQ – Hazard Quotient

EDIM – Estimated Daily Intake of Metal

R*f*D - Reference Dose (Cu - 0.04, Pb - 0.0035, Ni - 0.02 and Co - 0.012) Arigbede *et al.,* (2019) and Chijioke *et al.* (2020)

ABW - Average Body Weight (60 kg and 32.7 kg for Nigerian adult and children respectively) Arigbede *et al.,* 2019 and Chijioke *et al.* (2020).

Hazard Index (HI)

Potential risk to the population of the study area through the ingestion of more than one heavy metal in food crops was determined by the summation of all Target Hazard Quotient for individual heavy metals (NFPCSP Nutrition Fact Sheet, 2011).

$$HI = \Sigma HI_1 + HI_2 + ... + HQn \quad (3)$$

Where 1, 2 ...... are the hazard index for the individual heavy metals determined in the food crops.

Estimation of Carcinogenic Risk

Lifetime Cancer Risk (LCR) from the consumption to carcinogenic heavy metals in *M. oleifera* was determined using the equation below adopted from Onyedikachi *et al*. (2018):

$$CR = EDIM \ X \ CSF \quad (4)$$

Where CR - Carcinogenic Risk

EDIM - Estimated Daily Intake of Metal

CSF - Cancer Slope Factor (Ni- 1.7 and Pb – 0.01).

Data Analysis

Physicochemical parameters and levels of heavy metals in samples were determined statistically at 95% confidence level using Social Package for Social Sciences (SPSS) software application version 16.0. Other analysis and calculations were performed with Microsoft Excel 2007. Result were presented in bar charts and graphs.

RESULTS AND DISCUSSION

Irrigation water

Levels of Co in water sample ranged from 1.14ppm to 1.53ppm with highest levels obtained at Kofar Marusa and Kofar Sauri and the lowest level recorded at Kofar Durbi (1.14ppm) recorded at Kofar Durbi. The concentration recorded at each study area has exceeded the Food and Agricultural Organization 1992 permissible limit as shown in figure 2. High concentrations of Co obtained in each of the study site may be due to anthropogenic activities and solid waste containing Co into the irrigation water as most pollutants from the air are deposited in it. (Muhammad *et al.*, 2020).

Levels of Co reported in this work are low compared to the values reported by Priyanka and Jatinder, (2022) and all the values reported by Muhammad *et al.*, (2020). Cobalt is cytotoxic and have genotoxic effects when it accumulates in the heart, spleen human organs and tissues such as heart, liver, spleen, kidney and lymph nodes (Beniah and Christian, 2020).

Levels of Cu in the irrigation water showed that Kofar Marusa, Kofar Durbi and Kofar Sauri has concentrations as 0.97ppm, 0.91ppm and 0.90ppm respectively which are above the permissible level of FAO, 1992 and lower than the values reported by Muhammad (2020). Copper is found naturally in animals, plants, water, air and in rocks (Boadu, 2014), its occurrence in the earth crust amounts to approximately 50ppm (Emsley, 2003). In animals, copper is the key constituent of respiratory enzymes that produces haemoglobin. In plants, it helps in the production of seed, regulation of water and as a defense against diseases (Boadu, 2014). At high concentration, copper causes anemia, liver and kidney damage, intestinal and stomach irritation and neural complications (Boadu, 2014).

