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Assessment of Phyto-Remediation Quotient of Cultivated Waterleaf *Talinum Triangulare* on Soil Contaminated With Sodium Arsenate Pesticide

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ABSTRACT: *The potential of Waterleaf Talinum triangulare to abstract and consequently remediate As from As-pesticide contaminated soil was investigated by pot experiment and the concentration of As in the plant and soil was determined through mass plasma atomic emission spectroscopy (MP-AES). The composite soil in two separate groups of pots was spiked with 50 mg/kg and 70 mg/kg disodium arsenate pesticide and stands of Waterleaf were grown on them over a 9 weeks growth period. The plant parts (leave and root) and soil after harvesting were pretreated, acid digested and subjected to MP-AES analysis. The results depicted that As concentrations in the plant parts differ at all the treatment levels over the graded period of growth for frequency of harvest and time of harvest and exceeded the maximum permissible limit established by FAO/WHO, and makes the plant unfit for human and animal consumption. The experimental results revealed a statistically significant difference ($p<0.05$) in the bioaccumulation of As over the graded period of growth at all the treatment levels. The Bio-accumulation factor (BAF) and Bio-concentration factor (BCF) were generally greater than unity (BAF > 1 and BCF > 1) in all treatment levels at the time of harvest which indicates that Waterleaf is suitable for phytoextraction and phytostabilization. However, BAF < 1 and BCF < 1 were observed in the 3rd week and 9th week in the frequency of harvest. Furthermore, Translocation factor (TF <1 and TF > 1) were observed for frequency of harvest and time of harvest, implying that the plant can serve as a bad and good accumulator of As in contaminated soil for phytoremediation if the plant is frequently harvested or allowed to fallow. TF > 1 means that the vegetable will be suitable as a good phytoextractor. It can, therefore, be concluded that Waterleaf can be used for commercial and environmental friendly phytoremediation technology (Green technology) to clean up As polluted sites and environmental monitoring.*

KEY WORDS: BAF, BCF, TF, Phytoextraction and phytostabilization and As contamination

INTRODUCTION

Waterleaf *Talinum triangulare* is a perennial herb of tropical African descent that is widely grown and consumed as vegetables in West Africa and other parts of the earth like Asia, and South America (Enete & Okon, 2010, and Wilberforce, 2016). The plant contains important nutrients and phytochemicals such as flavonoids and polyphenols, crude protein, lipids, essential oils, cardiac glycosides, omega-3-fatty acids, minerals, soluble fibres and vitamins (Swarna & Ravindhran, 2013). This property of Waterleaf and its availability makes it one of the most sought vegetables. The plant is a short-duration crop that can be harvested within 35 to 45 days from planting and is known by several names by different ethnic groups in Nigeria. The Ibibio, Hausa and Igbos called it 'Momoiko', 'Alenyuw-a', 'Gborondi', 'Nte-oka or Inene' respectively. Anyaloggbu (2007), reported that the plant (leaves and young stem) is used in large quantities as a vegetable and softener when cooking a fibrous vegetable or thicker in sauce in the southern part of Nigeria.

This plant has been reported to abstract arsenic from contaminated composite soil samples and consequently remediate it (Anyalogbu, 2017; Onyia *et al*., 2020; Owonubi *et al*., 2022). This view was recognized by earlier researchers who claimed that certain plant species could accumulate high levels of arsenic from the soil while continuing to grow and proliferate normally (Udosen *et al*., 2006; Bhagure & Mirgane, 2010). However, other plants such as Alfalfa, sunflower, beetroot, lettuce, potato, radish and millet have been implicated in the uptake or abstraction of arsenic into plant matrices from soils exposed to arsenic (Warren *et al*., 2003; Smith *et al*., 2009; Daniel *et al*., 2012). The abstraction potential of Waterleaf for arsenic was corroborated in strong terms by Blum *et al*. (2017), who reported that the plant can abstract As into its matrix to a great extent over a graded period of growth. They discovered that as the time of harvest increases, so also the quantity of As absorbed by the plant increases, consequently leading to a quantity greater than the maximally permissible level. It was therefore concluded that some plants could be applied in phytoextraction or phytostabilization for phytoremediation purposes on As contaminated and polluted soil or environment.

