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Bioaccumulation of Arsenic by Cultivated Waterleaf *Talinum triangulare* from Soil Contaminated with Sodium Arsenate Pesticide and Health Risk Assessment

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ABSTRACT: *Through a pot experiment, the as concentration in the Waterleaf T. Triangulare plant grown on As-pesticide contaminated soil was examined at two treatment doses of 50 mg/kg and 70 mg/kg. After being harvested, the plant's leaves and roots as well as the soil around them were pre-treated, acid-digested, and analyzed for as content using mass plasma atomic emission spectroscopy (MP-AES Model 42100). The results from this analysis were used to determine the health risk posed by ingesting the crop. The study revealed that in all treatment levels for frequency of harvest and time of harvest, the concentration of as in the plant grew continuously over the graded period of growth (between 3 and 9 weeks after planting). Additionally, the increase in as content seen in the plant (leaf) exceeded the permissible limit of 0.1 mg/kg or 0.5mg/kg in vegetables as set by FAO/WHO/USEPA and EC/CODEX, from the third week of growth through the ninth week. The results of this investigation have demonstrated that Waterleaf's edible portions can bioaccumulate as to a great extent. Health risks results depicted that the estimated daily intake of as (EDIM) averages were below 1.0mg/kg/day and the FAO/WHO-recommended permissible tolerated daily intake of 0.002 mg/kg/day for inorganic arsenic. Similarly, the target health quotient (THQ) was below unity (THQ < 1), indicating that there is no expectation of a health danger from ingesting the plant. However, HRI or HQ was greater than unity (HRI or HQ > 1) and target cancer risk (TCR) was within the permissible predicted lifetime risk of getting cancer (1×10^{-6} to 1×10^{-4}), thus, suggesting the possibility of cancerous disease. In addition, the ratio of HRI to RFD indicates that consumption carries a very high health risk of cancer. The overall result indicates that a serious health issue for public health concern either now or in the future may arise from the consumption of waterleaf vegetable cultivated with high use of arsenate pesticides, based on the HRI and TCR which indicates the possibility of developing cancer during a lifetime.*

KEYWORDS: Bioaccumulation, Waterleaf plant, Arsenic Contaminated soil, Health Risks Assessment.

INTRODUCTION

Waterleaf *Talinum triangulare* is a very popular vegetable particularly grown in large scales by most rural and urban farmers in most African countries not just for personal consumption in the home gardens, but also as a means of livelihood due to its unique short harvest duration of 35-45 days after planting. It is easily propagated and mostly available in the wild as weeds but highly cherished for its succulent edible stems and leaves. Vegetables are important crops which constitute an essential part of the human and livestock diets. It is consumed mostly due to the presence of certain identified nutrients such as carbohydrate, protein, minerals, vitamins, and fibres etc. with varied concentrations from one vegetable to another (Mensah *et al*., 2008; Zhou *et al*., 2016). This herbaceous plant may be aromatic, bitter or tasteless and can be eaten as a supporting food or main dishes in our homes or eateries. Jolly *et al*. (2003) reported that vegetables have the potential of taking up heavy metals from the soil into its matrix and bio-accumulates either in large or small quantities in the edible parts of the plant. These heavy metals are non-biodegradable and regarded as persistent environmental contaminant which on deposition on the soil surface, are easily absorbed by plants into their tissues to a varied extent (Adesuyi *et al*., 2015; Adesuyi *et al*., 2016).

Heavy metals refer to any metallic element that has a relative density greater than 4g/cm^3, and they include; Lead (Pb), Cadmium (Cd), Arsenic (As), Zinc (Zn), Mercury (Hg), Silver (Ag) Iron (Fe) etc. (Adedakun *et al*., 2017). Rattan *et al*. (2005) and Sanayei *et al*. (2009) independently reported that most cultivated lands are contaminated with heavy metals contributed through anthropogenic activities such as vehicular emissions, biocides and fertilizers applications, industrial effluents, smelting and mining etc. This has resulted in the growth and harvesting of contaminated vegetables. Naturally, as can be released into the soil and water through weathering reactions, biological activities, geochemical reactions, volcanic emissions, etc. (Rasool *et al*., 2016). These sources tend to increase the as contents of the environment to exceed the WHO guideline of as in drinking water (0.01mg/L) and soil (20 mg/kg).

These contaminants have been reported to affect groundwater. Bramme & Ravenscroft (2009), reported as contamination of groundwater in over 70 countries with an estimated population of 150 million at health risk from as exposure around the globe. Shakoor *et al*. (2015) listed countries like Bangladesh, India, Taiwan, Mexico, Chile, Vietnam, Pakistan, France, Italy, New Zealand, and the USA to have underground water and soil to be as contaminated. Moreover, these large numbers of affected countries have attracted public health concerns because of food safety issues, and related health diseases such as keratosis, cancer of the liver, lung, skin etc. The form of as that is very toxic and causative agent to public health concern is the inorganic form as (III) and As (V). This view was corroborated by Vahter (2002), who reported that the inorganic as compounds are more hazardous than the organic as compounds.

