Original Research Article

EVALUATION OF HEAVY METAL CONTENT AND HUMAN HEALTH RISK ASSESSMENT OF WATER LEAF (*TALINUM TRIANGULARE*) GROWN ON ARSENIC SPIKED SOILS.

ABSTRACT

AIM: This study investigated the concentration of heavy metal (Arsenic), taken up by waterleaf plant cultivated in three contaminated soil type namely; sandy, loamy and clay. The health risk associated in exposing the waterleaf plants was accessed from the different soil types at different treatment levels of 40mg/kg (low) and 80mg/kg (high) of arsenic.

Methods: Three soil types namely; sandy, loamy and clay were spiked with 40 mg/kg and 80 mg/kg of arsenic. The waterleaf plant was grown on the spiked soil samples for the period of 2, 4, 6 and 8 weeks. Each soil sample was taken before the spiking of the soil to serve as the basal control soil sample. This will determine the content of arsenic in the soil. After the stipulated growth period, the soil sample was collected into a clean sample bottle likewise the waterleaf plant which was harvested, washed, oven dried and ground into a powdered form which was acid digested. The heavy metal content in both the plant and soils were determined by atomic absorption spectroscopy.

Results: The results of arsenic concentration in waterleaf from loamy soil at 40 mg/kg were 0.118 mg/kg, 0.189 mg/kg, 0.295 mg/kg, and 0.332 mg/kg at weeks 2, 4, 6, and 8 respectively. At 80 mg/kg, arsenic concentrations in loamy soil were 0.200 mg/kg, 0.388 mg/kg, 0.612 mg/kg and 0.693 mg/kg respectively. For sandy soil, the concentration of arsenic in the waterleaf plant treated at 40 mg/kg were 0.083 mg/kg, 0.219 mg/kg, 0.279 mg/kg, and 0.310 mg/kg for weeks 2, 4, 6 and 8 respectively and 0.159 mg/kg, 0.400mg/kg, 0.572 mg/kg, 0.632 mg/kg at 80 mg/kg respectively while in clay soil, the arsenic concentration was 0.079 mg/kg and 0.167 mg/kg for weeks 2 and 4 at 40 mg/kg and 0.190 mg/kg and 0.320mg/kg for 80 mg/kg for weeks 2 and 4 respectively. No growth was recorded in weeks 6 and 8. The mean values of the arsenic concentration in the plant spiked with 40 mg/kg and 80 mg/kg respectively were significantly different ($p<0.05$)from each other but that for the loamy soil at 40 mg/kg was not significantly different ($p>0.05$).The Estimated Daily Intake for arsenic was <1, Target Hazard Quotient was < 1 and the Health Risk Index for adults that would be exposed to arsenic was <1 except for the loamy soil which was ≥ 1 at week 4 and 6 for treatment level 80 mg/kg.

Conclusion: Arsenic concentration increases in the plant with increasing period of growth, while in the soil, the reverse occurs. The result shows that the waterleaf plant absorbed heavy metal (Arsenic) from the contaminated soils but at concentrations that do not pose any health risk within the experimental period of exposure. However, it is possible that with extended duration of exposure, consumption of plants exposed to contaminated soils with heavy metals could be hazardous.

Keywords: *Heavy metal, Health Risk Assessment, Arsenic Spiked soil, water leaves*

INTRODUCTION

Heavy metals are generally referred to as those metals which possess a specific density of more than 5 g/cm^3 and adversely affect the environment and living organisms [1]. They are elements that contaminate food and make them harmful to human health when present in quantities that are higher than the permissible limit [2]. Some trace elements such as arsenic (As), cadmium (Cd), mercury (Hg) and lead (Pb) are toxic even at small concentrations [3].

According to Ekwumemgbo [4], heavy metals though phytotoxic even at low concentration can be mopped up by certain species of plants in the soil. Earlier researchers recognized that certain species of plants could accumulate high levels of heavy metals from the soil. Waterleaf (*Talinum triangulare*), a portulaceace, is an erect glabrous perennial herb of tropical Africa descent, though widely grown in West Africa, Asia and South America [5]. It is known locally in Nigeria as 'Gborondi', 'Nte -oka' or Inene (Igbo), 'momoiko' (Ibibio), 'Gbure' (Yoruba), 'Alenyruwa' (Hausa) [6].

Soil contamination has become a serious problem in all industrialized areas of the world. Soil is equally regarded as the ultimate sink for the pollutants discharged into the environment [7]. Most plants and animals depend on the soil as a growth substrate for their sustained growth and development. In many instances, the sustenance of life in the soil matrix is adversely affected by the presence of deleterious substances or contaminants. The contamination of soils with heavy metals or micronutrients in phytotoxic concentrations generates adverse effects not only on plants but also poses risks to human health [8]. They might be transported from soil to ground waters or may be taken up by plants, including crops in which waterleaf is one [9]. It is well known that high industrial and traffic activities contribute high levels of heavy metals to the environments. Plants grown around such areas are likely to absorb these metals either from the soil through the roots or from atmospheric contaminants through the leaves [10]. Afterwards, the consumption of contaminated vegetables constitutes an important route of heavy metal exposure to animals and humans [11].

Abandoned waste dumpsites have been used extensively as fertile grounds for cultivating vegetables, though research has indicated that the vegetables are capable of accumulating high levels of heavy metals from contaminated and polluted soils [12, 13]. The soil contamination by heavy metals can transfer to food and ultimately to consumers. Plants accumulate heavy metals from contaminated soil without physical changes or visible indication, which could cause a potential risk for human and animal [14]. This study was thus designed to evaluate the heavy metal content and human risk assessment of waterleaf grown on soils spiked with different concentrations of arsenic.

MATERIALS AND METHODS

Soil

The soils used for the study were clay, loamy, and sandy soils which were collected from Rivers State University Agricultural Farm sites, in the Port Harcourt campus of the University. They were used without further treatment or modification. After the content of arsenic was determine in the basal samples collected before the experimentation.

Plant Material: Waterleaf (*Talinum triangulare*)

A locally adapted vegetable (Waterleaf), *Talinum triangulare* collected from the Rivers State University farm site was used to assess uptake of soil arsenic (As) through pot experiment. The plant was identified by the Plant morphologist of the agricultural farm in Rivers State University.

