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Heavy Metal Burden and Evaluation of Human Health Risks in Tomato Fruits Cultivated in Katsina State, North West Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Authors AIY and AJA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors AN, AIY, MB, AU and AI managed the analyses of the study. Authors IUM, SAY and IM managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

This study was conducted to determine the heavy metals concentration in Tomato fruits cultivated in Katsina State Nigeria. The objectives were mainly to detect the presence of heavy metals in the cultivated Tomato fruits in the study area, compare the concentration of heavy metals in samples in relation to the permissible limits specified by WHO/FAO/USEPA Standards. Samples of cultivated tomato fruits were collected in the year 2017 from the selected areas. Analysis for the

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concentration of these heavy metals; Cr, Cd, Fe, Ni, Mn, Pb and Zn was conducted by the use of AAS (by Atomic Absorption Spectrophotometry) method. The health risks to the local inhabitants from the consumption of the samples were evaluated based on the Target Hazard Quotient (THQ). The possibility of cancer risks in the samples through intake of carcinogenic heavy metals was estimated using the Incremental Lifetime Cancer Risk (ILCR). Results from this study has shown that with the exception of the mean values for the heavy metal Pb (1.171-1.21 mg/kg), the mean concentration (mg/kg) range values of Zn (0.558- 1.851), Fe (0.880-1.181), Mn (0.458-0.671) and Cd (0.054-0.062) were below the WHO/FAO maximum permissive limits. The results have indicated that the estimated daily intake (EDI) of the heavy metals were lower than the tolerable daily intake limit set by the USEPA in all the samples. All the studied tomato fruits showed the risk level (HI < 1). Risk level of Target Hazard Quotient (THQ < 1) was observed for all the evaluated heavy metals for both adults and children. The THQ for the samples were in the decreasing order Mn>Zn>Pb>Fe>Cd, for all the tomato fruits respectively. ILCR for Cd violated the threshold risk limit (>10⁻⁴) and ILCR for Pb reached the moderate risk limit (>10⁻³) in all the studied samples in adults, While in children ILCR for both Pb and Cd violated the risk. The sampling area trend of risk for developing cancer as a result of consuming the studied samples showed in decreasing order: Daura senatorial zone > Funtua senatorial zone> Katsina senatorial zone for both adult and children. Cumulative cancer risk (SILCR) of all the studied tomato fruits reached the moderate risk limit (>10⁻³) in adults, while in children it is above the moderate risk limit (>10⁻²). The study suggests that consumption of the studied tomato fruits in Katsina state is of public health concern as they may contribute to the population cancer burden.

Keywords: Tomato; heavy metals; target hazard quotient; health risk index; cancer risk.

1. INTRODUCTION

Vegetables play important roles in human nutrition and health, particularly as sources of vitamin C, thiamine, niacin, pyridoxine, folic acid, minerals, and dietary fiber [1]. Heavy metals are environmental contaminants capable of causing human health problems if excess amount is ingested through food they are non biodegradable and persistent, have a long biological half lives and can be bio-accumulated through biological chains [2]. Heavy metal toxicity may occur due to contamination of irrigation water, the application of fertilizer and metal based pesticides, industrial emission, harvesting process, transportation, storage or sale. Crops and vegetables grown in soils contaminated with heavy metals have greater accumulation than those grown in uncontaminated soils [3]. The toxicity of heavy metals most commonly involves the brain and kidney but other manifestations can occur in some other parts of the body for example arsenic is clearly capable of causing cancer, hypertension can result in individuals exposed to lead and renal toxicity in individual exposed to cadmium [4]. Tomato is a popular fruit vegetable produced and consumed in Nigeria as many people eat it in different forms in the preparation of stew, soup and food [5]. In Katsina State Nigeria, there is limited information on the levels of heavy metals in locally cultivated vegetables. This work there-fore seeks to bridge

that gap by providing information especially to the Katsina State populace on the levels of heavy metals of this most consumed vegetable. Information will further be provided on the heavy metals composition of the sources of these vegetables and the extent to which they are contaminated with these heavy metals for future studies and effective comparative analysis. Data on heavy metal in the cultivated tomato generated will give an insight on the level of metal contamination and by extension the impact on food safety standard and risk to consumers. The objective of this study therefore was to evaluate human exposure to some heavy metals through consumption of some locally cultivated vegetables in Katsina State, Nigeria.