Furthermore, the earlier work by Icaza *et al*. (2009), gave credence to the results obtained by Anyalogbu (2017), who reported a linear relationship between soil As and the plant matrix of rice, vegetables and fruits. However, plants vary considerably in their tolerance of As and in the quantity they can abstract from the soil and water environment respectively. A similar positive correlation between soil and plant As contents had been reported independently by works from other authors (Goyal *et al*., 2008; Feng *et al*., 2009; Malakootian *et al*., 2009 ; Urik *et al*., 2009), which was further corroborated by Zarolsalimi *et al*. (2011).

Ebong *et al*. (2007), studied heavy metal/metalloid abstraction and bio-accumulation by Waterleaf grown on dumpsites in Uyo metropolis, Akwa Ibom State, Nigeria. Parameters such as the relationship between heavy metal concentration and transfer ratio in soil-plant matrices were determined. Their result was in agreement with previously obtained results of other workers in the related discipline. Furthermore, this result is in agreement with Musam *et al.* (2005), and Odukoye *et al*. (2000), who did similar work on dumpsites in Obafemi Awolowo University, Ife, Nigeria. This review revealed that only limited work has been done on As abstraction by the Waterleaf plant in dumpsites and soils outside Rivers State.

The bioaccumulation potentials of brands of Rice in As-contaminated soil have been studied and reported to take up As from the surrounding soil to reach elevated levels (Juhasz *et al*., 2006; Williams *et al*., 2007). Furthermore, the amount of As in the edible parts of these vegetables can reach relatively high levels which could pose a health-related problem for heavy consumers of vegetables cultivated in As-contaminated soils due to their bioaccessibility. Generally, plants take up As through roots, but in certain plants, the uptake is through the leaves (Bravun *et al*., 2008). This ability of waterleaf to survive in contaminated environments has been exploited to remove other contaminants from the soil. However, there is paucity of information on the phytoremediation potential of *Talinum triangulare* of arsenic in Deltaic soils in Nigeria.

Important to mention that most mitigation systems are not environmentally friendly, thus rendering the soil unusable. The use of plant to remediate heavy metal contamination of the environment is suitable for the environmental conservation as there is no risks, side effects or hazards incurred in the process of mitigation or reversal of the effects of human activity on the environment. In looking for alternative that is environmentally friendly, researchers have resorted to the use of certain plants to mitigate soil pollution as an alternative.

Therefore, it is imperative to investigate the use of cultivated waterleaf *Talinum triangulare* to remove arsenic from arsenic-contaminated soil. Moreover, the result of this study will benefit the environmental chemist, crop production and add to the existing reference materials for researchers, farmers, teachers and the Government.

MATERIALS AND METHODS

Materials

The three major soil groups that exist in Rivers State, namely: the mangrove swamp alluvial soil (Chikoko marine clay), the marine and fluvo-marine deposit soils (Riverbed or River channel deposit) of the salt water swamp, and freshwater brown loam and sandy loam of the Niger delta were used without further treatment or modification. Furthermore, a locally adapted vegetable (Waterleaf),*Talinum triangulare* was used to assess the uptake of soil arsenic (As) through a pot experiment. The reagent used as a source of arsenic ($Na_2HAsO_4{:}7H_2O$) and organic fertilizer was

of an analytical grade that was purchased from a local chemical store in the Port Harcourt metropolis and dissolved in deionized water to form a solution of the desired concentration.

Soil Sample collection

Each sample consisted of four sub-samples or auger burrowing at a depth of 0-20cm, bulked together to form a representative soil of the three different soil types. The homogenate soil samples were transferred into polythene bags and transported to the chemistry laboratory of Rivers State University, Port Harcourt. The individual representative soils were air-dried, homogenized, and ground to pass through a 2mm nylon fibre sieve and stored in plastic bottles or bags for subsequent analysis and experiments. The soil types collected were namely: Freshwater brown loam and sandy loam, Riverbed or River channel deposit, and Chikoko marine clay which were coded SS1, SS2 and SS3 respectively.

Soil Sample Area

Three different alluvial soil types used for this research were collected from two sites at Isiokpo in Ikwerri Local Government Area (Longitude $4^0 54' 17''$ North and Latitude $6^0 53' 11''$East) and one site at Ogbakiri in Emuoha Local Government Area (Longitude $4^0 47' 47''$ North and Latitude $6^0 54' 46''$ East) all of Rivers State, (Niger Delta) Nigeria. The soils were collected in areas away from industrial sites (uncontaminated with Arsenic) from 22nd through 25th February 2018.