Reagents

The reagent used as a source of arsenic ($Na_2HAsO_4{:}7H_2O$) was purchased from the local chemical and agrochemical store Glo Chemicals located at AP22 Air- force Market, Rumuomasi in Port Harcourt, Rivers State.

Soil Sampled Areas.

The soil samples were taken at a uniform depth of 15cm with the aid of a hand trowel that had been pre-cleaned with concentrated nitric acid to prevent heavy metal contamination before analysis. The homogenate soil samples were transferred into polythene bags and transported to the laboratory. The soils samples were air-dried, homogenized, and ground to pass through a 2mm nylon fibre sieve and stored in plastic bottles (no preservative was added) for subsequent analysis.

Preparation of Arsenic Stock Solution.
0.416469g of (Na_2HAsO_4:$7H_2O$) Disodium hydrogen arsenate heptahydrate crystals was accurately weighed using Mettle Toledo weighing balance, Model PL 203 (New Zeeland) and transferred into a 50 mL volumetric flask, followed by the addition of a small quantity of deionized water to dissolve the salt. The solution formed was poured into a 100 mL volumetric flask and shaken vigorously to ensure complete dissolution, after which the solution was made up to the 100 mL mark with distilled water. This gives 1.3347 x 10^{-2} moles working solution of As (1000 ppm As^{5+}). Further working solutions were prepared by serial dilution of an appropriate volume of the stock solution with an appropriate volume of deionized water.

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(Australia).

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

Bioaccumulation of Arsenic (As) By Waterleaf (*Talinum triangulare)* Plant
Experimental Design
The design was a 2×5 factorial laid out in a randomized complete block design (RCBD). The design consisted of a basal sample, then four treatments (2 weeks, 4 weeks, 6 weeks and 8 weeks respectively) at two different treatment levels of 40 $mgkg^{-1}$ As (low) and 80 $mgkg^{-1}$ As (high), thus resulting to 10 treatment combinations.

Experimental Procedure
Planting and Harvesting of Waterleaf (*Talinum triangulare)*.
Two sets of 12 plastic pots containing accurately weighed 1kg of the composite or homogenate soil was added into each pot and spiked (contaminated) with 40 $mgkg^{-1}$ As and 80 mg kg^{-1} As solution and allowed to age for 2 weeks. The desired loading concentration of 40$mgkg^{-1}$ and 80$mgkg^{-1}$ is because safe levels of arsenic expected in soil is between 5-20$mgkg^{-1}$ [15].. The pots were placed in 4 groups of 3 (C1, L2, S3) for the two different treatment levels for each type of soil sample and labelled $C2_{wks}$, $L_{2wks,}$ S_{2wks}; $C4_{wks}$, $L4_{wks}$, S_{4wks}; C_{6wks}, L_{6wks}, S_{6wk} and C_{8wks}, L_{8wks}, S_{8wks} for the two treatment levels (40 mg/kg As and 80 mg/kg) As respectively. Furthermore, three other pots for each soil type containing Waterleaf *T. Triangulare* without arsenic were used as control. The plant in each group were harvested at two weeks interval.
On the second week, the first group of four (4) was sampled destructively (leave and soil), without sampling any other group. Destructive sampling (leave and soil) was carried out on the 2^{nd} group of 4, on the fourth, sixth and eight weeks respectively. Note that at each time the plant is sampled, the soil is also collected alongside.

Pre-treatment and Washing of Samples.
After collection, samples were brought to the laboratory and processed further for analysis. The edible portions and roots 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℃) to a constant weight. The plant materials were ground in a mill, powdered and digested before determining the residual arsenic content in it.

Acid Digestion Method

Soil samples were air-dried, crushed and sieved through a 2mm sieve; 1g of the samples were weighed, 10 mL of water samples were measured accurately and transferred to a 250 ml conical flask. 10 mL of chemical; perchloric, nitric and sulphuric acid in the ratio (1:2:2) was added into the sample and heated on a hot plate in a fume hood. The mixture was heated until a white fume was observed which signifies that digestion was complete. The sample was allowed to cool and 20 mL of distilled water was added to bring the metals into solution. The sample was allowed to cool to room temperature and filtered using a Whatman filter number 41 into a 100 mL volumetric flask and made up to the mark with distilled water, then transferred to100 mL plastic can for AAS analysis (Agilent 240 AA machine).

Statistical Analysis

Results obtained from evaluation of parameters were presented as Mean ± SD. The Statistical package used was the GraphPad Prism 8.02 version. Analysis of Variance (ANOVA) was used to compare the means using the Tukey multiple comparison test. Differences in mean values were considered significant at $p < 0.05$.

Calculation of Health Risk Assessment

To assess the possible health risk associated with the consumption of Waterleaf cultivated on soil spiked (contaminated) with sodium arsenate, the estimated daily intake of arsenic (EDI), Health Risk Index (HRI) and Target hazard quotient (THQ) were calculated using the appropriate equations [16,17]. 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)

$$\textbf{EDIM} = \frac{Cmetal \times Cfactor \times Cfoodintake}{Baverage\ Weight}$$

C_{Metal} = the As concentration in Waterleaf *Talinum triangulare* (mg/kg),

C_{Factor} = the conversion factor,

$C_{Foodintake}$ = the daily intake of vegetables and

$B_{Average}$ = 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} was assumed, while the average body weight of an adult vegetable consumer was 60kg for this study [11].

Health Risks Index (HRI).

$$\text{HRI} = \frac{EDIM}{RfD}$$

Where, EDI = the estimated daily intake of Arsenic and
R_fD = the oral reference dose of Arsenic which is 0.0003 mg/kg /day [18]

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 [16]. THQ values were calculated using the formula:

$$(THQ) = \frac{Efr \times ED \times FiR \times Cmetal}{RfD \times WAB \times TA} \times 10^{-3} \ [17].$$

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

RESULTS

The evaluation of heavy metal content and human health risk assessment of vegetables (waterleaf plant) grown on arsenic spiked soils was carried out and the results are highlighted thus; the physicochemical analysis of the soil are shown in Table 1

