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Research Article

Carcinogenic Potentials of Toxic Metals and Polycyclic Aromatic Hydrocarbons in *Telfairia occidentalis* and *Talinum triangulare* Impacted by Wastewater, Southern Nigeria

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Abstract: Health risks of toxic metals and polycyclic aromatic hydrocarbons (PAHs) associated with the consumption of *Telfairia occidentalis* and *Talinum triangulare* impacted by wastewater from water treatment plants within Uyo Metropolis, Southern Nigeria, were investigated using standard methods. The level of toxic metals and PAHs was higher in vegetables impacted by wastewater from food processing industries than in the other investigated sources. The levels of cadmium (Cd), lead (Pb), arsenic (As), and chromium (Cr) in the vegetables exceeded their recommended limits by the Food and Agriculture Organization (FAO) and the World Health Organization (WHO). Wastewater was confirmed as the major source of toxic metals in the vegetables. The estimated chronic daily intake of toxic metals through vegetables for both child and adult populations was lower than their tolerable daily intake. The hazard quotients of non-carcinogenic PAHs indicated higher values for children than adults. The vegetables contained all the priority PAHs except dibenz[a,h]anthracene. The estimated daily intake of PAHs was higher in children than in adults, except for phenanthrene. The threshold screening values for PAHs indicated lower non-carcinogenic health risks for consumers. The carcinogenic health risks of metals and PAHs via vegetables indicated a moderately high risk for both populations. The consumption of vegetables impacted by wastewater could result in serious health problems for both child and adult populations. This study has exposed the environmental and human health problems related to the use of wastewater for the irrigation of vegetable gardens, which hitherto were unavailable in the study area.

***Keywords*:** wastewater, vegetables, toxic metals, PAHs, carcinogenic and non-carcinogenic risks, Nigeria

1. Introduction

The high population density in urban areas within the developing countries of the world is associated with the scarcity of potable water. Consequently, farmers have resorted to the use of wastewater for the irrigation of their farmlands. In Nigeria, for instance, most farms, especially the ones cultivated with vegetables, are located close to water treatment plants for water accessibility. These farmers are also interested in the wastewater from these treatment plants for its high nutrient content [1-3]. However, literature has shown that wastewater is a good source of toxic metals, organic contaminants, and pathogens in the soil and subsequently in the plants cultivated there [4-8]. It has also been reported that wastewater from water treatment plants has high levels of polycyclic aromatic hydrocarbons (PAHs) [9-11]. These toxic organic substances may also be transferred to the soil environment and vegetables grown on it. Studies

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have shown that the high accumulation of PAHs in vegetables and their cancer-causing potential are becoming serious health problems [12-14]. High levels of toxic metals have been reported in vegetables cultivated in soils irrigated with wastewater from different sources [15-18]. The consumption of vegetables highly polluted with inorganic and organic toxic substances may also result in serious human health problems [19]. In Nigeria, vegetables, especially *Telfairia occidentalis* (fluted pumpkin) and *Talinum triangulare* (water leaf), are widely cultivated and consumed [20]. Hence, the cultivation and processing of these vegetables should be closely monitored to avoid serious health problems along the food chain. Studies have also established that leafy vegetables have the capacity of accumulating high levels of toxic substances in their edible parts and transferring them to consumers [21, 22]. Despite the intensive consumption of these vegetables, information on their processing, suitability, or otherwise for human consumption is still scanty. The use of untreated or partially treated wastewater for irrigation may result in several human health problems [23-25]. Nevertheless, the use of treated wastewater for farming is a good source of plant nutrients [26-28]. Several techniques could be applied by industries for the proper treatment and removal of emerging toxic contaminants from their wastewater, and these include liquid-liquid extraction, filtration and flotation, chemical precipitation, and ion exchange [29-31]. This study has indicated the impact of wastewater on the metal loads in vegetables and the human health problems associated with the consumption of vegetables impacted by wastewater within the study area. Previous studies in this area concentrated mainly on the characterization of wastewater from industries, but they never considered assessing the potential negative impacts of irrigating edible plants by the wastewater [32-34]. This study investigated the impact of irrigating vegetables with wastewater from water treatment plants and the human health risks associated with the consumption of contaminated vegetables. It focused on examining the accumulation of some selected toxic metals and PAHs in two vegetable types, i.e., *T. occidentalis* and *T. triangulare*, that are commonly consumed among locals. The study further assessed the carcinogenic health risks associated with this practice.

2. Materials and methods

2.1 *Description of study locations*

Three water treatment plants in Akwa Ibom State, Nigeria, namely: Ibom Hotels and Golf Resort wastewater treatment plant (Station 1), Champion Brewery wastewater treatment plant (Station 2), and Akwa Ibom Water Company wastewater treatment plant (Station 3), were used for this study. The descriptions of each of the stations including their coordinates were given below:

Station 1: The Ibom Hotels and Golf Resort is popularly known as a five-star hotel treatment plant that is located between latitude 5° 03’ 26.8” N and longitude 8° 02’ 20.7” E at Nwanaiba, Uruan, Akwa Ibom State. The hotel was established in 2006 and commenced full operation in 2008. It covers a land mass of 176 hectares and can treat 2,000 cubic meters of water per day for use in the hotel and the immediate environment.

Station 2: The plant is located between latitude 5° 04’ 55.7” N and longitude 9° 20’ 08.63” E along Aka Road, Uyo, Akwa Ibom State. The company was incorporated on the 31st of July 1979, and started operating in December with an initial capacity of 150,000 hectoliters of beer per annum. After a major upgrade in the year 2000, production capacity was increased to 500,000 hectoliters of beer per annum. The plant can treat about 6,000 cubic meters of water per day.

Station 3: The Akwa Ibom Water Company wastewater treatment plant is located between latitude 5° 02’ 1.1” N and longitude 7° 56’ 19.6” E at Dominic Utuk Avenue, Uyo, Akwa Ibom State. The water treatment facility was built in 1989 and expanded in 1992 to a capacity of 130,000 cubic meters per day before it began operation. It can provide treated water to urban, semi-urban, and rural communities in Akwa Ibom State.

The Control Station: The Control Station is a vegetable garden where tap water is used for irrigation. Hence, wastewater has no impact on the vegetables cultivated there. The farm is located between 5° 06’ 28.4” N and longitude 8° 04’ 22.5” E along Nwanaiba Road in Uyo Metropolis, Akwa Ibom State.