Plant Material

Land race of Waterleaf *Talinum triangulare* stands obtained from the Rivers State University Agricultural Farm were used as the plant material.

PREPARATION OF STOCK SOLUTION

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 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

NPK liquid organic fertilizer, Ag Zymer marketed by Zenith Energy – Enzymes Ltd used for the pot experiment, was obtained from an agrochemical store in the Port Harcourt metropolis. 5 mLs of the liquid organic fertilizer was added to 1 litre of distilled water and mixed properly. 100 mLs of the prepared solution was used to water each pot twice weekly until the time of harvest.

EXPERIMENTAL PROCEDURE

Soil Characterization

The pH of the soil was determined using the method of McLean (1982), while the method prescribed by Dellavale (1992) was used for the electrical conductance of the soil. Particle size analysis was determined by the Hydrometer method of Bouyocus (1951), and extrapolating on the Textural Triangle (USDA, 1951), while available phosphorus was determined using Bray and Kurtz No. 1 method as modified by Olsen and Sommers (1982). Furthermore, organic carbon was determined by Walkley and Black (1934) chromic acid and wet oxidation method as described and modified by Nelson and Sommers (1982), while the organic matter content was estimated by multiplying organic carbon concentration by 1.724.

Planting and Harvesting

Two sets of 15 plastic pots containing accurately weighed 1kg of the composite or homogenate soil were 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. Triangular* cuttings were 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 greenhouse and labelled A_{3wks}. B_{3wks}. $C_{3wks.;}$ A_{6wks}. B_{6wks}. $C_{6wks.;}$ A_{9wks}. B_{9wks}. C_{9wks}., and A_{6wks}. $B_{6wks}C_{6wks.}$; and 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 intervals.

In 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 (leaf, root and soil) was done on the only remaining group of 3. Note that each time the plant is destructively sampled, the soil is also collected alongside the root and leaves. For the 2 groups of 3, leaves, root, and soil was destructively sampled on the 6th and 9th weeks respectively. The pilot study gave no growth in SS2 and SS3 soil samples but stunted growth in SS1. Hence, 100 mL of liquid organic fertilizer was applied twice a week to the different soil types until the plant is due for harvest. It was only the plant in SS1 that gave good growth and yield, while those of SS2 and SS3 did not grow. Hence, the pot experiment was carried out only with the soil from Isiokpo (SS1).

Pretreatment 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 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 (90^0C) to a constant weight. The plant materials were ground in a mill, powdered and digested before determining the arsenic content in it.

Acid Digestion

Acid Digestion Method by Patrick-Iwuanyanwu & Chioma (2017) was uesd. 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 (5gm) of the samples was weighed and digested with 2mL of the mixed acid in each of the samples in a Kjeldahl 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 100 mL 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.

Statistical Analysis

The experimental results were expressed as mean ± Sd of triplicate determinations. Descriptive statistics of mean ± SD were used to describe the bioaccumulation potentials of the waterleaf plant.Analysis of variance for all the measured variables was performed by SPSS version 23 (Inc. IBM, USA) software and significant differences were shown at $P < 0.05$.

RESULT

Soil Characterization

The result of soil particle size analysis as presented in Table1 revealed that the particle sizes significantly differ. The soil has a 78.00% of Sand, 9.40% of silt and 12.60% of clay respectively. It further revealed that the sand content was the highest, followed by clay and silt as the least. In terms of textural classification, the soil was classified as sandy loam.

Table 1: Soil Particle Size Distribution

Soil Type	Soil Code	Sand%	Silt%	Clay%	Textural Class
Freshwater sandy loam and Sandy loam	SS1	78.00	9.40	12.60	Sandy Loam

The result of the physicochemical parameters presented in Table 2, revealed a soil pH of 6.3 with an electrical conductance of 240µS/cm and both organic carbon and matter as 1.99% and 3.43%, while the available phosphorous was 66.67mg/kg.