Table 1: Physicochemical Analysis of the Soil

S/N	Parameters	Sandy	Loamy	Clay
1	Soil pH 1:2.5	6.90	6.10	2.70
2	Electrical conductivity, µS/cm	138.74	4.12	5640.08
3	Available Phosphorus, mg/kg	21.05	136.84	3.51
4	Organic Carbon, %	0.31	3.61	4.17
5	Organic Matter, %	0.54	6.22	7.19
6	Total Nitrogen, %	0.01	0.09	0.03
7	Exchangeable K, cmol/kg	0.12	0.38	0.25
8	Exchangeable Na, cmol/kg	0.03	0.50	7.13
9	Exchangeable Ca, cmol/kg	1.24	4.96	2.40
10	Exchangeable Mg, cmol/kg	0.23	2.40	3.47
11	Total exchangeable acidity, cmol/kg	0.84	0.57	19.58
12	Effective cation exch. Capacity, cmol/kg	2.46	8.81	32.83
13	Sand, %	94.00	84.00	66.00
14	Silt, %	2.00	3.00	11.00
15	Clay, %	4.00	13.00	23.00

Table 1 highlighted the physicochemical properties of the soil samples. The pH of the sandy soil was 6.90, while that of the loamy and clay soils were 6.10 and 2.70 respectively. The total exchangeable acidity cmol/kg was highest in the clay soil (19.58 cmol/kg) and lowest in the loamy soil (0.57 cmol/kg).

The concentration of arsenic in the different soil samples before they were spiked is shown in table 2.

Table 2: Heavy Metal Analysis on Soil Samples (Basal sample)

Parameter	Dpr Limit	Clay	Loamy	Sandy
Arsenic, mg/kg	NS	≤ 0.01	≤ 0.01	≤ 0.01

In table 2 Arsenic content in clay, loam and sand was ≤ 0.01 mg/kg. However, the DPR limit was non-significant.

The result of the arsenic content by the waterleaf plant are shown in Figure 1a and 1b respectively indicating the different treatment levels.

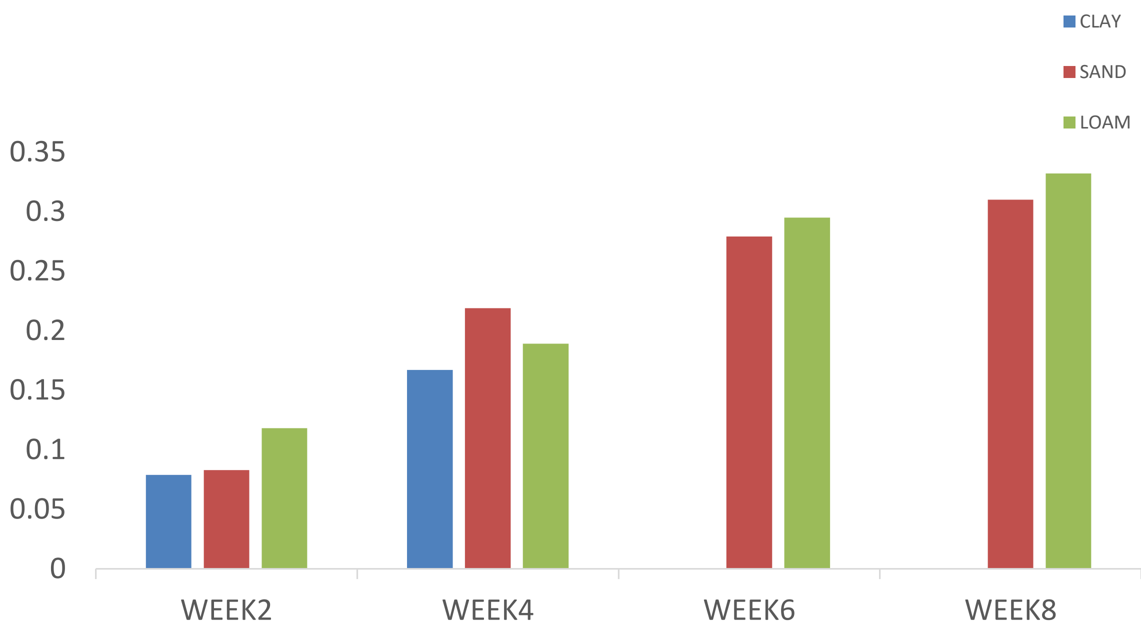

Figure. 1a: Summary of Bioaccumulation of Arsenic in Waterleaf Plant at 40 mg/kg Contaminated Soil

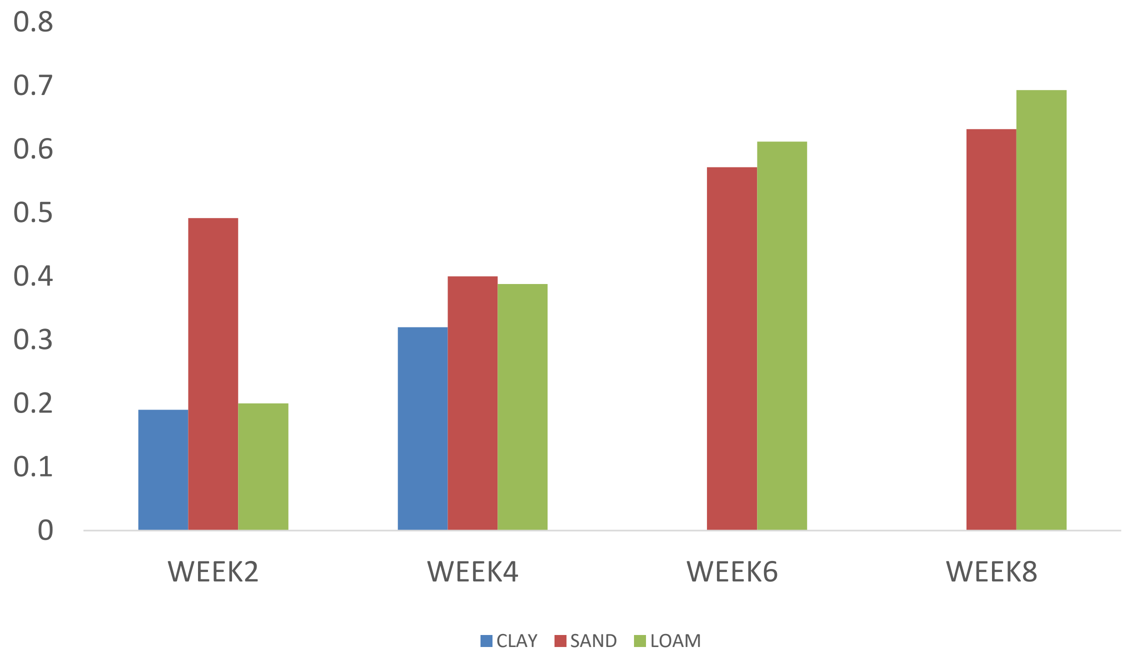

Figure. 1b: Summary of Bioaccumulation of Arsenic in Waterleaf Plant at 80 mg/kg Contaminated Soil

Sandy soil shows the highest concentration of accumulated arsenic while clay soil has the least value at week 2. At treatment in week 4, sandy soil has the highest value of arsenic concentration.
Arsenic content in waterleaf plants at 80 mg/kg as shown in fig. 1b illustrates that the arsenic accumulation in the waterleaf plant increases as the treatment week increase except in week 2 treatment where the sandy soil has the highest arsenic value.