2. MATERIALS AND METHODS

2.1 Study Area

The study was carried out in 2017 in Katsina State, Nigeria located between latitude 12°15'N and longitude 7°30'E in the North West Zone of Nigeria, with an area of 24,192 km2 (9,341 m²). Katsina State has two distinct seasons: Rainy and dry. The rainy season begins in April and ends in October, while the dry season starts in November and ends in March. This study was undertaken during the dry season. The average annual rainfall, temperature, and relative humidity of Katsina State are 1,312 mm, 27.3°C

and 50.2%, respectively [6]. The study was conducted within some catchment areas that cultivate tomatoes located within the 3 senatorial zones that make up the state (Katsina senatorial zone: Funtua senatorial zone; Daura senatorial zone). Sampling for this work was carried out by dividing the catchment areas into five [5] locations. In each of the locations, the plot where the tomatoes are cultivated was subdivided into twenty (20) sampling areas. Samples of tomato fruits were collected from each of the areas and combined to form bulk sample, from which a representative sample was obtained. The samples were code-named and stored in glass bottles with tight covers to protect them from moisture and contamination. They were then stored in the refrigerator at 4°C until ready for use.

2.2 Identification of Sample

The samples were identified in the herbarium of the Department of Biology of Umaru Musa Yar'adua University Katsina.

2.3 Sample Preparation

The collected samples were cleaned by using dry air to remove air borne pollutants, and the samples were fragmented with clean plastic spoon and knife and dried at ambient temperature. After drying, the seeds were removed from dried fruits. They were then stored in the refrigerator at 4°C until ready for used.

2.4 Heavy Metals Determination

5 g of each Sample was dried at 80°C for 2 hours in a Gallenkamp hotbox oven (CHF097XX2.5) and then blended in an electric blender. 0.5 g of each sample was weighed and ashed at 550°C for 24 hours in an electric muffle furnace (Thermolyne FB131DM Fisher Scientific). The ash was diluted with 4.5 ml concentrated hydrochloric acid (HCI) and concentrated nitric acid (HN0₃) mixed at ratio 3:1 the diluent is left for some minutes for proper digestion in a beaker. 50 ml of distilled water was added to the diluents to make up to 100 ml in a volumetric flask. The levels of heavy metals (Pb, Zn, Ni, Cd, Cr, Mn and Fe) were determined using AA210RAP BUCK Atomic Absorption Spectrometer flame emission spectrometer filter GLA-4B Graphite furnace (East Norwalk USA). Analytical blanks were run in the same way as the samples and concentrations were determined using standard solutions prepared in the same acid matrix. Standards for the instrument calibration were prepared on the basis of mono element certified reference solution ICP Standard (Merck). The potential contamination of the samples was evaluated by analyzing one acid blank in every batch. The instrument's setting and operational conditions were done in accordance with the manufacturer's specifications. The values of heavy metals (in triplicates) were calculated based on dry weights of the samples [7] and the results were given in (mg/kg).

2.5 Heavy Metal Health Risk Assessment

2.5.1 Daily Intake of Metals (DIM)

The daily intake of metals was calculated using the following equation:

$$\text{DIM} = \frac{C_{\text{metal}} * C_{\text{factor}} * D_{\text{intak}}}{B_{\text{weight}}}$$

Where, C_{metal} , C_{factor} , D_{intake} and B_{weight} represent the heavy metal concentrations in the tomato samples, the conversion factor, the daily intake of the sample and the average body weight, respectively. The conversion factor (CF) of 0.085 [8] was used for the conversion of the tomato samples to dry weights. The average daily intake of the tomatoes was 0.527 kg person⁻¹ d⁻¹ [9] and the average body weight for the adult and children population was 60 kg [10] and 24 kg [11] respectively; these values were used for the calculation of HRI as well.

2.5.2 Non-cancer risks

Non-carcinogenic risks for individual heavy metal for vegetable were evaluated by computing the target hazard quotient (THQ) using the following equation [12].