The evaluation of heavy metals content and human health risk assessment via consumption of vegetables from selected markets in Bayelsa State, Nigeria was investigated by Nganwuchu (2007). Four heavy metals (Pb, Ni, Cd, and Cr) content were evaluated via consumption of sixteen different vegetable samples comprising of Bitter leaves, curry leaves, scent leaves, Waterleaf, uziza, fluted pumpkin, okazi, and okra which were acid digested and analyzed for heavy metal contents using Solar Thermo Elemental Flame Atomic Absorption Spectrophotometer (STEF – AAS). The results of heavy metal content obtained, were used to estimate the health risks associated with the consumption of these vegetables contaminated with the aforementioned metals. It was revealed that the concentration of Pb was below the permissible limit recommended by WHO/FAO and EC/CODEX; while that of Ni exceeded the permissible limit in some of the vegetables from a particular market. Moreover, the hazard index (HI) values for all the samples under investigation were greater than 1 (HI > 1) which indicates that there is a potential health risk to those who consume these vegetables. This idea was supported by the results of the estimated daily intake (EDI) concentrations of Pb, Ni, and Cd which were above permissible tolerance daily intake (PTDI) limit as recommended by European food and safety agency (EFSA). This implies that those who consume this product may be at risk. They concluded that the consumption of vegetables from both markets under investigation in Bayelsa State could be one of the contributory factors to the heavy metal burden among consumers due to their frequent consumption.

Again, Arsenic accumulation in leafy vegetables of lettuce *Lactuca sativa L.* and broad bean *Viciafaba L.* crops and its potential risk for human consumption was studied by determining total dry biomass (TDB) and total as in roots, leaves, pods, seeds, and soils respectively (Yanez *et al.*,2019). The data obtained were used to estimate the translocation factor (TF) and bio-concentration factor (BCF), target hazard quotient (THQ) and carcinogenic risk (TCR). The results revealed that the THQ values were all greater than 1, thus suggesting a high risk of health to consumers of these vegetables. Furthermore, the TCR exceeded the acceptable 1×10^{-4} level. They, therefore, concluded that the consumption of these vegetables grown on as contaminated soil will pose a considerable health risk for residents who regularly consume them.

The posing of potential health risk by as contaminated vegetables was corroborated by Rafiq *et al.* (2017), who reported that as contamination levels have increased drastically in the last three decades, posing a potential risk to humans and the environment. This view was supported by Sadee *et al.* (2016), who reported high as concentrations in diets originating from both drinking and irrigation waters, from crops, vegetables and meat products. Be that as it may, research concerning the relationship between bioavailable as concentration in contaminated soils and crop production is not available. It is, therefore, necessary to conduct a pot experiment to examine the growth and accumulation of as in waterleaf grown in as contaminated soil, and the comparison of the obtained result with standards specified by WHO/FAO/USEPA and to assess the health risk associated with As intake via consumption, since it may accumulate in plants and animals and eventually be transferred to humans through the food chain (Rahman *et al*., 2008). This study aims to analyze waterleaf for the level of as in the plant and also carry out a health impact assessment to suggest necessary measures that can alleviate the effects of such environmental contaminant.

MATERIALS AND METHODS

Materials

The materials used for the pot or no-choice experiment in this study were indigenous soil, locally adapted Waterleaf *Talinum triangulare* plant obtained from the Rivers State University Agricultural Farm, an analar grade Na_2HAsO_4:$7H_20$ as a source of As and NPK liquid organic fertilizer, Ag Zymer marketed by Zenith Energy – Enzymes Ltd, gotten from a chemical store in Port Harcourt metropolis.

Preparation of Stock Solutions

Preparation of Arsenic Stock Solution

0.416469gm of Na_2HAsO_4:$7H_2O$ (Disodium hydrogen arsenate) crystals was accurately weighed and transferred into a 50mL volumetric flask, followed by the addition of a small quantity of distilled water to dissolve the salt. The solution formed was poured into a 100mL volumetric flask and shaken vigorously to ensure complete dissolution, after which the solution was made up to the 100mL mark with distilled water. This gives a 1.3347×10^{-2} moles working solution of as (1000ppm As^{5+}). Further working solutions were prepared by serial dilution of an appropriate volume of the stock solution with an appropriate volume of distilled water.

Preparation of Organic Fertilizer Solution

5mLs of the liquid organic fertilizer (NPK) was added to 1 litre of distilled water and mixed properly. 100mLs of the prepared solution was used to water each pot twice weekly until the time of harvest.

Collection and Preparation of Soil Sample

The soil sample was collected at Longitude $4^0$54’17’’ North and Latitude $6^0$53’11’’ East at Isiokpo in Ikwerri Local Government Area of Rivers State, Niger Delta, Nigeria in areas away from the industrial site (i.e. uncontaminated with As) on the 22nd of February, 2018. The soil was sampled randomly using a stainless steel soil sampling auger at a depth of 0 to 20 cm. The representative soil was gotten by bulking together four soil auger borrowings to form a composite soil. The collected soil was then placed into an appropriately labelled polyethene bag, and taken to the chemistry laboratory of Rivers State University, Nigeria. The soil sample was air-dried at

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room temperature and sieved through a 0.850 mm nylon sieve to remove coarse fragments to obtain < 2mm fraction.

Experimental Design

The design was a 2 × 3 factorial laid out in a randomized complete block design (RCBD) and consisted of three treatments (i.e. 3wks., 6 wks., and 9 wks. respectively) at two different treatment levels of 50 mg/kg as (low) and 70 mg/kg As (high), thus produced 6 treatment combinations and two blocks (Table 1).