Arsenic uptake by the different soil types at treatment level 40 mg/kg and 80 mg/kg are illustrated in fig. 2a and 2b respectively below. The arsenic taken up by the soil after treatment with 40 mg/kg as shown in fig. 2a was highest in clay followed by loam. The concentration of arsenic was high in treatment week 2 and decreased as the treatment week increases. This shows that the arsenic in the contaminated soil was successfully taken up by the waterleaf plant. The arsenic taken up by the soil at 80 mg/kg as shown in fig. 2b decreases as the treatment week increases. This is similar to the results in fig. 2a hence we can say that the arsenic content in the soil is inversely proportional to the treatment week while the arsenic content in the waterleaf plant is directly proportional to the treatment week.

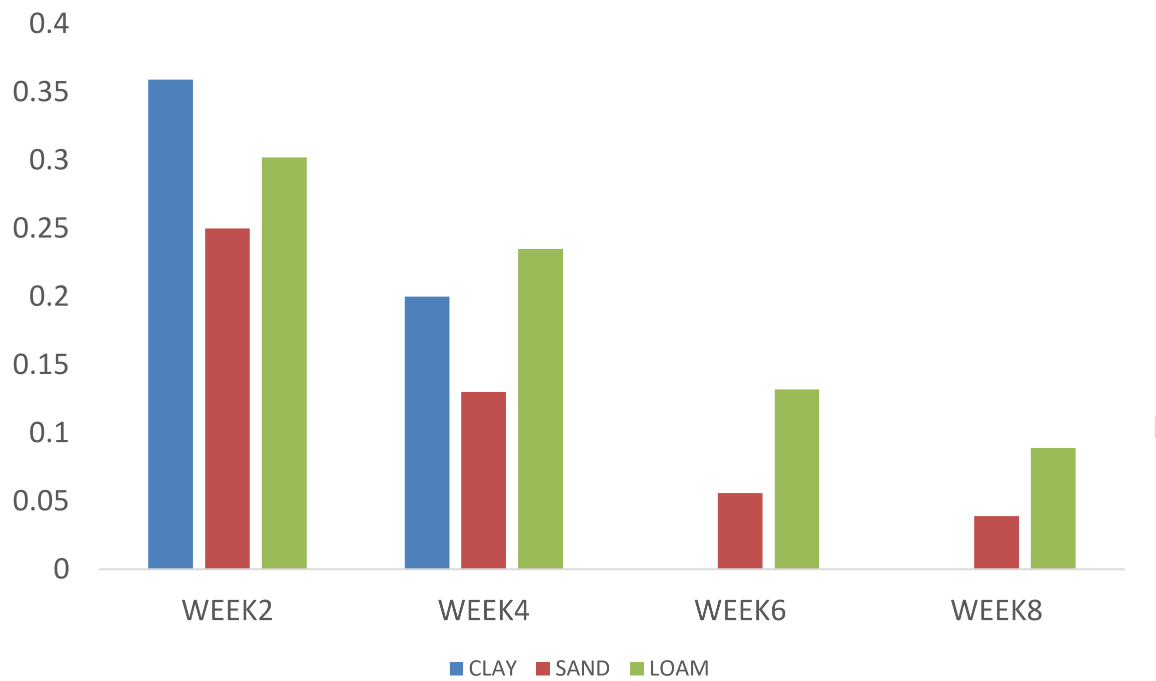

Fig. 2a: Arsenic Content in the Different Soil Types at Treatment Level 40 mg/kg

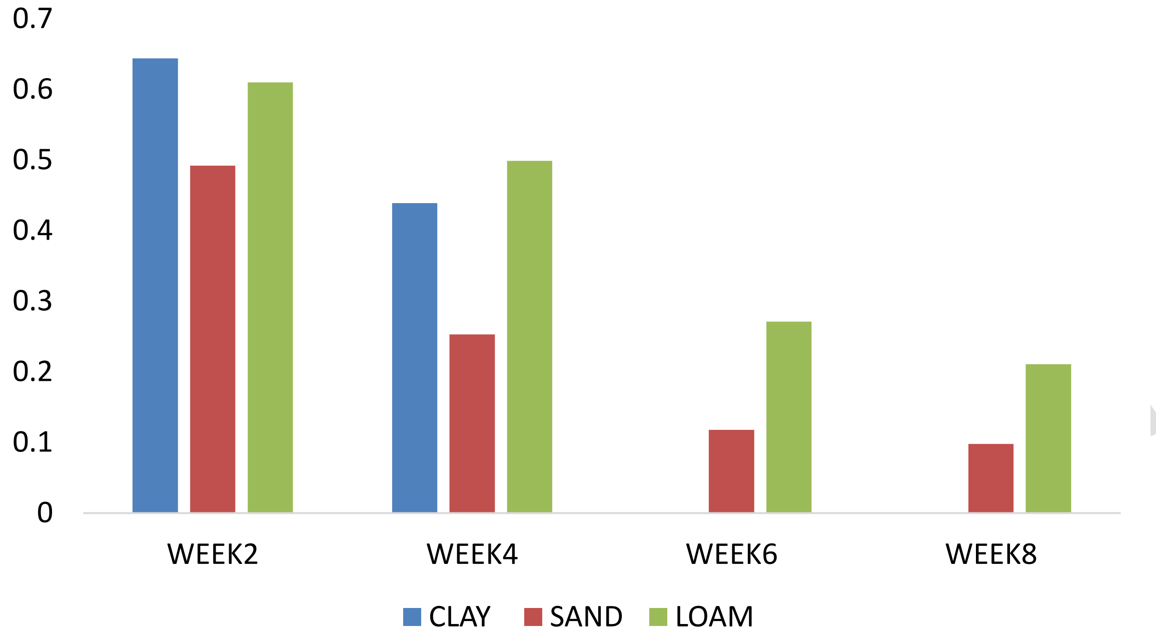

Fig. 2b: Arsenic Content in the Different Soil Types at Treatment Level 80 mg/kg

The arsenic taken up by the soil after treatment with 40 mg/kg as shown in fig. 2a was highest in clay soil followed by loamy soil. The concentration of arsenic was high in treatment week 2 and decreased as the treatment week increases. This shows that the arsenic in the contaminated soil was successfully taken up by the waterleaf plant. The arsenic taken up by the soil at 80 mg/kg as shown in fig. 2b decreases as the treatment week increases. This is similar to the results in fig. 2a hence it can be deduced that the arsenic content observed in the soils was inversely proportional to the treatment week while the arsenic content in the waterleaf plant was directly proportional to the treatment week.

Health Risk Assessment

Using the calculations and the formula [16,17] given: $EDI = \frac{Cmetal \times Cfactor \times Cfoodintake}{Baverage\ Weight}$, the estimated daily intake of arsenic in the waterleaf plants at treatment level 40 mg/kg was illustrated below in table 3.

Table 3a: Estimated Daily Intake (EDI) of Arsenic by Waterleaf Plant at 40mg/Kg

Soil type	2 Weeks	4 Weeks	6 Weeks	8 Weeks	Remarks
Clay	3.637×10^{-5}	7.689×10^{-5}	ND	ND	EDI < 1
Sand	3.822×10^{-4}	1.008×10^{-4}	1.285×10^{-4}	1.427×10^{-4}	EDI <1
Loam	5.433×10^{-5}	8.292×10^{-5}	1.358×10^{-4}	1.529×10^{-4}	EDI <1

ND- Not Detected

The estimated daily intake of arsenic in the waterleaf plant at concentration of 40 mg/kg was 3.637×10^{-5} and 7.689×10^{-5} for clay soil at treatment weeks 2 and 4 respectively; 3.822×10^{-4}, 1.008×10^{-4}, 1.285×10^{-4}, and 1.427×10^{-4} for sandy soil at treatment weeks 2, 4, 6, and 8 respectively, while loamy soil has EDI of 5.433×10^{-5}, 8.292×10^{-5}, 1.358×10^{-4}, 1.529×10^{-4}.

At 80 mg/kg the estimated daily intake of arsenic of waterleaf plant are given in table 3b