Table 2: **Physicochemical Properties of Soil**

Soil pH (1:2.5)	Electrical Conductivity (µS/cm)	Organic Carbon %	Organic Matter %	Available Phosphorous (Mg/kg))
6.3	240	1.99	3.43	66.67

Bioaccumulation of Arsenic by cultivated Waterleaf *Talinum triangulare*

The summary of As concentrations in waterleaf parts and soil obtained from the study is presented in Table 3. The result revealed that bioaccumulation of As in the plant parts differs at the two treatment levels and over the graded period of growth for both frequency of harvest and time of harvest. The As concentrations in the leaves recorded vary between 0.861 ± 0.009 to 1.116 ± 0.001 mg/kg, and 0.954 ± 0.021 to 1.136 ± 0.002 mg/kg, for frequency of harvest at 50 and 70 mg/kg As contaminated soil. A similar variation of As concentration for time of harvest was observed and recorded with values between 0.861 ± 0.009 to 1.135 ± 0.001mg/kg and 0.954 ± 0.021 to 2.339 ± 0.010mg/kg at 50 and 70mg/kg As contaminated soil. In the root, As concentration vary between 2.231 ± 0.010 to 2.323 ± 0.002 mg/kg, and 2.238 ± 0.014 to 2.339 ± 0.010 mg/kg for frequency of harvest at 50 and 70 mg/kg As contaminated soil. Similarly, for time of harvest, the results obtained at the same As contaminated rate vary between 2.231 ± 0.010 to 1.207 ± 0.001, and 2.238 ± 0.014 to 2.176 ± 0.003 mg/kg at 50 mg/kg and 70 mg/kg As contaminated soil. As in the soil vary between 1.353 ± 0.043 to 1.092 ± 0.009 mg/kg and 2.305 ± 0.001 to 2.215 ± 0.001 mg/kg at 50mg/kg and 70 mg/kg As contaminated soils, for frequency of harvest; whereas for time of harvest it vary between 1.353 ± 0.043 to 0.964 ± 0.023 and 2.305 ± 0.001 to 0.977 ± 0.010 mg/kg for 50 mg/kg and 70 mg/kg As contaminated soil.

Table 3: Statistical Evaluation of Arsenic (As) Concentration in Plant parts of Waterleaf *Talinumtriangulare* and Soil at Two Treatment Levels (n = 3).

Treatments (Weeks)	Frequency of Harvest (50 mg/Kg Soil)			Time of Harvest (50 mg/Kg Soil)		
	Leave	Root	Soil	Leave	Root	Soil
3	0.861± 0.009	2.231±0.010	1.353±0.043	0.861±0.009	2.231±0.010	1.353±0.043
6	0.882±0.010	2.118±0.001	1.228±0.004	1.118±0.001	1.107±0.001	0.988±0.010
9	1.116±0.001	2.323±0.002	1.092±0.009	1.135±0.001	1.207±0.001	0.964±0.023

Treatment (Weeks).	Frequency of Harvest (70 mg/Kg Soil)			Time of Harvest (70 mg/Kg Soil)		
	Leave	Root	Soil	Leave	Root	Soil
3	0.954 ± 0.021	2.238 ± 0.014	2.305 ± 0.001	0.954 ± 0.021	2.238 ± 0.014	2.305 ± 0.001
6	0.971 ± 0.008	2.318 ± 0.001	2.248 ± 0.017	2.228 ± 0.002	2.040 ± 0.014	1.138 ± 0.001
9	1.136 ± 0.002	2.339 ± 0.010	2.215 ± 0.001	2.339 ± 0.010	2.176 ± 0.003	0.977 ± 0.010

To ascertain if there is any statistically significant difference in the bioaccumulation of As by the leaf at the two treatment levels studied, the result was subjected to a one-way analysis of variance (ANOVA). The ANOVA result revealed that there was statistical significant difference in bioaccumulation over the graded period of growth and treatment levels [f (2, 6) =817.023, p = 0.000] and [f (2, 6) = 3.187.682, p = 0.000] for time of harvest at 50 mg/kg and 70 mg/kg soil respectively. A similar trend was observed for frequency of harvest as determined by one-way analysis of variance (ANOVA) [f (2, 6) = 326.199, p = 0.000] and [f (2, 6) = 59.756, p = 0.000] for 50 mg/kg and 70 mg/kg contaminated soil respectively. A Turkey Post Hoc multiple comparison tests were employed to identify where such statistical significant difference occurs and presented (Table 4).