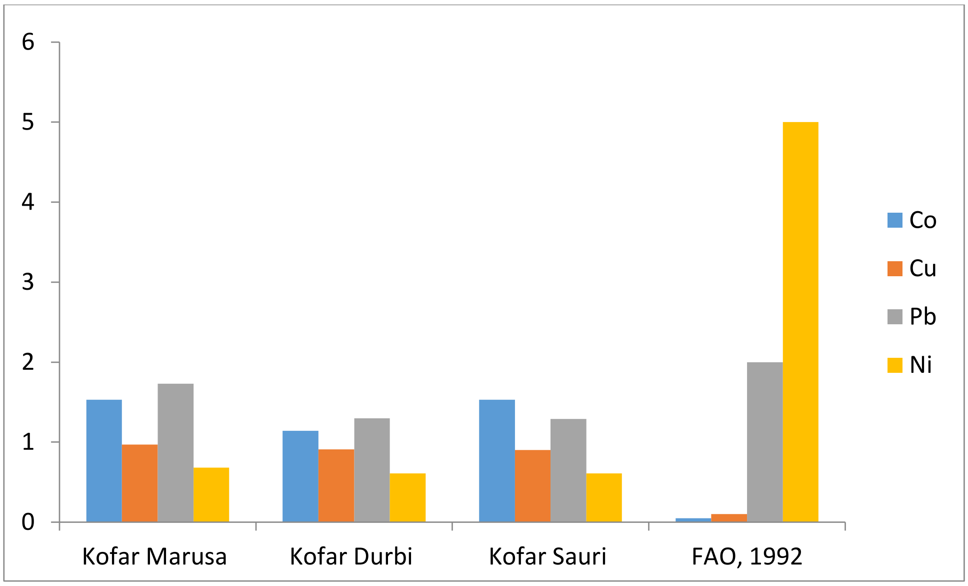

Figure 2: Concentrations of Heavy metals in irrigation water

Pollution indices of metalloids irrigation water sample
Result of heavy metals pollution indices of River Ginzo is presented in figure 3. The result showed the least pollution indices by Ni in Kofar Durbi sample (0.61) while the highest pollution indices (1.73) by Pb was recorded as slight pollution in Kofar Marusa sample.

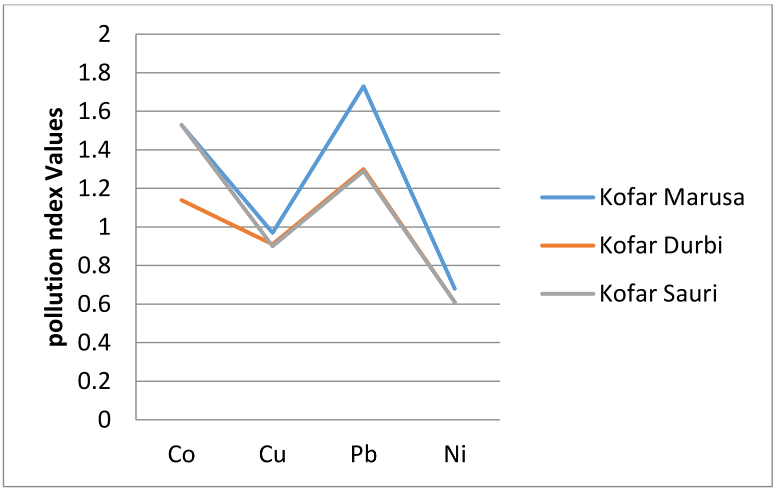

Figure 3: Pollution Indices of Heavy Metals in Irrigation Water

Concentration of heavy metals in soil samples
Among all metals analysed in soil sample, concentration of Co (1.13ppm) was found to be the highest recorded in Kofar Marusa soil sample while Ni was recorded to have the lowest concentration (0.46ppm) in Kofar Sauri sample as indicated in figure 4. Concentrations of Ni, Cu, Pb in all samples has exceeded the USEPA, (2000) permissible limit in agricultural soil.