Table 3b: Estimated Daily Intake of Arsenic by Waterleaf Plant at 80mg/kg

Soil Type	2 Weeks	4 Weeks	6 Weeks	8 Weeks	Remarks
Clay	8.749 x10^{-5}	1.473 x10^{-4}	ND	ND	EDI <1
Sandy	7.320 x10^{-5}	1.842 x10^{-4}	2.634 x10^{-4}	2.910 x10^{-4}	EDI <1
Loamy	9.208 x10^{-5}	1.786 x10^{-4}	2.818 x10^{-4}	3.191 x10^{-4}	EDI <1

ND-Not Detected

The estimated daily intake of arsenic by waterleaf plant at 80 mg/kg as seen in the table 3b for the soil types were less than 1. The health risk index (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 Health Risk Index (HRI) was calculated using the formula [19]

HRI$=\frac{EDIM}{RfD}$ and the results was shown in table 4 below.

Table 4a: Health Risk Index of Adults Exposed to Arsenic in Waterleaf Plant at 40mg/kg

Soil Type	2 Weeks	4 Weeks	6 Weeks	8 Weeks	Remarks
Clay	0.1212	0.2563	ND	ND	HRI < 1
Sandy	0.1274	0.3361	0.4282	0.4758	HRI <1
Loamy	0.1811	0.2764	0.4527	0.5095	HRI<1

ND-Not Detected

The health risk index of adults exposed to arsenic in waterleaf plant at 40 mg/kg was less than 1.

The health risk index for adults exposed to arsenic in waterleaf plants at 80 mg/kg is shown in table 4b

Table 4b: Health Risk Index of Adults Exposed to Arsenic in Waterleaf Plant at 80mg/kg

Soil Type	2 Weeks	4 Weeks	6 Weeks	8 Weeks	Remarks
Clay	0.2916	0.4911	ND	ND	HRI <1
Sandy	0.2440	0.6139	0.8779	0.9690	HR1 ≈1
Loamy	0.3069	0.5955	0.9393	1.0636	HR1 =1

ND-Not Detected

At treatment concentration of 80 mg/kg, health risk index of adults exposed to arsenic in waterleaf planted on loamy soil was higher followed by that on sandy soil. The health risk index in clay soil was less than 1, while in sandy and loamy, HRI was equals 1. It must also be noted that HRI increases as treatment week (exposure) increases. Non-carcinogenic risk estimation of heavy metal (As) was determined using Target Hazard Quotient (THQ) values, which is a ratio of the determined dose of a toxicant to a reference dose considered harmful. THQ values were calculated using the formula [17].

$(THQ) = \frac{Efr \times ED \times FiR \times Cmetal}{RfD \times WAB \times TA} \times 10^{-3}$ and the results are shown in Table 5 below

Table 5a: Target Hazard Quotient of Adults Exposed to Arsenic in Waterleaf Plant at 40 mg/kg

Soil Type	2 Weeks	4 Weeks	6 Weeks	8 Weeks	Remarks
Clay	1.368 $x10^{-3}$	2.891 $x10^{-3}$	ND	ND	THQ < 1
Sandy	1.437 $x10^{-3}$	3.792 $x10^{-3}$	4.831 $x10^{-3}$	5.367 $x10^{-3}$	THQ < 1
Loamy	2.043 $x10^{-3}$	3.272 $x10^{-3}$	5.108 $x10^{-3}$	5.748 $x10^{-3}$	THQ <1

ND-Not Detected

The target hazard quotient of adults exposed to arsenic in waterleaf plant in the three soil types were less than 1. This means that there is no carcinogenic risk due to exposure to arsenic in waterleaf plant at treatment level 40 mg/kg. The target hazard quotient of adults exposed to arsenic in waterleaf plant at 80mg/kg is shown in table 5b below.