THQ=CDI/R_fD

CDI is the chronic daily heavy metal intake (mg/kg/day) obtained from the previous section and R_fD is the oral reference dose (mg/kg/day) which is an estimation of the maximum permissible risk on human population through daily exposure, taking into consideration a sensitive group during a lifetime [13]. The following reference doses were used (Pb = 0.6, Cd = 0.5, Zn = 0.3, Fe = 0.7, Ni = 0.4, Mn = 0.014, Cr = 0.3) [14; 15]. To evaluate the potential risk to human health through more than one

heavy metal, chronic hazard index (HI) is obtained as the sum of all hazard quotients (THQ) calculated for individual heavy metals for a particular exposure pathway [16]. It is calculated as follows:

HI=THQ₁+THQ₂+···+THQ_n

Where, 1, 2..., n are the individual heavy metals for Tomato fruit samples.

It is assumed that the magnitude of the effect is proportional to the sum of the multiple metal exposures and that similar working mechanism linearly affects the target organ [17]. The calculated HI is compared to standard levels: the population is assumed to be safe when HI < 1 and in a level of concern when 1 < HI < 5 [18].

2.6 Cancer Risks

The possibility of cancer risks in the studied samples through intake of carcinogenic heavy metals were estimated using the Incremental Lifetime Cancer Risk (ILCR) [19].

ILCR= CDI×CSF

Where, CDI is chronic daily intake of chemical carcinogen, mg/kg BW/day which represents the lifetime average daily dose of exposure to the chemical carcinogen.

The US EPA ILCR is obtained using the cancer slope factor (CSF), which is the risk produced by a lifetime average dose of 1 mg/kg BW/day and is contaminant specific [12]. ILCR value in sample represents the probability of an individual's lifetime health risks from carcinogenic heavy metals' exposure [20]. The level of acceptable cancer risk (ILCR) for regulatory purposes is considered within the range of 10^{-6} to 10^{-4} [13]. The CDI value was calculated on the basis of the following equation and CSF values for carcinogenic heavy metals were used according to the literature [19].

CDI = (EDI × EFr × ED_{tot})/AT

Where, EDI is the estimated daily intake of metal via consumption of the tomato fruit; EFr is the exposure frequency (365 days/year); ED_{tot} is the exposure duration of 60 years, average lifetime for Nigerians; AT is the period of exposure for non-carcinogenic effects (EFr × ED_{tot}), and 60 years life time for carcinogenic effect [12]. The cumulative cancer risk as a result of exposure to

multiple carcinogenic heavy metals due to consumption of a particular type of food was assumed to be the sum of the individual heavy metal increment risks and calculated by the following equation [19].

 Σ ILCRn=ILCR₁+ILCR₂+···+ILCR_n

Where, n = 1, 2, ..., n is the individual carcinogenic heavy metal

3. RESULTS AND DISCUSSION

The present study investigated the presence of heavy metals in Tomato which is a major component of the diet among the population in Katsina state, Nigeria. A total of 3 composite samples were analyzed for the presence of heavy metals in this study. As shown in Table 1, among the heavy metals evaluated, the highest concentration (mg/kg) was observed for Zn (range: 0.558- 1.851), followed by Pb (range: 1.171-1.21), Fe (range: 0.880-1.181) and Mn (range: 0.458-0.671). While Cd has the lowest concentration (range: 0.054-0.062). The results for the heavy metals analysed in the sampled seeds is similar to that reported for heavy metals in beans and some beans products from some selected markets in Katsina state, Nigeria [21].

Lead was detected in all the samples, with 100% of samples seen to be higher than 0.01 mg/kg which is the maximum permissible limit set by WHO/FAO and also the maximum allowable concentration of 0.02 mg/kg by EU and 0.05 mg/kg limit set by USEPA [22]. The violation of the maximum permissible limits of Pb set by the WHO, EU, and US EPA is a cause for public health concern considering the frequency of exposure. The Pb concentration range for the tomato fruit samples in this study is lower than that reported for ginger (22 mg/kg) and in Negro pepper (5 mg/kg) in a study on the heavy metal content of spices in Abuja, Nigeria [23], that reported for leafy vegetables from Kaduna state Nigeria [24] and that reported for beans samples from Italy, Mexico, India, Japan, Ghana and Ivory Coast with a Pb concentration range of 4.084-14.475 ppm [25]. But the results are higher than the result reported for Pb in carrot and cucumber from Awka, Anambra state Nigeria [26].