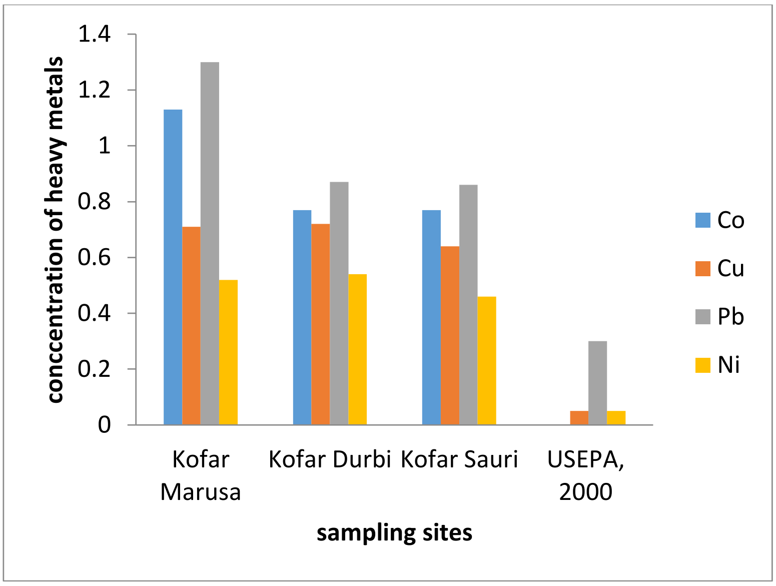

Figure 4: Concentrations of metals in soil samples

Pollution Indices of metals in soil samples

Figure 5 presents the pollution indices of heavy metals in soil samples of the research areas. The result showed lowest pollution indices by Ni with severe contamination in Kofar Marusa and Kofar Durbi and moderate contamination in Kofar Sauri soil sample. Lead was found to severely pollute Kofar Marusa sample. In Kofar Durbi and Kofar Sauri samples, both samples were very severely contaminated by Pb. This make pollution index by Pb to be highest in all samples.

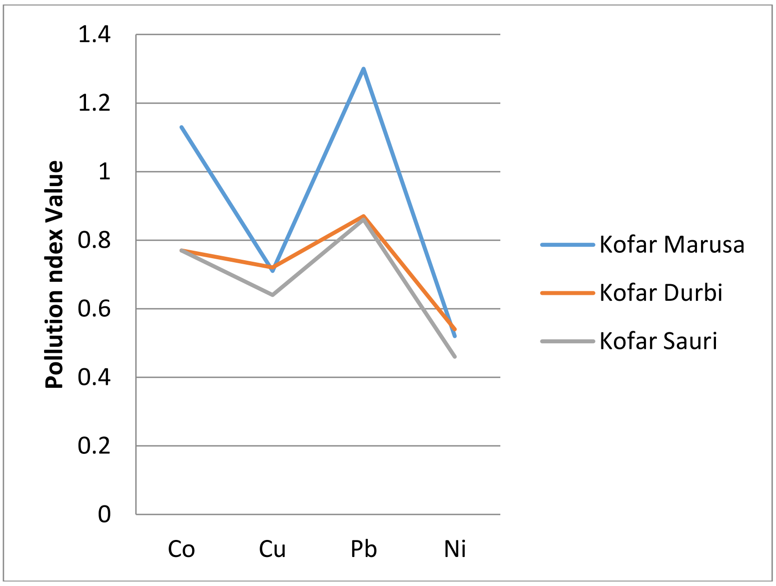

Figure 5: Pollution indices of metals in soil samples

Levels of heavy metals in leaves samples of *M. oleifera*

Determination of metals in leaves samples of *M. oleifera* showed that Co (0.77ppm) and Cu (0.23ppm) have the concentrations in leave samples of Kofar Sauri while Pb (0.12ppm) Ni (0.46ppm) have higher values in Kofar Marusa and Kofar Durbi respectively as shown in figure in figure 6. When compared with WHO/FAO (2014) permissible limit, only levels of Cu, Pb and Ni falls within the acceptable limit. Statistical analysis revealed significant difference in levels of Co, Cu and Pb at p>0.05.

A similar research conducted by Yarima and Labaran, (2017) revealed lowest concentration of Co in leaves of *M. oleifera contrary to the finding of these research.* In his research, Asieduet, (2014) reported a lower concentration of Cu in all leave sample of *M. oleifera* when compared to the values of the present research. On the contrary, research conducted by Gwana *et al.*, (2016), Pb was not detected in *M. oleifera* leave. On the contrary to the result of the present research work, Tsegaye *et al*., (2020) has not detected the presence of Pb in leave samples analysed for the presence of metals. The levels of Ni in leave samples of each sampling area are lower than the value reported by Yarima and Labaran, (2017).