2.2 *Analytical methods*

Fresh leaves of *T. occidentalis* and *T. triangulare* were collected from farmlands within the designated wastewater treatment plants using a stainless-steel knife. The collection of samples was done in the early hours of each day between 8:00 and 9:00 am. The leaves of *T. occidentalis* and *T. triangulare* were also collected from the Botanical Garden of the University of Uyo and used as controls. Both the studied vegetables and controls were packed into well-labeled polyethylene containers and transported to the laboratory. A total of 36 subsamples and 12 composite samples of *T. occidentalis* and *T. triangulare* were collected for toxic metals and PAHs determination. Six subsamples and two composite vegetables were obtained as controls for toxic metals determination. Meanwhile, six subsamples and two composite samples were also obtained as controls for the determination of PAHs. Samples for metal determination were dried in an oven at a temperature of 105 °C for six hours to eliminate water and other liquids. These samples were disaggregated to fine sizes using an electrical grinder (Binatone, Model BLG-402), and the resulting powdered samples were sieved mechanically to obtain fractions that were approximately 60 µm. The powdered samples were preserved in polyethylene containers for subsequent digestion and atomization. The samples and their controls meant for PAH analysis were preserved in a refrigerator at a temperature of 4 °C before analysis.

2.3 *Determination of toxic metals and polycyclic aromatic hydrocarbons in the vegetables*

2.3.1 *Determination of toxic metals in the vegetables*

One gram of each of the powdered samples was placed in a crucible with 10 mL of aqua regia (a mixture of hydrochloric acid (HCl) and nitric acid (HNO_3) in a ratio of 3:1). The solution was left to digest under reflux for 24 hours and then heated at 70 °C in a water bath for three hours. When cooled, the digest was decanted into a 20-mL standard flask and filled to the mark with de-ionized water. The concentration of toxic metals in the filtrate was obtained using an Agilent 710 inductively coupled plasma optical emission spectrophotometer (Perkin Elmer, Model Optima 5300 DV).

2.3.2 *Determination of polycyclic aromatic hydrocarbons in the vegetables*

PAH contents in studied vegetables were determined following a modified method by Tao et al. [35]. The homogenized air-dried sample was fortified with the surrogate standard solution and saponified with 45 mL of methanolic potassium hydroxide. After repeated extractions in n-hexane, further clean-up was carried out using a silica-SPE cartridge. The extract was concentrated to a 1 mL volume and analyzed by gas chromatography-mass spectrophotometer (GC-MS) using Agilent 7890 gas chromatography with a 5975C Inert Triple Axis Mass Selective Detector as described by Camargo and Toledo [13]. The instrument detection limits (IDLs) were obtained as the concentration of targeted compounds in a sample that results in peaks with a signal-to-noise (S/N) ratio of 3:1. The individual PAH IDLs for the GC-MS setup ranged from 0.01 and 0.03µg/L. The derivation of the limits of detection (LOD) and quantification (LOQ) was based on the standard deviation of the response (α) and the slope of the calibration curve (S) at levels approaching the limits according to the expressions: LOD = 3.3 (α/S) and LOQ = 10 (α/S). The LOD and LOQ values derived for standards of the target compounds were from 0.05 to 0.22 and 0.05 to 0.19 µg/L, respectively. Concentrations below the IDL were denoted as less than the method detection limit (<MDL).

2.4 *Health risk assessment of toxic metals and polycyclic aromatic hydrocarbons*

Cancer and non-cancer health risks associated with the consumption of *T. occidentalis* and *T. triangulare* impacted by wastewater from water treatment plants within Uyo Metropolis, Nigeria, for child and adult populations were assessed. The assessments were done for both toxic metals and PAHs using standard procedures as indicated in the equations below. The non-carcinogenic risk assessment for toxic metals and PAHs was carried out by determining the estimated chronic daily intake (*ECDI*), hazard quotient (*HQ*), total hazard index (*THI*), and screening value. Meanwhile, the carcinogenic health risk was assessed using an incremental lifetime cancer risk (*ILCR*) model. The estimated screening value and carcinogenic toxic equivalent (*TEQ*) for the PAHs were also carried out.

2.4.1 *Non-carcinogenic health risk*

The *ECDI*s for toxic metals and PAHs were computed using Equation 1:

$$ECDI = (MI \times MC)/BW \tag{1}$$

where *MI* is the estimated quantity of vegetables consumed in kg/person/day, *MC* is the mean concentration of each toxic metal and PAH in the vegetables expressed in mg kg^{-1}, and *BW* represents the average body weight for children and adults in kg. The estimated quantities of vegetables used in this study for computing *ECDI* for both child and adult populations are as reported by Nabulo et al. [36]. The *HQ* of individual toxic metals and PAHs was determined using Equation 2:

$$HQ = \frac{Ef \times EDtotal \times EDI}{RfDo \times BW \times AT} \times 10\text{E-03} \tag{2}$$

where *Ef* stands for exposure frequency, *EDtotal* represents the total exposure duration, *EDI* is the estimated daily intake of each metal, *RfDo* depicts the oral reference dose, and *AT* is the average time for non-carcinogens. The units for the parameters are indicated in Table 1. The *THI* for toxic metals and PAHs was calculated by summing up the *HQs* of the contaminant according to Equations 3 and 4:

$$THI = \Sigma HQ = HQCd + HQPb + HQAs + HQCr + HQNi \tag{3}$$

$$THI = \sum_{i=1}^{n} HQi(individual\ non\text{-}carcinogenic\ PAHs) \tag{4}$$

Table 1. Parameters used for estimating the risks associated with the consumption of *T. occidentalis* and *T. triangulare* and values used

No	Parameter	Value	Source
1.	Body weight (*BW*; kg)	Child = 15 kg and adult = 70 kg	[38, 39]
2.	Estimated consumption rate of studied vegetables (*MI*; kg/person/day)	Child = 0.118 and adult = 0.182	[36]
3.	Exposure frequency (*Ef*)	350 days/year	[40]
4.	Exposure duration (*EDtotal*)	Child = 6 years and adult = 30 years	[41]
5.	Maximum acceptable risk level (*RL*)	10^{-5}	[39]
6.	Estimated daily intake of toxic metals and PAHs (*EDI*)	Values in Tables 4 and 6	-
7.	Mean concentration of toxic metals in vegetables (*MC*; mg kg^{-1})	Values in Table 2	-
8.	Concentration of PAHs in vegetables (mg kg^{-1})	Values in Table 3	-
9.	Oral reference doses of toxic metals (*RfDo*; mg kg^{-1} day^{-1})	Cd = 0.004, Pb = 0.001, As = 0.0003, Cr = 0.001, and Ni = 0.02	[42]
10.	Oral reference doses of PAHs (*RfDo*; mg kg^{-1} day^{-1})	As indicated by the author	[43]
11.	Average time for non-carcinogens (*AT*; day/year)	365 days/year	[39]
12.	Maximum tolerable daily intake of toxic metals	Cd = 0.02 to 0.07, Pb = 0.21, As = 0.13, Cr = 0.035 to 0.2, and Ni = 0.04	[44-46]
13.	Toxicity equivalence factor (*TEF*)	As reported in these sources	[47, 48]
14.	Oral cancer slope factor of toxic metals (*CSF*)	Cd = 0.38, Pb = 0.0085, As = 1.5, Cr = 0.5, and Ni = 0.91	[49]
15.	Oral cancer slope factor of PAHs (*CSF*)	BaP = 7.3, BbFL = 0.73, BkFL = 0.073, Chr = 0.0073, BP = 0.023, DBA = 7.3, and Ind = 0.73	[39]
16.	Maximum incremental lifetime cancer risk limit (*ILCR*)	10^{-6} to 10^{-4}	[39]