The Cd concentration range for the samples in this study is lower than that reported for market sold legumes in eastern Nigeria, Europe, Asia and parts of West Africa [27,25], but the values are similar to that reported in a study for the Cadmium concentration range for both unprocessed and processed bean samples from Katsina state Nigeria [21], the Cd concentration in spice samples ranged from 0.45 mg/kg, in garlic, locust beans and onion and 0.3 mg/ kg in ginger reported in a study conducted on spices from Odo-Ori market Iwo, Nigeria [28] and the Cd in cucumber from Awka, Anambra state Nigeria [26]. The concentration of Cd (mg/kg) range from 0.054000 to 0.062000 in the tomato samples obtained in the present study are higher than the range (0.002 to 0.004 mg/kg) reported by Edem et al. in Wheat flours in 2009 [29]. These values are however, below the WHO (2005) safe limit for Cd (0.3 mg/kg) in spices [30].

In the present study, the mean Fe concentration in all the tomato samples is higher than the results reported by Fatoba et al. [5] in tomatoes, but is similar to that reported for market sold beans from Katsina, Nigeria [21] and lower than that reported in a study in eastern Nigeria [27] and that recorded by Zahir et al. [31] in a study conducted in Pakistan, the results for the study conducted by Di Bella et al. [25] on variety of beans from Mediterranean and Tropical areas and the result of Fe in tomato fruits conducted in Jordan [32] in a study conducted on heavy metals in spices that reported Fe concentrations of 56 mg/kg for cardamon and 650 mg/kg for mint [33] and the result for Fe in turmeric (840.69 mg/kg), red chili (807.60 mg/kg) and coriander (695.91 mg/kg) reported by Das et al. [34]. But the results are higher than the values reported for Fe in carrot and cabbage from Awka, Anambra state Nigeria [26].

The result for the heavy metal Mn concentrations in the present study is lower than the result of Mn levels in turmeric (76 mg/kg), red chilli (74.02 mg/kg) and coriander (52.91 mg/kg) reported by Das et al. [34] in their study conducted in Chittagong Metropolitan City, Bangladesh to evaluate heavy metals in spices and results of evaluation of heavy metals in various foods reported in other studies [27; 25]. But is similar to that reported by Yaradua *et al*, in a study of Mn levels in beans from Katsina state, Nigeria [21].

The heavy metal Zn values obtain in this study is similar to that reported in Zn levels in various foods in some studies [35,36,21], but are higher than the range (0.04 to 0.19 mg/kg) reported by Edem et al. in 2009 in wheat flours [29], the study conducted on heavy metals in tomato by Fatoba et al. [5] in llorin, but far below the Zn values reported for turmeric (75.5 mg/kg), red chilli (68.78 mg/kg) and coriander (87.89 mg/kg) by Das et al. [34] and the Zn range reported by Ahmed and Mohammed in 2005 (4.893 to 15.450 mg/kg) in foodstuff from Egyptian markets [37] and that reported in a study conducted by Sulyman et al. [38] in cereals from Kaduna state. These values also falls below the WHO permissible limit (100 mg/kg) for Zn in spices [30] and can also not provide for the required daily allowance for Zn which is 11mg/day for men and 8mg/day for women [39].

In the present study, an important finding was the absence of Cr and Ni in all the analyzed samples. There are several possible explanations for this result; e.g., low level of Cr and Ni in agricultural soil, limitation of Cr and Ni contamination sources and no intake or accumulation of Cr and Ni by the studied vegetables.

The degree for heavy metal toxicity to humans depends on daily consumption rate [40]. The results for the estimated daily intake (EDI) of the heavy metals on consumption of the samples were given in Tables 2 and 3. From the tables with the exception of the heavy metal Pb the estimated daily intake of the heavy metals (Zn,

 Table 1. Heavy metal concentration (mg/kg) in tomato fruit cultivated in the three senatorial zones of Katsina State

Zone	Pb	Cr	Zn	Ni	Fe	Mn	Cd
Katsina	1.171	BDL	1.156	BDL	0.880	0.458	0.055
	± 0.0004		± 0.0003		± 0.0014	± 0.0007	± 0.0001
Funtua	1.260	BDL	0.558	BDL	1.181	0.658	0.054
	± 0.0001		± 0.0003		± 0.0002	± 0.0001	± 0.0001
Daura	1.248	BDL	1.851	BDL	1.136	0.671	0.062
	± 0.0007		± 0.0001		± 0.0014	± 0.0003	± 0.0002

Values are expressed as Mean ± SD

Key: BDL (Below detection level)

Cd, Cr, Fe and Mn) in adults and children were lower than the tolerable daily intake limit set by the USEPA [41] in all the samples.