Table 5b: Target Hazard Quotient of Adult Exposed to Arsenic in Waterleaf Plant at 80mg/kg

Soil Type	2 Weeks	4 Weeks	6 Weeks	8 Weeks	Remarks
Clay	3.290 $x10^{-3}$	5.540 $x10^{-3}$	ND	ND	THQ < 1
Sandy	2.753 $x10^{-3}$	6.925 $x10^{-3}$	9.903 $x10^{-3}$	1.094 $x10^{-2}$	THQ <1
Loamy	3.463 $x10^{-3}$	6.718 $x10^{-3}$	1.060 $x10^{-3}$	1.200 $x10^{-2}$	THQ<1

ND-Not Detected

Table 5b shows that the target hazard quotient of adults exposed to arsenic in waterleaf plants at 80mg/kg was less than 1.

The mean ± SD of arsenic taken up by waterleaf plant in different soil types at different treatment periods at 40 mg/kg spiking is presented in table 6a below.

Table 6a: Arsenic Uptake by the Waterleaf Plant in Different Soil Types at Different Treatment Periods at 40mg/kg Spiking

Duration of Treatment (week)s	Soil Types		
	Clay (Mean ±SD)	Sandy (Mean ±SD)	Loamy (Mean ±SD)
0	0.002±0.0006^{a}	0.002±0.001^{a}	0.002±0.001^{a}
2	0.079±0.001^{b}	0.0827±0.005^{b}	0.118±0.001^{b}
4	0.167±0.0006^{c}	0.219±0.000^{c}	0.188±0.001^{c}
6	0.000±0.000^{df}	0.2787±0.002^{d}	0.295±0.000^{d}
8	0.000±0.000^{ef}	0.311±0.001^{e}	0.332±0.001^{e}
F value	49020	52263	134719
P value	<0.0001	<0.0001	<0.0001
Remarks	S	S	S

Key: S= significant, Mean ±SD of parameter along each column with different superscripts are significantly different from each other at *P*<0.05

In table 6a, the arsenic concentration significantly (*P*<0.0001) increased at the different treatment periods for clay soil, sandy soil and loamy soil respectively.

The mean ±SD of the arsenic concentration in the different soil types at different treatment periods at 40 mg/kg spiking is presented in table 6b below:

Table 6b: Arsenic Content in the Different Soil Types at Different Treatment Periods at 40mg/kg Spiking

Duration of Treatment (week)s	Soil Types		
	Clay (Mean± SD)	Sandy (Mean± SD)	Loamy (Mean± SD)
0	0.002±0.001^{a}	0.002±0.001^{a}	0.002±0.001
2	0.358±0.002^{b}	0.251±0.001^{b}	0.030±0.001
4	0.201±0.001^{c}	0.131±0.001^{c}	0.234±0.001
6	0.000±0.000^{a}	0.055±0.001^{d}	0.132±0.001
8	0.000±0.000^{a}	0.039±0.001^{e}	0.359±0.271
F value	108040	40004	1.375
P value	<0.0001	<0.0001	0.3101
Remarks	S	S	NS

Key: S= significant, NS=not significant. Mean± SD of parameter along each column with different superscripts are significantly different from each other at $P<0.05$

The mean values of arsenic concentration were significantly different ($P<0.0001$) in the clay and sandy soil types but the difference in loamy soil type was not significantly different ($P=0.3101$). Also, for the clay soil, the mean value for arsenic concentration in weeks 6 and 8 were the same as compared with week 0, the basal sample from the soil.

The mean ± SD of arsenic taken up by the waterleaf plant in different soil types at different treatment periods at 80 mg/kg spiking is presented in Table 7a below

Table 7a: Arsenic Uptake by the Water Leaf Plant in Different Soil Types at Different Treatment Periods at 80mg/kg Spiking

Duration of Treatment (week)s	Soil Types		
	Clay (Mean± SD)	Sandy (Mean± SD)	Loamy (Mean± SD)
0	0.002±0.001^{a}	0.001±0.000^{a}	0.001±0.001^{a}
2	0.200±0.001^{b}	0.159±0.000^{b}	0.200±0.001^{b}
4	0.321±0.001^{c}	0.401±0.001^{c}	0.388±0.000^{c}
6	0.000±0.000^{a}	0.572±0.001^{d}	0.612±0.001^{d}
8	0.000±0.000^{a}	0.632±0.001^{4}	0.692±0.001^{e}
F value	3272	813993	613496
P value	<0.0001	<0-0001	<0.0001
Remarks	S	S	S

Key: S= significant, Mean ± SD of parameter along each column with different superscripts are significantly different from each other at p<0.05

The mean values of arsenic in the different soil types were significantly different from each other at p<0.0001.

The mean ± SD of the Arsenic content in the different soil types at different treatment periods at 80 mg/kg spiking is shown in table 7b below.

Table 7b: Arsenic Content in the Different Soil Types at Different Treatment Periods at 80 mg/kg Spiking

Duration of Treatment (week)s	Soil Types		
	Clay (Mean± SD)	Sandy (Mean± SD)	Loamy (Mean± SD)
0	0.001±0.000^{a}	0.001±0.000^{a}	0.000±0.000^{a}
2	0.644±0.001^{b}	0.491±0.001^{b}	0.610±0.001^{b}
4	0.439±0.001^{c}	0.254±0.001^{c}	0.498±0.001^{c}
6	0.000±0.009^{df}	0.118±0.000^{d}	0.271±0.001^{d}
8	0.000±0.000^{ef}	0.098±0.001^{e}	0.210±0.001^{e}
F value	2092579	405014	8542
P value	<0.0001	<0.0001	<0.0001
Remarks	S	S	S

Key: S= significant, NS=not significant. Mean ±SD of parameter along each column with different superscripts are significantly different from each other at p<0.05

In Table 7b, there was significant ($p<0.0001$) decrease in the means of arsenic concentration in the different soil samples.

DISCUSSION

The pH of clay soil as indicated explains the reason for the stunted growth observed in the course of the experiment.

The pH concentration of the clay soil was acidic and accounts for the stunted growth experienced in the course of the experiment. The acceptable pH range for an agricultural soil is between 5.5 - 7.0 [20]. The sandy and loamy soil samples were within the acceptable pH limit of agricultural soil and this explains the reason for an appreciable growth observed in the experiment.