Note: BaP = benzo[a]pyrene; BbFL = benzo[b]fluoranthene; BkFL = benzo[k]fluoranthene; Chr = chyrsene; BP = benzo[ghi]perylene; DBA = dibenz[a,h]anthracene; and Ind = indino[1,2,3-c,d]pyrene

To further assess and identify potential chemical compounds of concern in the studied vegetables in terms of non-carcinogenic health problems, screening values of non-carcinogenic PAH compounds were estimated following the procedures of the U.S. Department of Health and Human Services [37] as indicated in Equation 5:

$$Screening\ value\ (non\text{-}carcinogenic) = \frac{RFD \times BW}{IFRvegetable} \tag{5}$$

where *RFD* is the reference dose of the PAHs and *IFRvegetable* represents the ingestion rates of *T. occidentalis* and *T. triangulare*. The values applied in the determination are listed in Table 1.

2.4.2 *Carcinogenic health risk*

The *ILCR* of toxic metals and PAHs through consumption of the studied vegetables was estimated using Equation 6:

$$ILCR = ECDI \times CSF \tag{6}$$

where *ECDI* is determined using Equation 1 and *CSF* is the oral cancer slope factor for toxic metals and carcinogenic PAHs. *CSF* for studied toxic metals and individual carcinogenic PAH are listed in Table 1. The cancer screening value which is the threshold concentration of the carcinogenic PAHs in the studied vegetables that are of public health concern, was evaluated using Equation 7:

$$Screening\ value\ (carcinogenic) = \frac{\frac{RL}{CSF} \times BW}{IFR} \tag{7}$$

where *RL* is the maximum acceptable risk level and *IFR* represents the consumption rate of studied vegetables. Values used for computation are shown in Table 1. The contribution of individual PAHs in the studied vegetables to the occurrence of carcinogenic health risks was examined using *TEQ*. The *TEQ* is the sum of the product of the concentration of individual PAH congeners and their toxicity equivalency factor, as shown in Equation 8, where *TEF* is the toxicity equivalence factor of each cancer-causing PAH.

$$TEQ = \Sigma PAHi \times TEF \tag{8}$$

2.5 *Statistical analysis*

The statistical analysis of the obtained results was performed using the IBM SPSS Statistics 20.0 (IBM USA) model. The principal component analyses were carried out by means of varimax factor analysis on five parameters and values of 0.669 and above were judged significant. The hierarchical cluster analysis (HCA) was performed using dendrograms to categorize homogeneous groups with common properties and sources.

3. Results and discussion

Concentrations of cadmium (Cd), lead (Pb), arsenic (As), chromium (Cr), and nickel (Ni) in *T. occidentalis* and *T. triangulare* are impacted by wastewater from some water treatment plants in the Uyo Metropolis and the control site and are presented in Table 2.

Table 2. Concentration (mg kg^{-1}) of toxic metals in vegetables

	T. occidentalis					*T. triangulare*				
	Cd	Pb	As	Cr	Ni	Cd	Pb	As	Cr	Ni
Station 1	0.19	0.36	0.13	1.67	2.26	0.18	0.36	0.24	2.03	3.46
Station 2	0.25	0.54	0.21	1.48	4.38	0.22	0.44	0.30	1.84	1.28
Station 3	0.14	0.28	0.17	1.24	1.51	0.12	0.32	0.16	1.52	2.32
Min	0.14	0.28	0.13	1.24	1.51	012	0.32	0.16	1.52	1.28
Max	0.25	0.54	0.21	1.67	4.38	0.22	0.44	0.30	2.03	3.46
Mean	0.19	0.39	0.17	1.46	2.71	0.17	0.37	0.23	1.79	2.35
SD	0.04	0.11	0.03	0.10	0.07	0.04	0.04	0.06	0.13	0.06
CTL	0.01	BDL	0.02	0.05	BDL	BDL	0.01	0.01	BDL	0.02

Note: SD = standard deviation; CTL = control; and BDL = below detectable limit

3.1 *Concentration of toxic metals in the vegetables*

The results obtained in the studied vegetables indicated appreciable levels of toxic metals, which varied between the two vegetables. Concentrations of Cd in the studied *T. occidentalis* and *T. triangulare* ranged from 0.14 to 0.25 mg/kg and 0.12 to 0.22 mg/kg, respectively. These ranges are higher than 0.01 to 0.07 mg/kg reported in vegetables by Mafuyai et al. [50], but lower than 0.34 to 5.44 mg/kg and 0.24 to 6.00 mg/kg obtained in vegetables by Akan et al. [51] and Lawal et al. [52], respectively. The highest level of Cd was obtained in *T. occidentalis* from Champion Breweries while the lowest was recorded in *T. triangulare* from Akwa Ibom Water Company (Table 2). The relatively high levels of Cd in *T. occidentalis* impacted by wastewater from Champion Breweries could be attributed to stainless steel equipment [53, 54]. The mean values of Cd recorded for the studied *T. occidentalis* and *T. triangulare* are relatively higher than the values reported for these vegetables at the control site (Table 2). This is consistent with the studies where higher concentrations of metals were recorded in the studied vegetables than in those from control sites by Al-Ansari et al. [55] and Etuk et al. [22]. This could be attributed to the impact of wastewater on the quality of these vegetables, as previously observed by Tiruneh et al. [56] and Dan et al. [57]. The mean values of Cd reported in the studied vegetables are higher than the 0.1 mg/kg recommended by the Food and Agriculture Organization (FAO) and World Health Organization (WHO) [58] for leafy vegetables. Hence, prolonged exposure may result in Cd toxicity and associated health problems, as reported by the United States Department of Health and Human Services (HHS) [59]. Thus, as a highly toxic element, routine monitoring of its availability in these widely consumed vegetables is encouraged.