Table 1: Treatment and Treatment Allocation

Treatments	Treatment Levels	
(Weeks)	$50mgkg^{-1}$ As(low)	$70mgkg^{-1}$ As (high)
3	3 Weeks	3 Weeks
6	6 Weeks	6 Weeks
9	9 Weeks	9 Weeks

Planting and Harvesting

Two sets of 15 plastic pots containing accurately weighed 1kg of the composite or homogenate soil was added into each pot and spiked with 50mg kg^{-1} As and 70mg kg^{-1} As solution and allowed to age for 2 weeks. A stand (one stick) of wild species of Waterleaf *T. triangulare* cuttings was planted and watered with $50cm^3$ of water twice daily as described by Yee *et al.*, (2013). The pots were placed in 3 groups of 3 (A, B, C) and 2 groups of 3 (A, B, C) each in a roofed house and labeled A_{3wks}., B_{3wks}., C_{3wks}., A_{6wks}., B_{6wks}., C_{6wks}; A_{9wks}., B_{9wks}., C_{9wks}. and A_{6wks}., B_{6wks}., C_{6wks}; A_{9wks}., B_{9wks}., C_{9wks}. for frequency of harvest and time of harvest respectively. The plant in each group was harvested at three weeks' interval. Another pot containing the plant, but without arsenic was also set up as control.

On the third week, the first group of 3 was sampled destructively (leave, root and soil), while only leaves were collected from the 2nd and 3rd groups. Destructive sampling (leave, root and soil) was carried out on the 2nd group of 3, while only leave was collected again from the 3rd group on the sixth week. But on the 9th week, only destructive sampling (leave, root and soil) was done on the only remaining group of 3. Note that in each time the plant is destructively sampled, the soil is also collected alongside the root and leaves. For the 2 groups of 3, leaves and roots, was destructively sampled and the soil also collected on the 6th and 9th weeks respectively at the same period the first group of 3 was collected at the 6th and 9th weeks. The pilot study gave a stunted growth; hence, 100mL of liquid organic fertilizer was applied twice a week to the soil until the plant was due for harvest.

Pretreatment and Washing of Samples

After collection, samples were brought to the laboratory and processed further for analysis. The edible portions (leave and root) of the samples were used while bruised or rotten portions were removed. Each stand of Waterleaf *T. Triangulare* from each pot was properly washed first under tap water and then in two changes of distilled water and air-dried under hygienic condition. The air-dried soil and plant samples were each dried in an air circulating oven ($90^{O}C$) to a constant weight. The plant materials were ground in a mill, powdered and digested before determining the arsenic content in it.

Acid Digestion Method (Patrick-Iwuanyanwu & Chioma, 2017).

A total of 100mL of H_2SO_4, HNO_3, and $HClO_4$ in the ratio of 40%: 40%:20% (2:2:1) was mixed. A portion of (1gm) of the samples was weighed and digested with a 2mL of the mixed acid to each of the samples in a Kjedahl flask. The samples were then digested in a fume cupboard with a hot plate until white fumes appeared. After that, the solution was then cooled, filtered and transferred into a 100mL volumetric flask and made up to mark with distilled water, and an aliquot aspirated into the MP-AES Agilent 4210 machine to determine the amount of As present in the samples.

Soil Characterization

Soil pH in Water (1: 2.5)

The pH of soil samples was determined in 1:2.5 soils: water ratio using HANNA pH meter, model 2211, according to the modified method of McLean (1982).

Electrical Conductivity

The electrical conductivity (EC) of the soil sample was determined on the filtrate after filtering the soil-water suspension used for the pH determination. The conductivity meter used was JENWAY 4510.

Particle Size Analysis

The particle size analysis of the soil sample was determined by the Hydrometer Method of Bouyoucos (1951), and extrapolating on the Textural Triangle (USDA, 1951).

Available Phosphorus

This was determined using Bray and Kurtz No.1 method as modified by Olsen and Sommers (1982). In this method, the absorbance of the prepared sample was measured on the spectrophotometer at 880nm wavelength.

Organic Carbon/Organic Matter

Organic carbon was determined by the Walkley and Black (1934) method as described and modified by Nelson and Sommers (1982). It is a rapid dichromate ion ($Cr_20_7^{-2}$) reduction and filtration technique. The organic matter content was estimated by multiplying organic carbon concentration by 1.724.

Exchangeable Bases (K^+, Na^+, Ca^{2+}, and Mg^{2+})

Exchangeable bases in the soil sample were extracted with neutral normal ammonium acetate buffered at pH 7.0 after shaking for 2 hours. Exchangeable K^+ and Na^+ were analyzed using the method of Knudsen *et al.*, (1982) with a flame photometer.

Exchangeable Ca^{2+} and Mg^{2+} (after extraction) were determined using EDTA titration method described by Lanyon and Heald (1982).

Total Exchangeable Acidity

Total exchangeable acidity was determined by extraction with unbuffered salts such as 1N KCl and titration with standard 0.01N NaOH (Thomas, 1982).

Effective Cation Exchange Capacity (ECEC)

The effective cation capacity of the soils was determined by the summation of the total exchangeable bases and the total exchangeable acidity.

Arsenic Health Risk Assessment Via Consumption of Waterleaf

To assess the possible health risk associated with the consumption of Waterleaf cultivated on soil spiked (contaminated) with sodium arsenate pesticide, the estimated daily intake of arsenic (EDIM), Health Risk Index (HRI) or Hazard Quotient (HQ), Target hazard quotient (THQ) and Target Cancer Risks (TCR) were calculated using the appropriate equations. These parameters do not depend solely on the intake amount of a contaminant, but also on the exposure frequency and duration, average body weight and oral reference dose (RfD).