A Turkey Post hoc multiple test for time of harvest at 50 mg/kg contaminated soil revealed that there is a statistical significant difference between the means of 3 Weeks (0.861 ± 0.009, p = 0.000) and 6 Weeks (1.118 ± 0.001, p = 0.000) and between 3 Weeks (0.862 ± 0.009, p = 0.000) and 9 Weeks (1.135 ± 0.001, p = 0.000). However, there was no such difference between the means of

6 Weeks (1.118 ± 0.001, p = 0.000) and 9 Weeks (1.135 ± 0.001, p = 0.000). For leaf from 70 mg/kg contaminated soil, statistical significant difference occurred between the means of 3 weeks (0.954 ± 0.021, p = 0.000) and 6 Weeks (2.228 ± 0.002, p = 0.000) and (2.339 ± 0.010, p = 0.000) respectively. A Turkey Post hoc multiple tests for frequency of harvest on the bioaccumulation of arsenic by Waterleaf plant from 50 and 70 mg/kg contaminated soil revealed that there was no statistically significant difference between 3 Weeks (0.861 ± 0.009, p = 0.000) and 6 weeks (0.882 ± 0.001, p = 0.000). However, significant difference was observed between 3 Weeks (0.861 ± 0.009, p = 0.000) and 9 Weeks (1.116 ± 0.001, p = 0.000). Similarly a statistical significant difference was observed between 6 Weeks (0.882 ± 0.001, p = 0.000) and 9 weeks (1.116 ± 0.001, p = 0.000) at 50 mg/kg contaminated soil. A similar result was observed at 70 mg/kg contaminated soil for the bioaccumulation of As in the Waterleaf plant.

Table 4: Post Hoc Multiple Comparison Test on Bioaccumulation of Arsenic by Waterleaf Grown on Pesticide Contaminated Soil

Treatment (Weeks)	**Control**	**Time of Harvest**		**Frequency of Harvest**	
		50 Mg/Kg Soil	70Mg/KgSoil	50Mg/Kg Soil	70 Mg/Kg Soil
3	0.000±0.000	0.861±0.009^{a}	0.954±0.021^{a}	0.861±0.009^{a}	0.954±0.021^{a}
6	0.000±0.000	1.118±0.001^{b}	2.228±0.002^{b}	0.882±0.010^{a}	0.971±0.008^{a}
9	0.000±0.000	1.135±0.001^{b}	2.339±0.010^{c}	1.116±0.001^{b}	1.136±0.002^{b}

Means with different superscripts are significantly different (Turkey HSD, p<0.05)

A further comparison test was conducted for frequency of harvest and time of harvest between the two treatment levels (50/70 mg/kg contaminated soils). The Turkey Post hoc tests revealed that bioaccumulation of As by Waterleaf showed no significant difference between 3 Weeks (0.907 ± 0.231, p = 0.000) and 6 Weeks (0.972 ± 0.021, p = 0.000) while these differences were observed between 3 Weeks (0.907 ± 0.231, p = 0.000) and 9 Weeks (1.125 ± 0.005, p = 0.000) for frequency of harvest (Table 5). However, statistical significant difference were observed between 3 Weeks (0.954 ± 0.013, p = 0.000), 6 weeks (1.673 ± 0.248, p = 0.000), and 9 weeks (1.737 ± 0.269, p = 0.000) respectively for time of harvest.

Table 5: Post Hoc Multiple Comparison Test on Bioaccumulation of Arsenic by waterleaf between Treatment Levels

Treatment (Weeks)	Control	Frequency of Harvest 50/70 Mg/Kg	Time of Harvest 50/70 Mg/Kg	Frequency of Harvest and Time of Harvest 50 Mg/Kg Soil	Frequency of Harvest and Time of Harvest 70 Mg/Kg Soil
3	0.000±0.000	0.907±0.231[a]	0.907±0.231[a]	0.861±0.006[a]	0.954±0.013[a]
6	0.000±0.000	0.927±0.021[a]	1.673±0.248[b]	0.990±0.058[b]	1.599±0.281[a]
9	0.000±0.000	1.126±0.005[b]	1.737±0.269[c]	1.125±0.004[c]	1.737±0.269[a]

Determination of Phytoremediation Quotient of waterleaf *Talinum triangulare*

The result for bioaccumulation was further accessed through three different parameters to establish the phytoremediation potentials and bio-availability of As to the plant. These parameters were bioaccumulation factor (BAF), bio-concentration factor (BCF) and translocation factors (TF) using the appropriate formula at two different treatment levels (50 mg/kg and 70 mg/kg soil As). They were used to evaluate the potential of waterleaf for phytoremediation, phytoextraction, and phytostabilization depending on if these parameters are greater than or less than one (Yoon *et al*., 2006; Cui *et al*., 2007 and Li *et al*., 2007). Furthermore, the effects of frequency of harvest and time of harvest on the aforementioned parameters were also studied (Figures 1 to 6).