Concentrations of Pb varied from 0.28 to 0.54 mg/kg and 0.32 to 0.44 mg/kg in the studied *T. occidentalis* and *T. triangulare*, respectively. These ranges are lower than the 1.22 to 4.18 mg/kg and 2.43 to 8.12 mg/kg reported in vegetables by Perveen et al. [60] and Najam et al. [61], respectively. The results in Table 2 also indicate higher levels of Pb in the studied vegetables than in vegetables from the control site. This is similar to the findings by Sayo et al. [62], which also obtained higher Pb in vegetables impacted by wastewater than in those from the control station. The higher Pb concentrations in the studied vegetables may be an indication of anthropogenic addition from wastewater used for irrigation. The general results in Table 2 indicate that the highest concentration of Pb was obtained in *T. occidentalis* from Champion Breweries, while the lowest Pb level was also reported in *T. occidentalis* but from Akwa Ibom Water Company. Hence, wastewater from a food processing industry (brewery) could contain higher levels of Pb than that from a water treatment plant, as previously observed by Khanal et al. [63] and Rai et al. [64]. The mean values of Pb in Table 2 are higher than the acceptable limit of 0.30 mg/kg for leafy vegetables set by the FAO and WHO [65]. Consequently, the consumption of *T. occidentalis* and *T. triangulare* harvested from the studied location may result in Pb-toxicity and associated health problems over time [66]. It has been reported that prolonged exposure to Pb could result in health problems associated with the kidney, liver, spleen, and lungs, with the child population being more vulnerable [67].

The concentrations of As in the studied vegetables ranged from 0.13 to 0.21 mg/kg and 0.16 to 0.30 mg/kg in *T. occidentalis* and *T. triangulare*, respectively. The reported ranges are higher than 0.05 to 0.11 mg/kg and 0.03 to 0.09

mg/kg obtained in vegetables by Tom et al. [68] and Chaoua et al. [69], respectively. However, the ranges obtained in this study are lower than the 1.93 to 5.73 mg/kg reported in vegetables by Gebeyehu and Bayissa [70]. Higher mean values of As were obtained in the studied vegetables than in those from the control site. This could be attributed to the impact of wastewater on the quality of the studied vegetables [56, 71]. The general concentrations of As in the studied vegetables ranged from 0.13 to 0.30 mg/kg. The highest level was obtained in *T. triangulare* impacted by wastewater from Champion Breweries and the lowest in *T. occidentalis* affected by wastewater from The Ibom Hotels and Golf Resort. The high content of As in *T. triangulare* impacted by wastewater from Champion Breweries validates the negative impacts of industrial wastewater on the quality of the studied vegetables [72, 73]. The results of this study also revealed that the mean values of As in the studied vegetables were higher than the recommended 0.1 mg/kg safe limit for leafy vegetables by FAO and WHO [65]. Consequently, the consumption of *T. occidentalis* and *T. triangulare* impacted by wastewater could result in health risks related to As-toxicity, with children being more susceptible, as reported by Wani et al. [74].

Results in Table 2 show the ranges of Cr in the studied *T. occidentalis* and *T. triangulare* as 1.24 to 1.67 mg/kg and 1.52 to 2.03 mg/kg, respectively. The ranges of Cr obtained in the studied vegetables are consistent with the findings by Gebeyehu and Bayissa [70], higher than the 0.28 to 1.16 mg/kg reported by Khan et al. [75], but lower than the 2.67 to 4.82 mg/kg obtained by Mahmood and Malik [76]. The results in Table 2 also indicate higher mean values of Cr in the studied vegetables than in those from the control site. This agrees with the findings by Mahmood and Malik [76], who also reported higher Cr in the studied vegetables than in those from the control site. This could be attributed to the anthropogenic inputs of Cr from the wastewater used for irrigation. The highest concentration of Cr was reported in *T. triangulare* impacted by wastewater from a food processing company (The Ibom Hotels and Golf Resort), whereas the lowest level was obtained in *T. occidentalis* affected by wastewater from Akwa Ibom Water Company (Table 2). This high level of Cr in the sample impacted by wastewater from a food processing company may be caused by the stainless steel used as cooking and packaging apparatus [77, 78]. The mean values of Cr in the studied vegetables were also higher than the safe limit of 1.00 mg/kg for leafy vegetables established by FAO and WHO [65]. The consumption of these vegetables could result in health problems associated with high Cr in consumers over time [79, 80].

The concentrations of Ni in the studied *T. occidentalis* and *T. triangulare* ranged from 1.51 to 4.38 mg/kg and 1.28 to 3.46 mg/kg, respectively. The obtained ranges are lower than the 8.47 to 11.28 mg/kg reported by Ismail et al. [81] but higher than the 0.18 to 0.34 mg/kg reported in leafy vegetables by Lone et al. [82]. The mean values of Ni in the studied vegetables were higher than those obtained in similar vegetables from the control site. This could be attributed to the influence of wastewater on Ni accumulation in the studied vegetables from the experimental locations. The results in Table 2 indicate the highest level of Ni in *T. occidentalis* from Champion Breweries, while the lowest level is in *T. triangulare*, also impacted by wastewater from Champion Breweries. Consequently, this study has revealed a relatively higher tendency for *T. occidentalis* to absorb Ni from a contaminated environment than *T. triangulare*. Hence, *T. occidentalis* could be utilized for phytoremediation of Ni-impacted soil, as reported by Animashaun et al. [83]. The high contents of Ni in wastewater from a food processing company (brewery) as reported by Ashraf et al. [84] and Cempel and Nikel [85], have also been confirmed. However, the mean values of Ni reported in the studied vegetables were lower than the recommended 10.00 mg/kg safe limit for leafy vegetables by FAO and WHO [65]. Hence, the consumption of *T. occidentalis* and *T. triangulare* from the studied locations may not result in health risks related to Ni-toxicity. Nevertheless, since Ni can bioaccumulate in living cells with time, its presence in the food chain should be closely observed to forestall problems related to Ni [86, 87].

Generally, it was observed that *T. occidentalis* accumulated higher levels of Cd, Pb, and Ni than *T. triangulare*. Meanwhile, *T. triangulare* showed a higher potential for absorbing As and Cr than *T. occidentalis*. The potentials of these vegetables to bioaccumulate metals are in the following order: Ni > Cr > Pb > Cd > As for *T. occidentalis*, and Ni > Cr > Pb > As > Cd for *T. triangulare*. This indicates that both *T. occidentalis* and *T. triangulare* have a high ability to bio-accumulate Cr and Ni from contaminated soil.