In plants, absorption and distribution of Co is dependents on the plant species and is controlled by different mechanisms (Bakkaus *et al*., 2005). Even though there is limited information on the phytotoxicity of excess Co on plants, studies on Co in oilseed rape (*Brassica napus* L.), Barley (*Hordeum vulgare* L.) and tomato (*Lycopersicon esculentum* L.) showed serious effect on biomass and shoot growth (Li *et*

al., 2009), catalyse activity in leaves of cauliflower, restriction of the Fe concentration, proteins and chlorophyll.
Copper is potentially toxic but an essential element for normal plant growth. Apart from been a micronutrient for plant, Cu has an effect on photosynthesis pigmentation secretions in many leaves of different trees (Mohnish & Kumar, 2015) and leaf chlorosis (Jyotish, 2015).
Lead is one of the universally distributed and abundant toxic metals in soil. One of the means through which Pb contaminated plant is through air deposition (Kankara *et al.*, 2021). It is known to exert adverse effects on photosynthetic processes of plants and leaf development in Allium barley and *Raphanus sativas*. In *Thespesia populnea*, reduction in leaves and leaf surface area was reported due to contamination by Pb (Kabir *et al*., 2009).
Availability of Ni in soil is determined by mining activity, use of pesticides and phosphate fertilizers in farmlands, burning of oil and coal etc. In *Cajanus cajan*, Ni causes a reduction in stomata conductance and chlorophyll. Leave samples of the crops under this study reported acceptable values of Ni in line with the FAO/WHO (2014). This may be associated with the limited engagement of activities that causes the release of Ni into the environment within and around the study area.

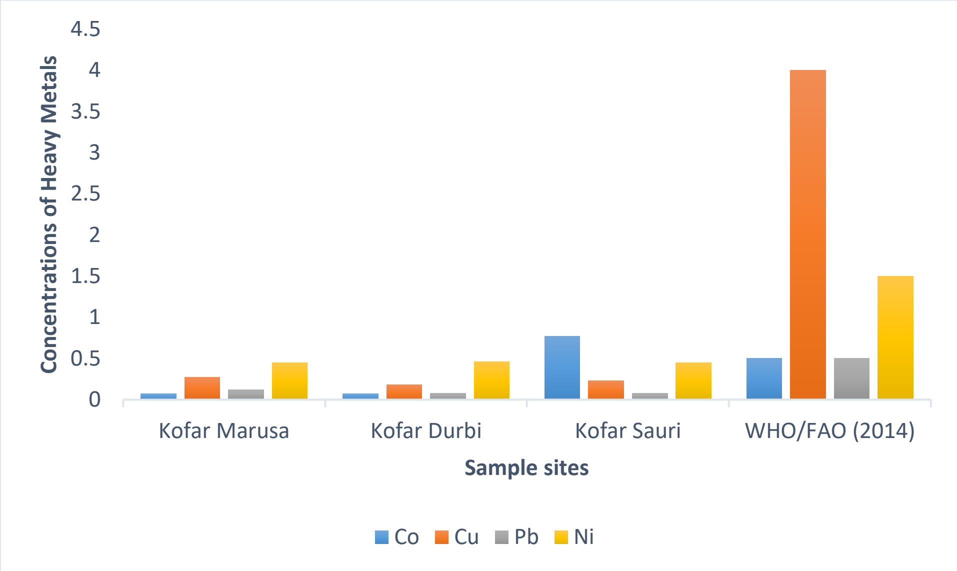

Figure 6: Levels of heavy metals in leaves of *M. oleifera*

Pollution Index of metals in Leaves of *M. oleifera*
Heavy metals pollution index in leaves of *M. oleifera* is presented in figure 7. The result showed lowest pollution index (Very Slight Contamination; 0.07) in the leave samples of Kofar Marusa and Kofar Durbi. Highest pollution index was recorded as Slight Contamination (0.46) by Ni in Kofar Durbi samples. Co in Kofar to have Very Slight was found to showed that samples of Kofar Marusa and Kofar Durbi have the least pollution index of very slight contaminated while all samples were moderately contaminated by Ni making it to be the highest pollution in leave samples.