Bio-accumulation Factor.

The bioaccumulationfactor (BAF) which is a ratio of shoot arsenic concentration to that in the soil, was calculated using the formula BAF = As [Shoot]/As [Soil] (Li *et al*., 2007). The result for frequency of harvest at 50 mg/kg recorded were 0.636, 0.716, and 1,022 for 3 weeks, 6 weeks and 9 Weeks respectively; whereas, for time of harvest the values obtained were 0.636, 1.132, and 1.177 for 3 Weeks, 6 Weeks and 9 weeks respectively. Similar variation existed or was observed from 70 mg/kg contaminated soil for frequency of harvest and time of harvest. The results obtained were 0.414, 0.432, and 0.513 for frequency of harvest; 0.414, 1.958, and 2.394 for time of harvest over the graded period of growth i.e. 3, 6 and 9 weeks respectively (Figure 1 and 2).

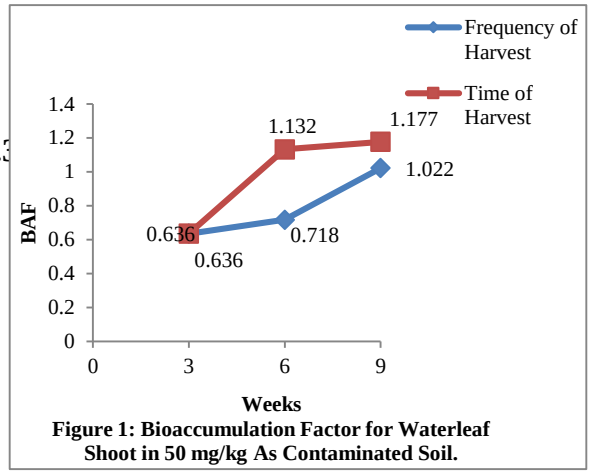

Figure 1: Bioaccumulation Factor for Waterleaf Shoot in 50 mg/kg As Contaminated Soil.

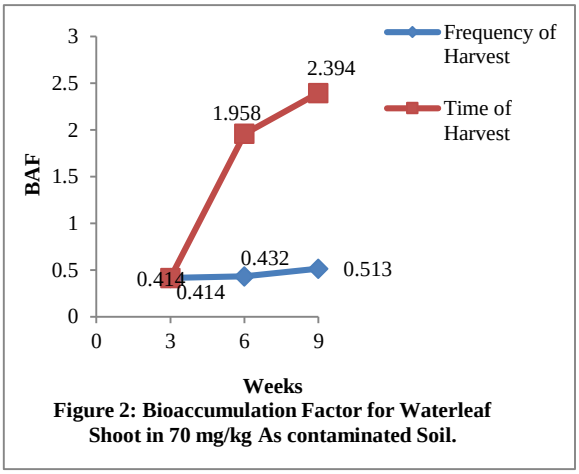

Figure 2: Bioaccumulation Factor for Waterleaf Shoot in 70 mg/kg As contaminated Soil.

Bio-concentration Factor

The bio-concentration factor (BCF) which relates to the amount of arsenic abstracted from the soil by the plant was calculated using the formula: BCF = As [Root] / As [Soil] (Yoon *et al*., 2006). The bio-concentration (root/soil) values for frequency of harvest at 50 mg/kg contaminated soil recorded were 1.649, 1.725, and 2.127 over the graded period of growth (i.e. 3,6, and 9 Weeks respectively), while for time of harvest the values obtained were 1.649, 1.120, and 1.252 over the same graded period of growth (Figure 3 and 4).