3.2 *Levels of polycyclic aromatic hydrocarbons in the studied vegetables and control*

Results in Table 3 show the levels of PAHs in *T. occidentalis* and *T. triangulare* irrigated with wastewater from some water treatment plants within the Uyo Metropolis, Southern Nigeria, and their control site. For this study, all 16 priority PAHs were determined in the vegetables and their controls. Interestingly, the studied vegetables showed variable levels of these PAHs based on stations except for dibenz[a,h]anthracene (DBA) which was not detected in either vegetable at all stations. The total amount of PAHs found in *T. occidentalis* were 6.53, 7.33, and 5.12 mg/kg for Stations 1, 2, and 3, respectively. Meanwhile, the total PAHs in *T. triangulare* from Stations 1, 2, and 3 were 4.31, 5.19, and 3.16 mg/kg, respectively. The values of total PAHs reported in this study are higher than the 1.54 to 3.62 mg/

kg obtained by Khan et al. [75] but lower than the 8.24 to 11.36 mg/kg reported by Song et al. [88]. The total PAHs in *T. occidentalis* and *T. triangulare* from the control site were 0.39 and 0.33 mg/kg, respectively. The results obtained showed that *T. occidentalis* has a higher potential for accumulating PAHs than *T. triangulare*. Hence, the ability of vegetables to accumulate PAHs from contaminated soil is dependent upon the species of the plant, as observed by Inam et al. [89]. The variations in the accumulation of PAHs by these vegetables at different locations could also be attributed to disparities in the concentration of these hydrocarbons in the wastewater used for irrigation, in addition to the physical and chemical nature of the soil [88]. The relatively lower total PAHs in the vegetables from the control site shows the impact of wastewater on the accumulation of PAHs in the studied soils and vegetables. The higher PAH contents in the vegetables impacted by wastewater compared to the controls are consistent with the findings by Inam et al. [89] and Ashraf and Salam [90]. According to Tao et al. [35], the variations in the results of PAHs reported at different locations could be attributed to the differences in the composition of wastewater, species of plants, soil chemistry, and plant parts used. The results of individual PAHs in the studied vegetables revealed higher concentrations of low-molecular-weight hydrocarbons such as NaP, AcPY, AcP, Flu, and Phe than the higher-molecular-weight of Ind, BP, BaP, and BkFL. The results also indicated that the total PAHs in the studied vegetables were mainly contributed by the two and three rings than the four, five, and six rings PAHs. This is similar to the reports by Sojinu et al. [91] and Zhang et al. [92], who also reported higher concentrations of two to three ring PAHs in wastewater-impacted vegetables than in the higher ring PAHs. This could be attributed to the high water and fewer oil contents of vegetables, increasing PAHs' lipophilicity with increased ring size, hence more of the less lipophilic PAHs will be favored [93]. The study also showed that concentrations of most of the higher molecular weight PAHs, such as BaA, Chr, BbFL, and BkFL, were below the detectable limit of the equipment used. According to the USEPA [94], there are no stipulated limits for PAHs in vegetables and other food items. However, it has been reported that several health problems are associated with long-term exposure to total PAHs at a level higher than 5.00 mg/kg [95].

Table 3. Level (mg kg^{-1}) of PAHs in *T. occidentalis* and *T. triangulare*

	T. occidentalis				*T. triangulare*			
	Station 1	Station 2	Station 3	CTL	Station 1	Station 2	Station 3	CTL
NaP	2.14	1.80	1.24	0.17	0.93	1.14	0.66	0.11
AcPY	2.06	2.77	1.51	0.08	1.54	1.73	0.83	0.04
AcP	0.74	0.82	0.91	0.05	0.48	0.73	0.54	0.08
Flu	0.80	0.92	0.74	0.01	0.53	0.54	0.41	0.03
Phe	0.13	0.18	0.15	0.04	0.19	0.24	0.28	0.02
Ant	0.08	0.03	0.05	0.02	0.10	0.06	0.09	0.01
FL	0.08	0.06	0.06	0.01	0.08	0.04	0.02	0.01
Pyr	0.02	0.05	0.03	0.01	0.03	0.07	0.03	0.02
BaA	0.04	0.06	0.06	BDL	0.02	0.04	0.03	0.01
Chr	0.07	0.11	0.04	BDL	0.03	0.06	0.01	BDL
BbFL	0.12	0.16	0.08	BDL	0.09	0.13	0.06	BDL
BkFL	0.10	0.17	0.08	BDL	0.11	0.11	0.07	BDL
BaP	0.01	0.03	0.01	BDL	0.02	0.02	0.04	0.01
BP	0.04	0.03	BDL	BDL	0.06	0.04	0.03	BDL
DBA	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Ind	0.10	0.14	0.16	BDL	0.09	0.12	0.06	BDL
Total	6.53	7.33	5.12	0.39	4.31	5.19	3.16	0.33

Note: BDL = below detectable limit; CTL = control; NaP = naphthalene; AcPY = acenaphthylene; AcP = acenaphthene; Flu = fluorene; Phe = phenanthrene; Ant = anthracene; FL = fluoranthene; Pyr = pyrene; BaA = benz[a]anthracene; Chr = chyrsene; BbFL = benzo[b]fluoranthene; BkFL = benzo[k]fluoranthene; BaP = benzo[a]pyrene; BP = benzo[ghi]perylene; DBA = dibenz[a,h]anthracene; and Ind = indino[1,2,3-c,d]pyrene

3.3 *Results of multivariate analysis of toxic metals in the vegetables*

Principal component analysis (PCA) was utilized for the identification of the definite factors responsible for the accumulation of these toxic metals in the studied vegetables [96]. Table 4 reveals that *T. occidentalis* and *T. triangulare* each has two principal factors with eigenvalues greater than one that account for 100% of the total variance. In the PCA of *T. occidentalis*, factor one added 71.2% to the total variance with significant positive loadings on Cd, Pb, As, and Ni (Table 5). This represents the negative influence of wastewater on the quality of the studied *T. occidentalis* [15]. Whereas factor two contributed 28.8% of the total variance, with a strong negative loading on As and a significant positive loading on Cr (Table 5). This shows the impacts of both natural and anthropogenic (wastewater) sources [97, 98]. The PCA of the studied *T. triangulare* in Table 4 also reveals two major factors. Factor one contributed 71.2% of the total variance, with strong positive loadings on Cd, Pb, and As (Table 5). This indicates the impact of anthropogenic sources (wastewater) on the quality of the studied vegetable [99]. Factor two added 28.8% to the total variance with significant positive loadings on Cr and Ni (Table 5). This signifies the impacts of wastewater from the food processing industry on the metal accumulation in *T. triangulare* [66, 72].