The non-cancer risks (THQ) of the investigated heavy metals through the consumption of tomato fruits for both adults and children inhabitants of the study area were determined and presented in Tables 2 and 3. The THQ has been recognized as a useful parameter for evaluating the risk associated with the consumption of metalcontaminated foods [42]. THQ is interpreted as either greater than 1 (>1) or less than 1 (<1), where THQ >1 shows human health risk concern [43]. Bhalkhair and Ashraf [9] in their study have put forward the suggestion that the ingested dose of heavy metals is not equal to the absorbed pollutant dose in reality because a fraction of the ingested heavy metals may be excreted, with the remainder being accumulated in body tissues where they can affect human health. Risk level of Target Hazard Quotient (THQ < 1) was observed for all the evaluated heavy metals for both adults and children. It indicates that intake of these heavy metals through consumption of the tomato fruits does not poses a considerable non-cancer risk. The THQ for the samples was in the decreasing order Mn>Zn>Pb>Fe>Cd, for all the tomato fruits respectively. The sequence of risk was the same for both adults and children although the children had higher THQ values in all cases. Similar observations have been reported previously by Mahfuza et al. [44], Micheal et al. [12] and Liu et al. [19].

Furthermore, the non-cancer risks for each type of the tomato fruits were expressed as the cumulative HI, which is the sum of individual metal THQ. All the studied tomato fruits showed the risk level (HI < 1) with highest in tomato fruit

sample from Daura senatorial zone and lowest in tomato fruit from Katsina senatorial zone.

Cd and Pb are classified by the IARC as being carcinogenic agents [45,46]. Chronic exposure to low doses of Cd, and Pb could therefore result into many types of cancers [47]. US-EPA recommended the safe limit for cancer risk is below about 1 chance in 1,000,000 lifetime exposure (ILCR < 10^{-6}) and threshold risk limit $(ILCR > 10^{-4})$ for chance of cancer is above 1 in 10,000 exposure where remedial measures are considerable and moderate risk level (ILCR > 10^{-3}) is above 1 in 1,000 where public health safety consideration is more important [20,48]. ILCR for Cd violated the threshold risk limit $(>10^{-4})$ and ILCR for Pb reached the moderate risk limit (> 10^{-3}) in all the studied samples in adults, While in children ILCR for both Pb and Cd violated the risk. The sampling area trend of risk for developing cancer as a result of consuming the studied samples showed: Daura senatorial zone > Funtua senatorial zone> Katsina senatorial zone for both adult and children (Tables 4 and 5).

Moreover, cumulative cancer risk (\sum ILCR) of all the studied tomato fruits reached the moderate risk limit (>10⁻³) in adults, while in children it is above the moderate risk limit (>10⁻²). Further, among all the studied samples, tomato sample from Daura senatorial zone has the highest chances of cancer risks (ILCR 6.56425 × 10⁻³ in adults; ILCR 1.641064 × 10⁻² in children) and tomato sample from Katsina senatorial zone has the lowest chances of cancer risk (ILCR 6.123718 × 10⁻³ adults; ILCR 1.530924 × 10⁻² in children). These risk values indicate that consumption of the tomato sample from Daura senatorial would result in an excess of 66 cancer cases per 10,000 people exposure in adults and

Table 2. Daily metal intake target hazard quotient and health risk index in adults from consumption of tomato fruit cultivated in the three senatorial zones of Katsina State

Heavy metal	Daily intake of metal			Target hazard quotient		
	Katsina	Funtua	Daura	Katsina	Funtua	Daura
Mn	0.000342	0.000491	0.000501	0.024424	0.035089	0.035783
Zn	0.000863	0.000417	0.001382	0.002877	0.001389	0.004607
Pb	0.000874	0.000941	0.000932	0.001457	0.001568	0.001853
Cd	0.000049	0.000040	0.000046	0.000068	0.000983	0.001002
Ni	BDL	BDL	BDL	BDL	BDL	BDL
Fe	0.000657	0.000882	0.000848	0.000939	0.001260	0.001212
Cr	BDL	BDL	BDL	BDL	BDL	BDL
Health Risk Index				0.038827	0.040288	0.044156