Estimated Daily intake of metal (As)

$$\mathrm{EDIM} = \frac{\mathrm{Cmetal} \times \mathrm{Cfactor} \times \mathrm{Cfoodintake}}{\mathrm{Baverage\ Weight}} \ldots\ldots\ldots \text{equation 1 (Chary } et\ al., 2008).$$

Where, Cmetal is the As concentration in Waterleaf *Talinum triangulare* (mg/kg), Cfactor is the conversion factor, Cfood intake is the daily intake of vegetables and Baverage is the average body weight for the adult vegetable consumer. The conversion factor 0.085 was used to convert fresh vegetable for adult and the average daily intake of vegetable recommended by WHO is between 300 to 350g. But in this study an average of 325g $person^{-1}$ $day^{-1}$is assumed, while the average body weight of an adult vegetable consumer was 60kg for this study (Tsafe *et al.*, 2012).

Health Risks Index (HRI)

The HRI for the consumption of contaminated vegetable (Waterleaf) was estimated as the ratio of the daily intake of metal (As) to the oral reference dose (RfD) for As. The HRI < 1 means the exposed population is safe and vice visa.The Health Risk Index (HRI) was calculated using the Formula:

$$\mathrm{HRI} = \frac{\mathrm{EDIM}}{\mathrm{RfD}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{equation 2(Jan } et\ al., 2010).$$

Where EDIM is the estimated daily intake of As and R_fD is the oral reference dose of As which is 0.0003 mg/kg /day (EPA, 2002; IRIS, 2007). An HRI > 1 for As in food crop indicates that the consumer population faces a health risk and vice visa.

The Target Hazard Quotient

Non-carcinogenic risk estimation of heavy metal (As) was determined using THQ values, which is a ratio of the determined dose of a toxicant to a reference dose considered harmful. THQ is a dimensionless quantity (Harmanescu *et al*., 2011). If the ratio is equal to or greater than 1, an exposed population is at risk and vice visa. THQ values were calculated using the formula:

$$(\text{THQ}) = \frac{\text{Efr} \times \text{ED} \times \text{FiR} \times \text{Cmetal}}{\text{RfD} \times \text{WAB} \times \text{TA}} \times 10^{-3} \ldots\ldots\ldots\text{equation 3} \quad (\text{Adedokun } et\ al., 2017).$$

Where Efr is the exposure frequency in 350 days/year, ED is the exposure duration in 54 years equivalent an average lifetime of the Nigerian population, FiR is the average daily food intake rate in Kg/person/day (0.325 Kg), Cmetal is the concentration of metal in food sample in Mg/Kg, R_fD is the oral reference dose in Mg/Kg/day and TA is the average exposure time for non-carcinogen in days (ED × 365 days/year).

Target Cancer Risks (TCR)

TCR is used to assess the potential risk associated with the exposure to a carcinogenic agent throughout the lifetime exposure period. Here, instead of an oral reference dose, as was used in the determination of THQ, an oral cancer slope factor for inorganic arsenic (CpSo) is used. This factor determines, along with the dose of the carcinogen, the probability of excess cancer risk over the lifetime of the exposed individual.

$$\text{TCR} = \frac{Efr \times ED \times FiR \times Cmetal \times CpSo}{WAB \times TA} \times 10^{-3} \ldots\ldots\text{equation 4 (Joahnn } et\ al\text{., 2017)}$$

Statistical Methods and Analysis

All statistical analyses were performed using IBM SPSS Version 25.0. Analysis of variance (ANOVA) was used to analyze the uptake of arsenic by Waterleaf plant and means were separated using Turkey's test at $p < 0.05$ (significant) over the graded period of growth at all treatment levels. Descriptive statistic of mean ± Sd was also used to demonstrate the bioaccumulation of As by the plant parts.

RESULTS

Soil Characterization

The soil was characterized using parameters such as particle size analysis, physicochemical properties and heavy metals concentrations. The result of soil particle analysis revealed that the percentages of sand, silt, and clay determined were 78.00%, 9.40%, and 12.60% respectively (Table 2). Furthermore, the percentage of sand was observed to be greater than clay, followed by silt which is the lowest.

Table 2: Soil Particle Size

Sand %	Silt %	Clay %	Sand/Clay Ratio	Textural Class
78.00	9.40	12.60	6.19	Sandy loam

The Physiocochemical properties of the soil are presented in Table 3. The pH of the soil is observed to be 6.3; while the electrical conductivity had a value of 240µS/cm. Organic matter and organic carbon content had values of 3.43% and 1.99% respectively and were found to be high. The available phosphorus level was observed to be high (66.67 mg/kg) and is at a level that is suitable for uptake of certain metals by plants and vegetable growth. The result revealed that this high phosphorus content is responsible for the high percentage composition of sand in the soil investigated.

Table 3: Physicochemical Properties of the Soil

Soil pH(1:2.5)	Electrical conductivity (µS/cm)	Organic Carbon (%)	Organic Matter (%)	Available Phosphorus (mg/kg)	Exchangeable Cation Exchange Capacity (ECEC) Cmol/kg
6.3	240	1.99	3.43	66.67	14.6

The heavy metals results (Table 4) differ in their concentrations as 0.3142, 6,003, and 52.421 mg/kg for aluminum, iron and manganese respectively. Furthermore, iron has the highest content followed by manganese, while aluminum is the least amongst these metals.

Table 4: Heavy Metals Content of the Soil

Aluminum Al (mg/kg)	Iron (Fe) (mg/kg)	Manganese (Mn) (mg/kg)
0.3142	6,033	52.421

Uptake of Arsenic by Waterleaf *Talinum triangulare* in As contaminated Soil

The uptake of arsenic by Waterleaf into the leaf and root of the plant part was investigated via the effect of frequency of harvest and time of harvest at the two treatment levels, and the result obtained presented (Tables 5 and 6).