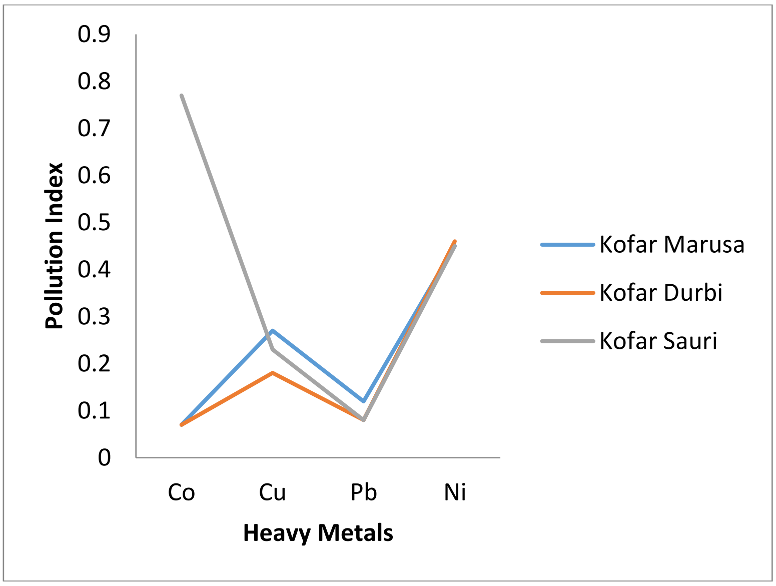

Figure 7: Level of heavy metals Pollution in leave samples of *M. oleifera*

Daily Intake of heavy metals (EDIM) by residents of the research area from the ingestion of *M. oliefera*

Table 1 presents the EDIM from the consumption of leaves of *M. oleifera* by residents of the study areas. The result further shows that intake of Ni from the consumption of *M. oleifera* leave by both adults and children is higher than the intake of Co, Cu and Pb with corresponding values as 2.12E-4 and 2.02E-4 respectively while the intake of Co in has the lowest value of 1.34E-5 (1.12%). While comparing this finding with previous research, Flora *et al.*, (2023) reported 1.30E-3 daily intake of Ni on M. *oleifera* higher than the values reported in this research.

The finding of this research on the EDIM implies no potential health risk to the population as values of the EDIM analysed from the consumption of *M. oleifera* are lower than the WHO/FAO, (2003) Tolerable Daily Intake, although there was no apparent health risk to the inhabitants of the study area due to consumption of these elements found in the leaf samples. However, potential risk could still exist through bioaccumulation after several years of exposure

Table 1: Estimated Daily Intake of Metals (ppm

Metals	Level and % Intake of *M. Oleifera*				WHO/FAO, (2003) TDI
	Adults		Children		
	Leaves	% Intake	Leaves	% Intake	
Co	1.46E-4	1.22	1.34E-5	1.12	1.20E-2
Cu	1.12E-4	0.28	1.03E-4	0.25	4.00E-2
Pb	4.39E-5	1.26	4.04E-5	1.16	3.50E-3
Ni	2.12E-4	1.09	2.02E-4	1.01	2.00E-2

Source: Field Study, 2020. Co = Cobalt, Cu = Copper, Pb = Lead, Ni = Nickel. E = Exponential, PPM = Part Per Million, WHO = World Health Organization, FAO = Food and Agricultural Organization, TDI = Tolerable Daily Intake

Non-Cancer Risk

Target Hazard Quotient

Table 2 depicts the non-cancer risk (THQ) and HI from the consumption of leaves of *M. oleifera*. Calculated THQ of the individual elements in *M. oleifera* samples were obtained to be <1 with lowest values obtained in Cu both in adults and children study population (0.0028 and 0.0025). This implies that populace of Katsina metropolis are safe to consume *M. oleifera* leaves grown in the study areas and has no obvious human risk with respect to heavy metals analyzed. This is because, both THQ an HI are <1 in all cases (USEPA, 2002). Harmanescu *et al*., (2011) reported values of THQ and HI of Pb, Cd, Mn, and Fe to be >1contrary to the result of the present research work which implies no health risk.