Key: BDL (Below detection level)

Heavy metal	Daily intake of metal			Target hazard quotient		
	Katsina	Funtua	Daura	Katsina	Funtua	Daura
Mn	0.000855	0.001228	0.001253	0.061060	0.087724	0.089457
Zn	0.002158	0.003472	0.003455	0.002193	0.003472	0.011516
Pb	0.002186	0.002352	0.002329	0.003642	0.003920	0.003882
Cd	0.000103	0.000110	0.000114	0.000205	0.000220	0.000231
Ni	BDL	BDL	BDL	BDL	BDL	BDL
Fe	0.001643	0.002204	0.002120	0.002346	0.003149	0.003029
Cr	BDL	BDL	BDL	BDL	BDL	BDL
Health Risk Index				0.074442	0.098484	0.108115

Table 3. Daily metal intake target hazard quotient and health risk index in children from consumption of tomato fruit cultivated in the three senatorial zones of Katsina State

Key: BDL (Below detection level)

Table 4. Incremental life time cancer risk in children from consuming of tomato fruit cultivated in the three senatorial zones of Katsina State

Zone	ILCF	∑ILCR	
	Pb	Cd	
Katsina	1.376942E-02	1.539825E-03	1.530924E-02
Funtua	1.481594E-02	1.651815E-03	1.636776E-02
Daura	1.467484E-02	1.735800E-03	1.641064E-02

Table 5. Incremental life time cancer risk in adults from consuming of tomato fruit cultivated in the three senatorial zones of Katsina State

Zone	ILC	∑ILCR	
	Pb	Cd	
Katsina	5.507768E-03	6.159300E-04	6.123718E-03
Funtua	5.926378E-03	6.047250E-04	6.531103E-03
Daura	5.869936E-03	6.943200E-04	6.564256E-03

16 cancer cases per 1,000 people exposure in children, while consumption of the tomato sample from Katsina senatorial zone would result in an excess of 61 cancer cases per 10,000 people exposure in adults and 15 cancer cases in children per 1,000 people exposure [40]. Prompt action should be needed to control the excess use of heavy metal-based fertilizer and pesticides and also emission of heavy metal exhaust from automobiles should be checked to save the population from cancer risk.

4. CONCLUSION

This study determines the heavy metals concentration in tomato fruits from the 3 senatorial zones (Katsina, Funtua and Daura) of Katsina state Nigeria. Results from this study has shown that concentration values of Mn, Zn, Pb, Cd and Fe in the samples were generally lower than the USEPA, WHO/FAO maximum permissive limits. The results have indicated that the estimated daily intake of the heavy metals were lower than the tolerable daily intake limit set by the USEPA (2013) in both samples. All the studied tomato fruits showed the risk level (HI < 1). Risk level of Target Hazard Quotient (THQ < 1) was observed for all the evaluated heavy metals for both adults and children. It indicates that intake of these heavy metals through consumption of the local fruits does not poses a considerable non-cancer risk. Therefore the intake of individual heavy metals through consumption of tomato fruits in this area is safe for the inhabitants. The THQ for the samples was in the decreasing order Mn>Zn>Pb>Fe>Cd, for all the tomato fruits respectively. ILCR for Cd violated the threshold risk limit $(>10^{-4})$ and ILCR for Pb reached the moderate risk limit (> 10^{-3}) in all the studied samples in adults, While in children ILCR for both Pb and Cd violated the risk. The sampling area trend of risk for developing cancer as a result of consuming the studied samples showed: Daura senatorial zone > Funtua senatorial zone> Katsina senatorial zone for both adult and children. Cumulative cancer risk (*SILCR*) of all the studied tomato fruits reached the moderate risk limit $(>10^{-3})$ in adults, while in children it is above the moderate risk limit (> 10^{-2}). The study suggests that consumption of the studied tomato fruits in Katsina state is of public health concern as they may contribute to the population cancer burden.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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