Table 5: Statistical Evaluation of Arsenic (As) Concentration in Plant Parts of waterleaf *Talinum triangulare* and Accumulation Factors (n = 3)

		Frequency of Harvest		Time of Harvest	
Treatment Levels (Mg/Kg)	**Treatments**	**Leaf**	**Root**	**Leaf**	**Root**
50 Mg/Kg Soil	3	0.861 ± 0.009	2.231 ± 0.010	0.861 ± 0.009	2.231± 0.010
	6	0.882± 0.010	2.118 ± 0.001	1.118± 0.001	1.107 ± 0.001
	9	1.116 ± 0.001	2.323 ± 0.002	1.135 ± 0.010	1.207 ± 0.001
70 Mg/Kg Soil	3	0.954 ± 0.021	2.238 ± 0.014	0.954± 0.021	2.238 ± 0.014
	6	0.971 ± 0.008	2.318 ± 0.001	2.228 ± 0.002	2.040 ± 0.014
	9	1.136± 0.002	2.339 ± 0.010	2.339± 0.010	2.176 ± 0.003

The results showed that variation of as concentration in Waterleaf plant exist in all the treatment levels (50 and 70 mg/kg contaminated soil) and treatments (weeks) studied. As concentration vary between 0.861 ± 0.009 to 1.116 ±0.001 and 0.954 ± 0.021 to 1.136 ± 0.002mg/kg at 50mg/kg and 70mg/kg treatment levels over the graded period of growth for frequency of harvest. A similar variation in as concentration was observed for time of harvest and ranged between 0.861 ± 0.009 to 1.135 ± 0.010 mg/kg and 0.954 ± 0.021 to 2.339 ± 0.010 mg/kg at 50 mg/kg and 70 mg/kg treatment levels over the graded period of growth.

The amount of as abstracted into the root from the soil and subsequent translocation into the leaf for frequency of harvest and time of harvest were calculated and presented in Tables (Tables 5 and 6). As concentration vary between 2.231 ± 0.010 to 2.323 ± 0.002 mg/kg and 2.238 ± 0.014 to 2.339 ± 0.010mg/kg at 50 mg/kg and 70 mg/kg treatment levels for frequency of harvest. Similarly, it vary between 2.231 ± 0.010 to 1.207 ± 0.001 mg/kg and 2.238 ± 0.014 to 2.176 ± 0.003mg/kg at 50 mg/kg and 70 mg/kg treatment levels for time of harvest. The overall result depicts that as concentration in the root is generally higher than that in the leaf.

Health Risk Assessment by Consumption of Waterleaf Grown on as Contaminated Soil
3.3.1: Estimated Daily Intake of as (EDIM)
The results of EDIM for adults (60kg) are shown in Table 6. It was observed that the calculated EDIM for leave and root were affected by the frequency of harvest and time of harvest at the treatment levels studied. EDIM vary from 3.964×10^{-4} to 5.2303×10^{-4} and 3.9642×10^{-4} to

1.0769×10^{-3} for frequency of harvest and time of harvest across the treatment levels in the leaf. In the root, EDIM vary from 1.0272×10^{-3} to 9.7516×10^{-3} and 5.0968×10^{-4} to 9.3925×10^{-3} for frequency of harvest and time of harvest across the treatment levels.

Table 6: Estimated Daily Intake [EDIM] of metal (As) in mg/kg for Adults (60Kg) from Consumption of Waterleaf *Talinum triangulare*

Treatment Levels (mg/kg).	Treatments (Weeks)	Leaf		Root	
		FH	TH	FH	TH
50 Mg / Kg	3	3.9642×10^{-4}	3.9642×10^{-4}	1.0272×10^{-3}	1.0272×10^{-3}
	6	4.0610×10^{-4}	5.1475×10^{-4}	9.7516×10^{-3}	5.0968×10^{-4}
	9	5.1303×10^{-4}	5.2260×10^{-4}	1.0695×10^{-3}	5.5572×10^{-4}
70 Mg / Kg					
	3	4.3920×10^{-4}	4.3920×10^{-4}	1.0304×10^{-3}	1.0304×10^{-3}
	6	4.4706×10^{-4}	1.0258×10^{-3}	1.0672×10^{-3}	9.3925×10^{-3}
	9	5.2303×10^{-4}	1.0769×10^{-3}	1.0769×10^{-3}	1.0019×10^{-3}

FH: Frequency of Harvest and TH: Time of Harvest

Health Risk Index (HRI) or Hazard Quotient (HQ)

To further assess the health risk that might arise by consuming the vegetable, the health risks index (HRI) was calculated and presented in Table 7. The HRI vary from 1.3214 to 1.7434 and 1.3214 to 3.5897 for frequency of harvest and time of harvest across the treatment levels in the leaf; Whereas, in the root it vary from 3.2440 to 3.5897 and 1.6989 to 3.4347 for frequency of harvest and time of harvest across the treatment levels.

Table 7: Hazard Quotient or Health Risk Index (HRI) for Adults Exposed to Waterleaf Contaminated with Sodium Arsenate Pesticide

Treatment Levels (mg/kg)	Treatments (Weeks)	Leaf		Root	
		FH	TH	FH	TH
50 Mg / Kg	3	1.3214	1.3214	3.4240	3.4240
	6	1.3537	1.7158	3.2505	1.6989
	9	1.7128	1.7420	3.5650	1.8524
70 Mg / Kg					
	3	1.4641	1.4641	3.4347	3.4347
	6	1.4902	3.4193	3.5573	3.1308
	9	1.7434	3.5897	3.5897	3.3397

FH: Frequency of Harvest and TH: Time of Harvest

Target Hazard Quotient (THQ)

The result of the non-cancer parameter (THQ) vary from 0.0140970 to 0.0404964 and 0.0140970 to 0.0376743 for frequency of harvest and time of harvest across the treatment levels in the leave, whereas, in the root it varies from 0.0196682 to 0.0402194 and 0.0165191 to 0.0404964 for frequency of harvest and time of harvest across the treatment levels over the graded period of growth (Table 8). It was also observed that the THQ increased over the graded period of growth and was dependent on the treatment level.