Table 2: Result of calculated Target Hazard Qoutient and Hazard Index from consumption of *M. oleifera* leaves

Heavy metal	THQ (Adults)	THQ (Children)	THQ Standards for Both Adult and Children (USEPA, 2002)
Co	-	-	-
Cu	0.0028	0.0025	<1
Pb	0.0125	0.0113	<1
Ni	0.0109	0.0101	<1
HI=∑THQ	0.1366	0.0239	<1
HI Standards (USEPA, 2002)	<1	<1	

Estimated Cancer Risk

Table 3 shows the ECR of the study population from lifelong consumption of leaves of *M. oleifera* and *L. sativum*. The result shows that Ni has the highest value of 2.16E-2 among carcinogenic metals analysed for cancer risk. The finding further showed that ECR from the long-time consumption of Pb in leaves of *M. oleifera* falls within the negligible and acceptable range ($1x10^{-6}$) with the exception of ECR for Ni whose value falls within the range suggesting cancer risk to the study population with percentage of 0.0216%. This may be associated with the high Cancer Slope Factor for Ni (1.70) which is higher than that of Pb (0.01). This finding is similar to that of Nyabuti (2019) and Azeez *et al*., (2020) where it was reported that Ni have the potential of causing cancer as values does not fall within the acceptable and negligible range.

As reported by Sylla and Wild, (2011) cancer has become the major source of morbidity and mortality globally. Ferlay *et al.,* (2008) reported that in 2008, there were 7.6 million cancer-related deaths and 12.7 million new cases mostly in developing countries amounting to 57% and is expected to be 70% in 2030. Boyle and Levin (2008) stated that the increased incidence may be associated to the growth in population and an increased life expectancy (Lyerly *et al.*, 2011).

In Nigeria, a yearly fatal ratio of 100,000 is reported (Ferlay *et al.*, 2008). The country represents approximately 20% population of West Africa and as reported by Sylla and Wild, (2011) Nigeria had 15% of the 681,000 new cancer cases that occurred in Africa in 2008. However, this may be related to the consumption of green leafy vegetables irrigated with wastewater containing carcinogenic elements as reported by ATSDR, 2004.

Table 3: Incremental Lifetime Cancer Risk

Heavy metal	Estimated Cancer Risk (ECR)
Co	-
Cu	-
Pb	2.63E-5
Ni	2.16E-2
USEPA Standard	$< 10^{-4}$ to 10^{-6}

Co = Cobalt, Cu = Copper, Pb = Lead, Ni = Nickel. E = Exponential

CONCLUSION

Food contamination by heavy metals has become a global concern. This research determined the pollution of irrigation water and agricultural soil and *M. oleifera* cultivated along River Ginzo, Katsina with a focus on carcinogenic and non-carcinogenic health risk assessment caused by Co, Cu, Pb and Ni. The outcome of the research showed the presence of these metals in all samples analysed. This is related to the irrigation practice using heavy metals polluted water and subsequent absorption and accumulation of the elements in both the soil and *M. oleifera* cultivated in the study area. The result further suggests that long time consumption of *M. oleifera* leaves from each of the study areas are not suitable and safe for humans owing to its tendency of accumulating heavy metals and ability to cause cancer. It is recommended that waste containing heavy metals should be disposed on government designated dump site rather than the irrigation water for human safety.

ACKNOWLEDGMENT

The researchers would like to appreciate the management and staff of Umaru Musa Yar' adua University Katsina for providing professional guidance and support during the conduct of this research.

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