The heavy metal content of waterleaf plant from the three different soil types at treatment levels 40 mg/kg and 80 mg/kg in fig. 1a and 1b shows that bioaccumulation of arsenic concentration in waterleaf plant ranges from 0.079 to 0.320 for clay soil, 0.083 to 0.632 for sandy soil and 0.118 to 0.693 for loamy soil. It was observed that waterleaf plant in the loamy soil had the highest metal concentration followed by sandy soil, while clay soil had the lowest. That is loamy soil > sandy soil > clay soil. Generally, the waterleaf has shown bioaccumulation potential for the studied metal into their tissues at different concentrations. This observation is in strong agreement with the report of [21] who did a similar study on bioaccumulation of heavy metal in plant.

It was also observed that the amount of arsenic taken up by the waterleaf plant increased as the treatment week increased as shown in table 6a and 7a indicating that the increase in arsenic uptake by the waterleaf plant was 2 weeks < 4 weeks < 6 weeks < 8 weeks. This could be that the waterleaf plant is capable of bioaccumulating heavy metals from the surrounding environment. Hence the longer the planting duration, the higher the heavy metal (arsenic) uptake by the plant which might pose health risk when consumed. This observation is similar to a study on uptake of arsenic by the lettuce plant where high Arsenic concentration in soil may have contributed to high arsenic uptake by lettuce [22,23].

The arsenic content of the different soil types generally decreased as the treatment week increased. The decrease in the arsenic soil level is accounted for by the uptake by the waterleaf plants as seen in the results. The arsenic in the soil is higher in clay soil, followed by loamy and sandy soil soils respectively. This is because of the porous characteristics of the soil types. Clay being the least porous soil holds in the arsenic spiked into the soil, followed by loamy soil. Sandy soil which is the most porous loses its spiked arsenic during watering. This explains the reason for the least concentration of arsenic in the sandy soil illustrated in fig. 2a and 2b. A significant ($P<0.0001$) decrease in the arsenic content of the soil was also observed in table 7b. Thus, the trend of decrease in the arsenic concentration of the soil can be represented as 2 weeks > 4 weeks > 6 weeks > 8 weeks.

The estimated daily intake of metals by waterleaf sourced from three soil samples as calculated by standard methodology indicated values that were < 1 which suggest that at the present level of exposure in the clay, sandy, and loamy respectively lower than the regulatory permissible limit (0.1mg/kg) prescribed by FAO/WHO (2011) [24,25]. Furthermore, the result depicted that all the EDI was lower than 1. This implies that health risk associated with consumption of the plant at the present level of exposure might not arise and hence will not pose any health risk if consumed.

The present result agrees with the work of these researchers [26,27,28] respectively who investigated EDI in vegetables.

Table 4a reveals the health risk index obtained after comparing the EDI with their individual metal oral referral dose. HRI obtained ranged between 0.12 to 0.49, 0.13 to 0.97 and 0.18 to 1.1 for clay, sandy and loamy soils spiked at 40 mg/kg respectively This implies that it may not pose any health risk to exposed adult at this treatment level. However, the overall result in table 4a shows that the HRI of soil types was lower than 1 (HRI < 1) and might not pose any metal toxicity to consumers of waterleaf plant grown on clay soil with arsenic concentration within the range encountered in this study. The result in table 4b shows that the HRI was > 1 or approximately equals to 1 for the soil types spiked at 80 mg/kg indicating the likelihood of metal toxicity to consumers of waterleaf plants grown on the contaminated soil with increased arsenic contamination. This implies that at a higher treatment concentration, exposure of adults to arsenic in waterleaf plant on contaminated soil could pose health risk. The HRI obtained in this study is in accordance with the values obtained separately by [27, 29] who reported HRI > 1.

To determine if the population exposed to these metals via consumption of the sourced waterleaf plant on contaminated soil, the bio-accumulated amount was used to calculate the target hazard quotient (THQ) as presented in table 5a and 5b. The overall result depicted that all the THQ was less than 1. This implies that there will be no carcinogenic risk resulting from exposure via consumption of contaminated vegetables. The result is similar to that reported by Islam *et al*. [30],[31 & 32] also stated that THQ not exceeding 1 does not pose any alarm for public health concern. However, it is important to state while in the finding in the present may not imply the possibility of carcinogenic implication or any systemic effect in one's lifetime via consumption of vegetable planted on contaminated soil might at the concentration used in the study, the health risk implication of arsenic consumption through the food chain should not be underestimated.

CONCLUSION

The study has shown that vegetables such as waterleaf planted on contaminated soil of different types can bio-accumulate the heavy metal (Arsenic) in their tissues. Although the concentrations of arsenic obtained in the vegetable plant in this study did not indicated potential health risk at the concentration with which the soil samples were spiked, the health risk associated with consumption of food sources such as vegetable contaminated with high concentrations of arsenic should not be ignored.

REFERENCES

1. Yeboah Shi G & Shi W. Effects of heavy metal contamination on soil enzymes activities, Journal of Geoscience and Environment Protection, 2021; 9: 135 -154.
2. Waribo HA, Bartimaeus, ES & Onuoha, IC Assessment of some heavy metal content of Dried crayfish sold in Creek Road market, Borokiri, Port Harcourt, Nigeria. Asian Journal fisherman and Aquatic Research, 2019; 5(1):1-6.
3. Divrikli U. Horzum N. Soylak M. & ElciL Trace Heavy Metal Contents of Some Spices and Herbal Plants from Western Anatolia, Turkey International Journal of Food Science Technology, 2006; 14:712-716.
4. Ekwumemgbo, PA Eddy, NO. & Omoniyi, IK. Decontamination of heavy metals in polluted soil by phytoremediation using Bryophyllum pinnatum, Phytoremediation .2013. pp.39-43.
5. Enete, AA. & Okon. UE. Economics of Waterleaf (*Talinum triangulare*) Production in Akwa Ibom State, Nigeria, Field Actions Science Reports: The Journal of Field Actions, 2010; 4:1-6.
6. Ibeawuchi, II Nwufo, MI. Oti, NN; Opara, CC. & Eshett, ET. Productivity of Intercropped Green (*Amaranthus cruentus*)/ Waterleaf (*Talinum triangulare*) with poultry manure rates in South Eastern Nigeria. Journal of Plant Science.2007; 2(2):222-227.
7. Shokoohi, R., Saghi, MH., Ghafari, HR. & Hadi, M. Bio-absorption of iron from Aqueous Solution by dried bio mass of activated sludge. Iran Journal of Environmental Health Science and Engineering, 2009; 6(2):107-114.
8. Murugesan, AG., Maheshwari, S. & Bagirath, G. Biosorption of cadmium by Live and Cells of Spirulinaplatensis, Journal of Environmental Resource ,2008; 2(3): 307-312.
9. Opaluma OD Aremu MO Ogbo LO Abiola KA Odiba IE Abubakar MM &Nweze NO. Heavy Metal concentration in soils, plants, leaves and crops grown around dump around dumpsites in Lafia metropolis, Nasarawa state, Nigeria. Advances in Applied Science Research,2012;3(2):780-784.
10. Fifield, W.& Haina, I. Environmental Analytical Chemistry. Blackie Academic and Professional. Chapman and Hall, Boundary Row, London. 1997, pp.56-60.
11. Tsafe, AI. Hassan, LG. Sahabi, DM., Alhassan, Yand Bala, BM. Evaluation of Heavy Metal Uptake and Risk Assessment of Vegetables Grown in Yargadama of Northern Nigeria, Journal of Basic and Applied Scientific Research,2012; 2(7): 607-674.