Table 8: Target Hazard Quotient (THQ) for Adults Exposed to waterleaf Contaminated with Arsenic

Treatment Levels (mg/kg)	Treatments (Weeks)	Leaf		Root	
		FH	TH	FH	TH
50 Mg / Kg	3	0.0140970	0.0140970	0.0386265	0.0386265
	6	0.0152705	0.0193565	0.0366701	0.0191661
	9	0.0193219	0.0196509	0.0402194	0.0208974
70 Mg / Kg					
	3	0.0387477	0.0387477	0.0165191	0.0165191
	6	0.0401328	0.0353196	0.0168115	0.0385746
	9	0.0404964	0.0376743	0.0196682	0.0404964

FH: Frequency of Harvest and TH: Time of Harvest

Target Cancer Risk

The potential risk associated with the exposure to carcinogenic agent throughout the lifetime exposure to consumers of Waterleaf grown on As contaminated soil was studied through the target cancer risk (TCR), and the result obtained presented in Table 10. This factor determines, along with the dose of the carcinogen, the probability of excess cancer risk over the lifetime of the exposed individual.

Table 10: Target Cancer Risk (TCR) for As Analyzed Waterleaf *T. triangulare*Grown in As Contaminated Soil

Treatment Levels (mg/kg)	Treatments (Weeks)	Leaf		Root	
		FH	TH	FH	TH
50 Mg / Kg	3	6.7081×10^{-6}	6.7081×10^{-6}	1.7382×10^{-5}	1.7382×10^{-5}
	6	6.8717×10^{-6}	8.7104×10^{-6}	1.6502×10^{-5}	8.6247×10^{-6}
	9	8.6949×10^{-6}	8.8429×10^{-6}	1.8099×10^{-5}	9.4039×10^{-6}
70 Mg / Kg					
	3	7.4327×10^{-6}	7.4327×10^{-6}	1.7436×10^{-5}	1.7436×10^{-5}
	6	7.5652×10^{-6}	1.7358×10^{-6}	1.8060×10^{-5}	1.5894×10^{-5}
	9	8.8507×10^{-6}	1.8223×10^{-6}	1.8223×10^{-5}	1.6953×10^{-5}

FH: Frequency of Harvest and TH: Time of Harvest

In the leaf, the TCR vary between 6.7081×10^{-6} to $8.6949 \times10^{-6}$and $6.7081\times10^{-6}$to $8.8429\times10^{-6}$for frequency of harvest and time of harvest at 50 mg/kg contaminated soil. Again, the TCR vary between $7.4327 \times 10^{-6}$to $8.8507 \times 10^{-6}$and $7.4327 \times 10^{-6}$to 1.8223×10^{-5} for frequency of harvest and time of harvest at 70 mg/kg contaminated soil. The root also showed a similar variation of the leaf and vary between 1.7382×10^{-5} to1.8099×10^{-6} and 1.7382×10^{-5} to 9.4039×10^{-6} for frequency of harvest and time of harvest at 50 mg/kg contaminated soil; whereas, it varies between 1.7436×10^{-5} to 1.8223×10^{-5} and 1.7436×10^{-5} to 1.6953×10^{-5} for frequency of harvest and time of harvest at 70 mg/kg contaminated soil.

DISCUSSION

Physicochemical Parameters and Bioaccumulation of Arsenic (As) by Waterleaf Plant

The result of Soil particle analysis displayed in Table 2 revealed variation in their particles with sand having the highest percentage. The result of our current study is in agreement with the report of Mohammed *et al.* (2017) who reported a high sand content in their sampled soil. However, the high sand content is at variance with the reports of Ebuchua *et al.* (2013), George (2014), and George and Amah (2015) who reported low sand content. Their results was supported independently by Rahaman *et al.* (2013) and Shahri and Chan (2015). Texturally, the soil could be classified as sandy loam due to the high sand content and such soil has been reported to be suitable for plant growth and yield. It could therefore be inferred that the vegetable may give good growth and yield as a function of its texture. The pH (6.3) is an indication of the soil being slightly acidic. This pH range is found to be within the pH range suitable for most vegetable growth and speciation of arsenic compounds, depending on the oxidative state. The optimum pH range for most plants growth has been observed to be between the ranges of 5.5-7.0. However, many plants have adapted to thrive at pH values outside this range. Waterleaf *T. triangular* thrives between pH of 6.1 to 7.8 for maximal growth and yield. Furthermore, the soil has high electrical conductance. This view was supported by Khamla and Pantawalt (2015) who reported high electrical conductance in their result. The high electrical conductance implies that the soil contained high dissolved mineral salts, and this is in agreement with the separate works of Karaca (2004) and Arias *et al.* (2005).