Table 4. Explanation of total variance for toxic metals determined in the vegetables

	Initial eigenvalues			Extraction sums of squared loadings			Rotation sums of squared loadings		
Component	Total	Percentage of variance	Cumulative percentage	Total	Percentage of variance	Cumulative percentage	Total	Percentage of variance	Cumulative percentage
T. occidentalis									
1	3.56	71.2	71.2	3.56	71.2	71.2	3.55	71.1	71.7
2	1.44	28.8	100.0	1.44	28.8	100.0	1.45	28.9	100.0
T. triangulare									
1	3.56	71.2	71.2	3.56	71.2	71.2	3.50	70.0	70.0
2	1.44	28.8	100.0	1.44	28.8	100.0	1.502	30.0	100.0

Table 5. Result of principal component analysis representing relative loading for metals in the studied vegetables

	T. occidentalis		*T. triangulare*	
Component	1	2	1	2
Cd	0.982	0.187	0.997	0.078
Pb	1.000	0.022	0.974	-0.225
As	0.692	-0.722	0.999	0.046
Cr	0.343	0.939	0.649	0.761
Ni	1.000	-0.028	-0.444	0.896

The HCA was used to identify the similarity and common sources among the toxic metals determined in the studied vegetables [100]. The results for HCA of both *T. occidentalis* and *T. triangulare* revealed a similar dendrogram with two main clusters, as shown in Figure 1. This corroborates the two major factors identified by PCA in Table 5. Cluster one links Cd, As, and Pb together as identified by factor one for both vegetables. This also confirms that these three metals originated from a common source [101]. Cluster two links Cr and Ni together as reported by Factor 2 in the PCA of *T. triangulare*. This validates a common source (stainless steel in wastewater from food processing industries) for Cr and Ni in the studied vegetables [78, 84].

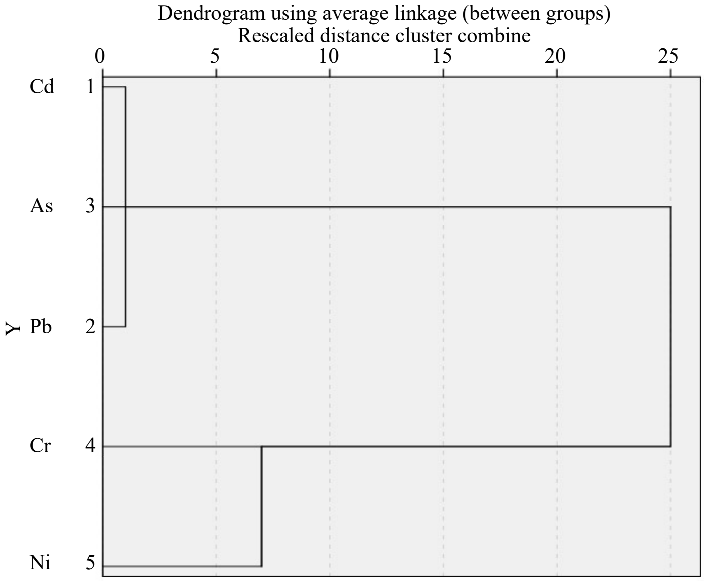

Figure 1. Hierarchical clusters of the trace metals determined in the vegetables

3.4 *Health risk assessment of toxic metals and polycyclic aromatic hydrocarbons through vegetable consumption*

3.4.1 *Estimated chronic daily intake, hazard quotient, and indices for non-carcinogenic risk of toxic metals*

Results for the *ECDI*, *HQ*, and indices of toxic metals in vegetables impacted by wastewater are presented in Table 6. These parameters were computed for child and adult populations. The result indicates variable daily intake values for the toxic metals for both populations. All the toxic metals determined in *T. occidentalis* and *T. triangulare* indicated higher estimated daily intake values for children than adults, with a range of 4.0E-04 to 2.0E-02. None of the metals recorded *ECDI* values higher than their respective maximum tolerable daily intake, as indicated in Table 1. The *HQs* for *T. occidentalis* and *T. triangulare* ranged from 1.4E-04 to 4.4E-03 for both child and adult populations, with *HQ* values for children being higher than those of the adult population. The *THI* of toxic metals in the studied vegetables were lower than one, implying low health risks to consumers. However, the relatively lower *EDI* and *HQ* obtained in this study should not be overlooked as these health risk indicators can increase over time. The *EDI*, *HQ*, and *THI* were estimated based on the estimated daily consumption of *T. occidentalis* and *T. triangulare*, taken as 182 g/person/day and 118 g/person/day for adults and children, respectively (Table 1). There is a possibility that the computed indicators for non-carcinogenic health risk may change depending on the daily consumption rate of these vegetables, whose values fluctuate with time, location, and other factors.

Table 6. Estimated chronic daily intake, hazard quotient and indices of toxic metal

	Estimated chronic daily intake (*ECDI*)				**Hazard quotient (*HQ*)**			
	T. occidentalis		*T. triangulare*		*T. occidentalis*		*T. triangulare*	
	Children	Adults	Children	Adults	Children	Adults	Children	Adults
Cd	0.0020	0.0005	0.0010	0.0004	5.8E-04	2.1E-04	4.9E-04	1.7E-04
Pb	0.0030	0.0010	0.0030	0.0010	2.9E-04	1.0E-04	2.9E-04	1.0E-04
As	0.0010	0.0004	0.0020	0.0006	1.7E-03	5.5E-04	2.6E-03	8.2E-04
Cr	0.0100	0.0040	0.0141	0.0050	4.4E-03	1.6E-03	5.0E-03	2.1E-03
Ni	0.0200	0.0070	0.0185	0.0062	4.0E-04	1.4E-04	3.6E-04	1.3E-04
THI					7.4E-03	4.5E-03	8.7E-03	6.0E-03

3.4.2 *Cancer health risk of toxic metals through vegetable consumption*

The cancer health risk of toxic metals through the consumption of *T. occidentalis* and *T. triangulare* was estimated using the *ILCR* model, and the results are presented in Table 6. The *ILCR* values were higher in *T. occidentalis* than *T. triangulare* for both child and adult populations, with the child population recording higher levels of the metals. The range of *ILCR* obtained for both vegetables for both child and adult populations varied from 8.5E-06 to 1.9E-02 which is higher than the 1.0E-06 to 1.0E-04 stipulated by USEPA [102]. However, the *ILCR* values obtained for Pb in both vegetables for adults were within the acceptable range. Consequently, consuming *T. occidentalis* and *T. triangulare* from the studied locations can pose a carcinogenic risk to consumers due to Cd, As, Ni, and Cr toxicity. The *ILCR* range obtained in this study is higher than the 9.1E-08 to 6.7E-06 reported by Shaheen et al. [45] but consistent with the 2.3E-04 to 2.2E-03 obtained by Gebeyehu and Bayissa [70]. Among the five toxic metals appraised for cancer risk through the consumption of these vegetables, all were capable of stimulating cancer risk in the child population, while Cd, As, Ni, and Cr represented 80% of the studied metals that could cause cancer in the adult population.