12. Cobb, GP. Sands, K., Waters, M. Wixson, BG. & Dorward king, E. Accumulation of Heavy metals by vegetables grown in mine waste. Environmental Toxicology and Chemistry,2000; 19(3), 600-607.
13. Benson, NU. & Ebong, GA. Heavy metals in vegetables commonly grown in a Tropical garden. Journal of Sustainable Tropical Agricultural Research, 2005; 16,77-80.
14. Osma, E., Serin, M., Leblebici, Z. & Aksoy A. Heavy Metals Accumulation in Some Vegetables and Soils in Istanbul. Ekoloji, 2012; 21(82):1-8.
15. Rekha, B. Arsenic Toxicity: an overview. Horticulture International Journal,2019;3(1):20-22.

16 Oves, M., Khan, M.S., Zaidi, A., Ahmad, E. Soil contamination, nutritive value, and human health risk assessment of heavy metals: an overview. In: Zaidi, A., Wani, P.A., Khan, M.S. (Eds.), Toxicity of Heavy Metals to Legumes and Bioremediation. Springer, New York, 2012, pp. 1–27
17 Harmanescu M Alda LM Borden DM Gogoasa L& Gergen L. Heavy metal health risk assessment for population via consumption of vegetables grown in old mining area; a case study: Banat County, Romania. Chemistry Central Journal,2011; 5:64-68.

18 Jahnn MRA Leslie AHF &Charles NG. Assessment of Potential health risk associated with the aluminum, arsenic, cadmium and lead content in selected fruits and vegetables grown in Jamaica. Toxicology Reports,2017; 4:181-187.
19 Adedokun A Njoku K Akinola M Adesuyi AA & Jolaoso AO. Heavy metal content and the potential health risk assessment of some leafy vegetables cultivated in some flood plains and farmlands in Lagos, Nigeria. FUNAI Journal of Science and Technology,2017; 3(1):30-47.
20 Neina D. The role of soil pH in plant nutrition and soil remediation. Applied and Environmental Soil Science, 2019; 5794869:1-9.
21 Benard, B.B., Silverleen, D.C & Chidima, I.J. Human health assessment of consuming heavy metals in oyster (Crassostrea virginica) from different markets in port Harcourt, State Earth and Environmental Science Research and Review, 2020; 3(2):67-72.
22 Cao, X. & Ma, L.Q. Effects of compost and phosphate on plant arsenic accumulation from soils near pressure-treated wood. Environmental Pollution, 2004; 132: 435-442
23 de Oliveira, L.M., Suchismita, D. Arsenic uptake by lettuce from As-contaminated soil remediated with Pteris vittata and organic amendment. Chemosphere 2017; 176, 249–254
24 FDA (Food and Drug Administration) Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc. Report of the Panel on Micronutrients. National Academy Press, Washington, DC Food and Drug Administration, Dietary supplements, Center for Food Safety and Applied Nutrition. 2001.
25 FAO/WHO. Codex Alimentarius Commission. Joint FAO/WHO Food Standards Programme Codex Committee on Contaminants in Foods. Fifth Session. 21-25 March 2011. Working Document for Information and Use in Discussions Related to Contaminants and Toxins in the GSCTFF (Prepared by Japan and the Netherlands) CF/5 INF/1. The Hague, The Netherlands, 2011; pp 89-102.

26 Akande F. O. & Ajayi S.A. Assessment of heavy metals levels in soil and vegetables grown in Peri-Urban farms around Osun State and the associated human health risk.), International Journal of Environment, Agriculture and Biotechnology (IJEAB 2017; 2(6): 3250-3261.
27 Zhuang, P., Zou, B & Li, Z. A Heavy metal contamination in soils and food crops around Dabaoshan Mine in Guangdong, China. Implication for human health. Environmental Geochemistry and Health, 2009;31:707-715.
28 Sharma, R.K., Agrawal M. & Marshal, F.M Heavy metal contamination in vegetables grown on waste water irrigated areas of Varanasi, India. Bulletin of Environmental Contamination and Toxicology. 2005; 77:311-318.
29 Ikeda, M.S; Zhhang, Z.W; Shimbo, S.; Watanabe, T.; Nakatsuka, H., Moon, C.S.; Matsuda-Inuguchi, N. & Higashikawa, k. Urban population exposure to lead and cadmium in East and South East Asia. Science of Total Environment, 2000; 249:373-384.

30 Islam, M.S.; Ahmed, M.K.; AL-Mamum, M.H; Islam, K.N.; Ibrahim, M & Masunaga, S. Arsenic and lead in foods: a potential threat to human in Bangladesh. Food Additives and Contaminants: part A. 2014;31(12):1982-1992.
31 Mehjbeen J. & Nazura U. Accumulation of heavy metals and human health risk assessment via the consumption of freshwater fish *Mastacembelus armatus* inhabiting, thermal power plant effluent loaded canal, Springerplus,2016, 5(1):776
32 George, DMC Bills, US, Sydney -Jack, T. & Taylor SP. Assessment of Heavy Metal Contents of Oyster (Crassostrea virginica) and Associated Health Risks in Rivers State Journal of Health, Applied Sciences and Management,2020; 4:1-10