The high organic carbon and matter were observed to be high and could be attributed to the solid municipal waste that is constantly dumped at the bank of the freshwater stream at Isiokpo from where the soil was obtained. Another contributory factor is the bonding effect of the minerals found in the soil with certain minerals like smectite, kaolinite, chlorite, and montmorillonite with organic matter. In this form, organic matter is preserved without depletion and hence the presence of high organic matter observed in the soil (Kalantri & Haul, 2008).This view was corroborated separately by Tei *et al.*, (2013) and Yunus *et al.*, (2015), who reported that organic matter can be adsorbed onto clay minerals and preserved organic matter in rocks. The organic matter affects the quality and quantity of soil fertility and stabilizes soil pH. The aluminium, manganese and iron content are displayed in Table 4. These metals are responsible for plague formation upon which arsenic can be adsorbed or precipitated to accumulate in the root part of the plant. When this happen the translocation power of the plant is reduced as observed in the result for the two treatment levels.

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Uptake of Arsenic by Waterleaf *Talinum triangulare* in as contaminated Soil

The uptake of as by Waterleaf plant has shown that generally, the as concentrations obtained were all higher than the 0.002 mg/kg or 2µg/kg body weight prescribed by JECFA provisional tolerable daily intake for inorganic as (FAO/WHO,1983). It was also revealed from the results obtained that as the number of weeks over the graded period increase, there is a corresponding increase in the amount of as abstracted into the leaf at the two treatment levels (50mg/kg and 70 mg/kg) for both frequencies of harvest and time of harvest.

The overall result revealed that over the graded period of growth and at all treatment levels studied, the amount of As abstracted into the leaf exceeded the maximal permissible limit of 0.1 mg/kg and 0.5 mg/kg prescribed by FAO/WHO (1999), and Lombi and Nokan (2005) respectively. This makes the Waterleaf grown on this soil to be unfit for human consumption and the need to prevent the latent health issues of public health concerns that may arise (ATSDR, 2010). This view was in accord with the result of ecotoxicological studies of D' Amore *et al*., (2005), which showed that soil contaminated with heavy metals/metalloids is still a major and growing public health and environmental concern owing to their ability to impair human life and the environment.

Furthermore, the greatest amount of as was found in time of harvest than for frequency of harvest at all the treatment levels over the graded period of growth. This means that if the plant is allowed to fallow over the graded period of growth, more as will be accumulated than when it is frequently harvested. Moreover, the degree of as abstracted into the leaf was shown to be dependent on the graded period of growth. The result confirmed the independent reports of Anyalobu *et al.,* (2007) and Blum *et al.,* (2017) who claimed that Waterleaf can abstract arsenic into its matrix at varying degrees and over the graded period of growth. This means that Waterleaf has the potential to bioaccumulate as at vary degrees (Table 5). This could be attributed to the presence of water-soluble and mobile as fraction in the soil where the Waterleaf was grown. This view had the backing of several authors who had done similar studies and reported that water-soluble as fractions are the most ecologically relevant since they are more readily mobile and made bioavailable. In this form plants roots can absorbAs and subsequently sequester it into other parts of the plant e.g. leaf and stem (Mench *et al*., 2009; Kabata – Pendias & Pendias, 2011; Beesley & Marmiroli, 2011). Further support was given by Radulescu *et al.,* (2013), who reported the formation of soluble complexes by as with the sap component of Waterleaf and their subsequent transfer from the soil to the aerial parts of the plant.

The pH of the soil on which the plant was grown is another key factor for the degree of bioaccumulation of as in the plant. Soil pH is responsible for changes in the surface charge of adsorbents and determines as speciation with the most thermodynamically stable oxidative state and also affects the solubility and bioavailability of soil As. The soil pH (6.3) is within the optimal pH (6.1-7.8) for most vegetable growth like Waterleaf. Moreover, it is within the optimum pH (4.0-8.0) for the formation of the most thermodynamically stable forms of As ($H_2AsO_4^-$ and $HAs_2O_4^{2-)}$. At this pH, phosphorus is made available by isomorphous substitution of as when more phosphate will be bonded to the soil active sites with subsequent release of As into the plant matrix.

The variety of the Waterleaf plant used in this study could also be implicated for the high content of as in the leaf and other plant parts. This view was supported by Alam and Rahman (2003) who observed that as accumulation in rice was dependent on the rice variety used for the experiment. They were supported by Liu *et al* (2006), and Zavala and Duxbury (2008) which demonstrated that different rice cultivars showed significant difference for as concentration in straw, husk, and grain. It could, therefore, be inferred that the variety of Wild species of Waterleaf plant used was responsible for the degree of as accumulation in the leaf of the plant. Apart from the aforementioned contributory factors, the age and part of the plant can also affect As uptake (Singh *et al*., (2007).

The soil texture plays an important role in the solubility and bioavailability of as for plant uptake into its matrix. Generally, soils with clayey textures have less availability of as compared with sandy and loamy soils. Clay and silt are characterized by a large surface area which tends to increase sorption (Bussen & Frimmel, 2003; Sahoo & Kin, 2013). The soil texture upon which the plant was grown is typically sandy loam which will permit a very low amount of As to be held by the soil, while the majority will be made available for abstraction by waterleaf. Hence, the observed as concentration in the leaf of the plant.

Also, the result depicts that as concentration in the root is generally higher than that in the leaf, which could be attributed to the suitable pH (6.3) of the soil for the growth of the plant and the formation of iron (Fe), manganese (Mn) and Aluminum (Al) plague and their hydroxides on the surface of the root with the stable species of As. The formation of the plague is made possible because of the presence of Fe, Mn, and Al in the soil. These plagues serve as a good adsorption surface upon which as can co-precipitate or precipitate to increase as in the root (Smeldey & Kimburgh, 2011; Martin *et al*., 2012; Mench *et al*., 2009; Kabata – Pendias & Pendias, 2011; Beesley & Marmiroli, 2011). Further support was given by Radulescu *et al.*, (2013). The adsorbed as can be desorbed and leached in its mobile phase as a result of watering, and thus be abstracted into the plant matrix. In addition, the activity of phosphate transporter in the soil at a suitable pH which was able to present as in the mobile and soluble form that can be easily transported to the root of the plant could be implicated.