Table 7. Incremental lifetime cancer risk of toxic metals

	T. occidentalis		*T. triangulare*	
	Children	Adults	Children	Adults
Cd	6.0E-04	2.0E-04	5.0E-04	1.6E-04
Pb	3.0E-05	8.5E-06	2.6E-05	8.5E-06
As	2.0E-03	6.0E-04	3.0E-03	9.0E-04
Cr	6.1E-03	2.0E-03	7.1E-03	2.5E-03
Ni	1.9E-02	7.0E-03	1.7E-02	5.7E-03

3.4.3 *Estimated chronic daily intake, hazard quotient, indices, and screening value for non-carcinogenic risk of polycyclic aromatic hydrocarbons*

Table 8 shows the *EDI* of PAHs through the consumption of *T. occidentalis* and *T. triangulare* impacted by wastewater for both child and adult populations. For all the PAHs, the *EDI* values varied with vegetables and population. For both vegetables, all the PAHs recorded higher daily intake values for children than adults, except for Phe. The *EDI* for DBA was not computed for the vegetables because their concentration was below the detectable limit, as indicated in Table 3. The non-carcinogenic *HQ* of PAHs through the consumption of studied vegetables for child and adult populations is presented in Table 8. Out of the nine non-carcinogenic PAHs, *HQ* was computed for only six, namely: NaP, AcPY, Flu, Phe, Pyr, and BaA for both vegetables due to a lack of information about the *RfDo* of the other three. Generally, *HQ* values for both vegetables and populations ranged from 1.8E-06 to 1.4E-04. The *HQ* values were higher for children than the adult population for all the PAHs computed except for Flu (Table 8). This is due to the higher *EDI* values obtained for children; however, none of the six PAHs recorded *HQ* values close to or higher than one. The *THI* obtained as the sum of individual non-carcinogenic *HQ* for both populations and vegetables are presented in Table 8. The hazard indices ranged from 4.9E-05 to 1.9E-04, with none being close to one, implying that exposure to either individual PAHs or complex PAH mixtures through the consumption of studied vegetables may not result in an immediate non-carcinogenic health risk in the consumers. To further estimate the threshold level of each of the six non-carcinogenic PAHs, non-carcinogenic screening values were determined using Equation 5 and the results are presented in Figure 2. The calculation was done based on the consumption rates for child and adult populations. As shown in Figure 2, the screening values of the PAHs range from 9.01 to 35.59, with a higher value recorded for the children than the adult population. The concentrations of PAHs in the studied vegetables were lower than the estimated threshold screening values. Consequently, a low risk of non-carcinogenic health challenges regarding the consumption of these vegetables was envisaged [103]. However, the level of PAHs in these vegetables should be closely monitored to avoid bioaccumulation over time since consumers are already exposed to some levels of these toxic hydrocarbons, as indicated in the results obtained. The non-carcinogenic health risks to both child and adult populations may likely increase with an increased consumption rate if the level of PAHs in these vegetables is elevated.

Table 8. Estimated daily intake, hazard quotient, and indices of PAHs of *T. occidentalis* and *T. triangulare*

	Estimated chronic daily intake (*ECDI*)				Hazard quotient (*HQ*)			
	T. occidentalis		*T. triangulare*		*T. occidentalis*		*T. triangulare*	
	Children	Adults	Children	Adults	Children	Adults	Children	Adults
NaP	0.020	0.005	0.01	0.002	2.1E-05	5.7E-06	1.1E-05	2.3E-06
AcPY	0.030	0.005	0.02	0.004	1.4E-04	2.6E-05	9.5E-05	2.1E-05
AcP	0.010	0.002	0.01	0.001	-	-	-	-
Flu	0.010	0.002	0.01	0.002	7.6E-06	1.6E-05	7.6E-06	1.6E-05
Phe	0.002	0.004	0.003	0.001	3.3E-05	2.7E-05	1.9E-05	6.8E-06
Ant	0.001	0.0001	0.001	0.0002	-	-	-	-
FL	0.001	0.0002	0.001	0.0001	-	-	-	-
Pyr	0.0004	0.0000-	0.001	0.0001	7.7E-06	1.8E-06	1.9E-05	2.1E-06
BaA	0.0007	0.0001	0.0004	0.0001	8.9E-06	1.4E-06	5.1E-06	1.4E-06
Chr	0.001	0.0002	0.0004	0.00009	-	-	-	-
BbFL	0.001	0.0003	0.001	0.0002	-	-	-	-
BkFL	0.001	0.0003	0.001	0.0002	-	-	-	-
BaP	0.0002	0.00004	0.0003	0.00007	-	-	-	-
BP	0.0003	0.00006	0.001	0.0001	-	-	-	-
DBA	-	-	-	-	-	-	-	-
Ind	0.002	0.0004	0.001	0.0002	-	-	-	-
THI					1.9E-04	8.7E-05	1.5E-04	4.9E-05

Note: *THI* = total hazard indix; NaP = naphthalene; AcPY = acenaphthylene; AcP = acenaphthene; Flu = fluorene; Phe = phenanthrene; Ant = anthracene; FL = fluoranthene; Pyr = pyrene; BaA = benz[a]anthracene; Chr = chyrsene; BbFL = benzo[b]fluoranthene; BkFL = benzo[k]fluoranthene; BaP = benzo[a]pyrene; BP = benzo[ghi]perylene; DBA = dibenz[a,h]anthracene; and Ind = indino[1,2,3-cd]pyrene