Health Risk Assessment

The estimated daily intake of metal (EDIM) is displayed in Table 7. The result depicted that highest and lowest EDIM was observed in the root and leave for frequency of harvest and time of harvest across the treatment levels, which could be attributed to the presence of high levels of As in the root (Tables 5), due to adsorption of As on plague formed by Fe, Mn, and Al (Smeldey & Kimburgh, 2011; Martin *et al*., 2012). Again, the ability of phosphate transporters in the soil to transfer the water-soluble and mobile form of As into the root is another reason. Generally, the EDIM of both the leave and root, were very low and lower than the recommended tolerable daily intake of 0.002 mg/kg/body/day by FAO/WHO (1983). It was also lower than unity (EDIM < 1), which indicates that no undue health issue of public health concern is expected.

Again, health risk index result depicted that the HRI increased as the graded period of growth increased from 3 weeks to 9 weeks except in the root in 6th and 9th weeks respectively. Generally, the HRI is greater than unity (HRI > 1), and is in accord with the independent views of Ikeda *et al*. (2000) and Zhuang *et al*. (2007). The HRI could be considered safe or unsafe depending on if the HRI is greater or lower than unity. HRI > 1 is considered unsafe, while HRI < 1 is considered safe. The HRI >1in this study revealed that this vegetable is unfit for consumption. In order to classify the health risks associated with the consumption of this vegetable, the HRI was compared with the RFD and it was found that the ratio was 10 times greater than the HRI. This value according to NYSDOH (2007) is considered to cause very serious health issue to a population that is practically vegetarian and those that take it as a staple food (Khan *et al*., 2008; Zhou *et al*., 2016). The study has shown that the use of arsenical pesticide in growing vegetable is a contributor to soil arsenic and subsequent abstraction into the plant matrix.

Furthermore, the overall result of target hazard quotient showed that the THQ for the root was greater than that of the leave across the treatment levels. Furthermore, all the THQ values were less than unity (THQ < 1). This is in accord with the separate views of Islam *et al*., (2014) and Zodape (2014) who argued that THQ should not be greater than unity in order not to arouse health issues of public health concern. This was further confirmed by the report of Maheshwari and Jasrotia (2015) and Adedokun *et al*., (2016 & 2017). The THQ < 1 indicates a level of no concern that the population may be at systemic health risk. This implies that long time exposure to as via consumption of this vegetable, may not pose any obvious adverse health effect for the period of life expectancy considered. However, there are reports of several authors whose results were at variance (THQ > 1) with the result of this present study. THQ > 1 in heavy metals in had been reported by Cui et al., (2004), and was supported by Singh *et al.*, (2010) when they investigated the THQ in vegetable from wastewater irrigated area. Credence was given to the earlier reports by Udeme *et al.*, (2016), Zhou *et al.*, 2016, and Ogbo and Patrick-Iwanyanwu (2019) who all had THQ > 1.

The general result for target cancer risks depicted that all the TCRs obtained from this study were all lower than the recommended upper limit of 1×10^{-4} used for acceptable cancer risk (Joahnn *et al*., 2017). The result of this study is in agreement with the results of Joahnn *et al*., (2017) who had TCR values less than 1×10^{-4}. However, this is at variance with the result of Yanez *et al.*,

(2019) who had TCR that exceeded that of our current result. Literature has it that a TCR lower than 10^{-6} is considered negligible; one between 10^{-6} and 10^{-4} is generally considered acceptable (average risk of developing cancer over a human lifetime is 1 in 100,000), whereas one above 10^{-4} is held as dangerous and indicates high potential cancer risk. This implies that as has the low potential to develop cancer among the exposed population during lifetime (USEPA, 2010 and Monferra *et al*., 2016). It can therefore be inferred that since the TCR for this study is less than the upper limit of 10^{-4}, and slightly above the lower limit of 1×10^{-6}, As hold alow potentiality to develop cancer among the exposed population during a lifetime.

CONCLUSION

The study has shown that Waterleaf cultivated on as pesticide contaminated soil, have the potential to bioaccumulate as and sequester it through the plant matrix. The extent of bioaccumulation was dependent on the levels of contamination and the graded period of growth, for both frequencies of harvest and time of harvest. The results of this study have shown that the concentration of as in the plant matrix was high and above the permissible limit (0.1 mg/kg) of FAO/WHO. Thus, suggesting that waterleaf grown on arsenical pesticide should be eaten in moderation due to possible health hazard that may arise from consumption. Health risks assessment has revealed that the EDIM and THQ pose no possible undue health risks since their values were all less than unity. However, the HRI and TCR indicates that consumers may be exposed to as toxicity now or in the future and may likely develop cancer since the TCR is within the permissible limit of developing cancer. The overall result suggest that indiscriminate pesticide application is a major source of soil and plant contamination and its consumption can cause possible health issues of public health concern. This means vegetarians are at high risks of suffering health challenges in the future if not now. The study has also provided a brief insight into the current scenario of vegetable crop contamination and possible future health risks estimate. Therefore, to prevent or protect against as toxicity, consumers need vegetable specific and site-specific information, and should pay serious attention to the kind and type of pesticides used in its cultivation. There is also need for continuous monitoring of as levels in vegetables grown with arsenical pesticides.

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