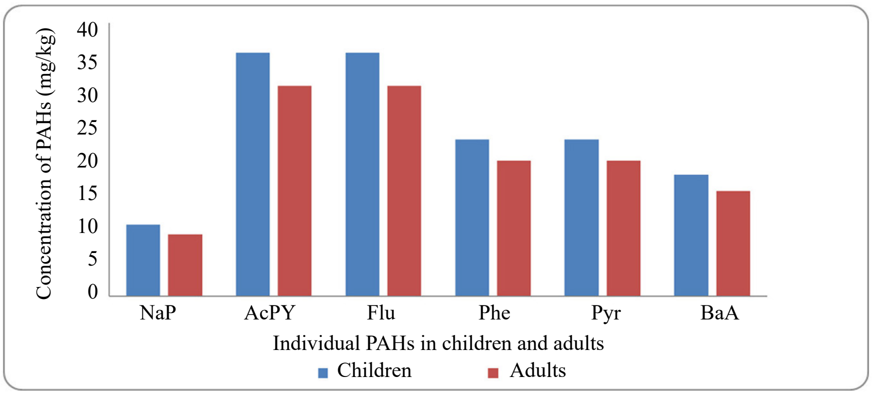

Figure 2. Non-carcinogenic threshold screening value of vegetables for children and adults

3.4.4 *Cancer health risk of polycyclic aromatic hydrocarbons through vegetable consumption*

The cancer health risk of PAHs in child and adult populations through the consumption of vegetables impacted by wastewater was determined using the *ILCR* model in terms of the carcinogenic hazard quotient. Results for the carcinogenic hazard quotients and indices of seven carcinogenic PAHs recognized by the USEPA [39] are presented in Table 9. For both vegetables, the *HQ* and indices were higher for child than adults, with none of the PAHs being closer to one. The range for the *HQ* for both vegetables in child and adult populations was 6.3E-08 to 2.2E-03. The hazard indices for *T. occidentalis* and *T. triangulare* in the case of both child and adult populations were very close. This is due to the closeness of their *EDI* values, as shown in Table 8. For individual carcinogenic potencies of PAHs for both populations, variable values were obtained, with BbFL, BkFL, and Ind congeners showing higher cancer potencies for both vegetables. The potencies of the seven carcinogenic PAHs combined to cause health challenges to consumers of the studied vegetables were further tested by determining the *TEQ* shown in Figure 3. The results showed a *TEQ* value of 1.06 for *T. occidentalis* and 0.87 for *T. triangulare*. The *TEQ* values obtained for both vegetables were higher than their carcinogenic screening values presented in Table 9. This is an indication of the threshold concentration of the contaminants in the studied vegetables that is of public health concern [104]. These results imply that the cancer-causing PAHs assessed in the studied vegetables are of public concern and that the tendency to cause cancer in consumers is moderately high.

Table 9. Carcinogenic hazard quotient, total hazard indices, and carcinogenic screening values of PAHs for child and adult populations

	ILCR measured as carcinogenic hazard quotient					
	T. occidentalis		*T. triangulare*		Carcinogenic screening value	
	Children	Adults	Children	Adults	Children	Adults
Chr	7.3E-06	1.5E-06	2.9E-06	6.3E-08	1.7E-01	5.2E-01
BbFL	7.3E-04	2.2E-04	7.3E-04	1.5E-04	2.0E-03	5.0E-03
BkFL	7.3E-05	2.2E-05	7.3E-05	1.5E-05	2.0E-02	5.0E-02
BaP	1.5E-03	3.1E-04	2.2E-03	5.1E-04	2.0E-02	5.0E-04
BP	6.9E-06	1.4E-07	2.3E-05	2.3E-06	2.0E-04	1.7E-01
DBA	-	-	-	-	-	-
Ind	1.5E-03	2.9E-04	7.3E-04	1.5E-04	2.0E- 03	5.0E-03
THI	4.2E-03	8.0E-04	3.8E-03	8.3E-04		

Note: *ILCR* = incremental lifetime cancer risk; *THI* = total hazard indices; Chr = chyrsene; BbFL = benzo[b]fluoranthene; BkFL = benzo[k]fluoranthene; BaP = benzo[a]pyrene; BP = benzo[ghi]perylene; DBA = dibenz[a,h]anthracene; and Ind = indino[1,2,3-cd]pyrene

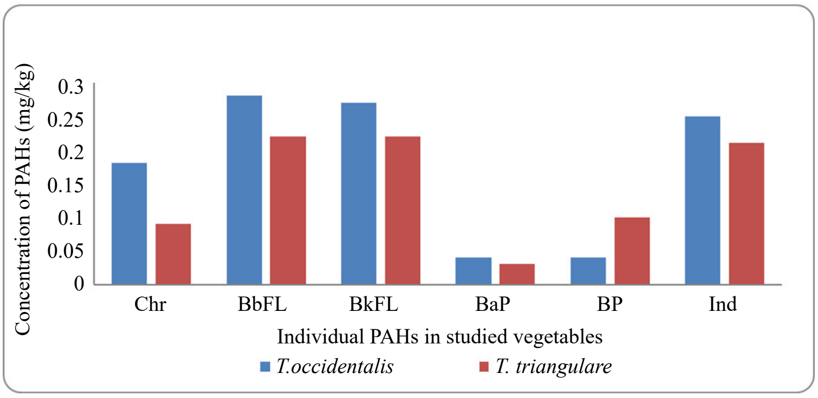

Figure 3. Carcinogenic toxic equivalent of PAHs

4. Conclusion

Based on the results of this study, vegetables irrigated with wastewater from water treatment plants within Uyo Metropolis accumulate very high concentrations of toxic metals and PAHs. Hence, modern and standard techniques should be employed for the treatment of wastewater by industries before discharging it into the environment. The PCA and HCA analyses have confirmed that wastewater from food processing industries used for irrigation was the fundamental source of these toxic substances for the vegetables. However, the *EDI* rate of toxic metals via the consumption of these vegetables in child and adult populations was below the maximum tolerable daily limits. An increase in the concentration of PAHs in the studied vegetables resulted in increased daily intake, non-carcinogenic *HQs*, indices, and other health risk indicators. The cancer risk to both child and adult populations for toxic metals and PAHs due to the consumption of the studied vegetables exceeded the maximum threshold values for all the metals and the seven carcinogenic PAHs. It could therefore be concluded that there is a need for strict regulations on the use of wastewater for irrigation as it can result in the excessive accumulation of toxic metals and PAHs in the soil and edible plants cultivated. Incidentally, the use of untreated wastewater from food processing industries for irrigation may cause serious human health problems for both child and adult populations.

4.1 *Future plans*

Based on the outcome of this study and the comments of the reviewers, subsequent studies in similar areas shall consider other toxic metals not examined in this work. Wastewater from the industries shall be analyzed to ascertain their levels of toxic substances, and the results obtained shall be correlated with those of the media impacted by the wastewater. The removal and quantification of toxic substances shall be one of the procedures of future studies. Proper and current techniques used for emerging contaminants shall be recommended for industries concerned with forestalling associated health and environmental problems along the food chain.

Conflict of interest

There is no conflict of interest for this study.

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