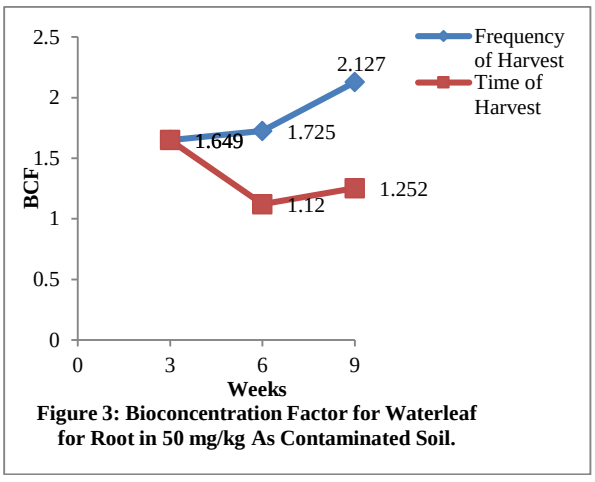

Figure 3: Bioconcentration Factor for Waterleaf for Root in 50 mg/kg As Contaminated Soil.

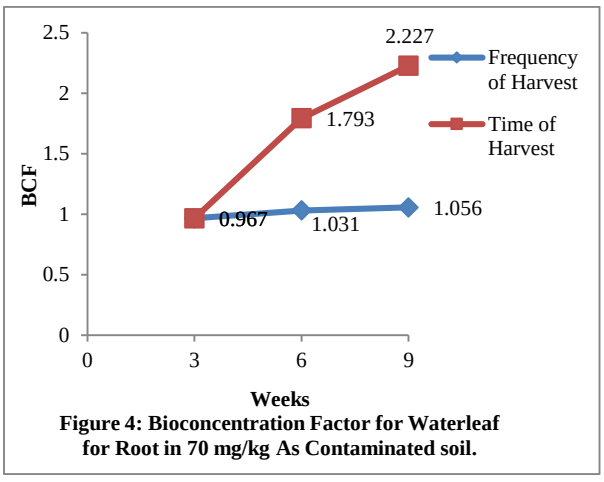

Figure 4: Bioconcentration Factor for Waterleaf for Root in 70 mg/kg As Contaminated soil.

Translocation Factor

Thetranslocation factor defined as the ratio of arsenic in the shoot to that in the plant root was calculated using the formula: TF = As [Shoot] / As [root] (Cui *et al*., 2007). The translocation factor (TF) determined over the graded period of growth revealed that the TF values vary between

< 1 and > 1 in the frequency of harvest and time of harvest at 50 mg/kg and 70 mg/kg contaminated soil (Figure 5 and 6).

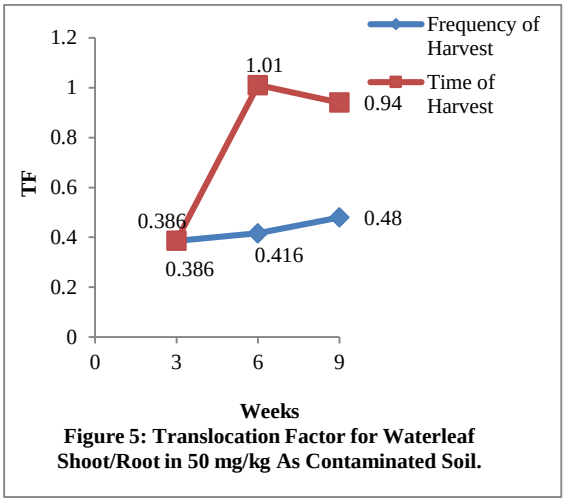

Figure 5: Translocation Factor for Waterleaf Shoot/Root in 50 mg/kg As Contaminated Soil.

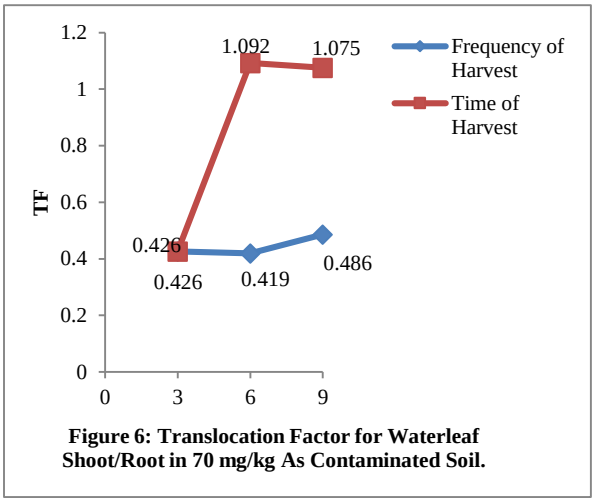

Figure 6: Translocation Factor for Waterleaf Shoot/Root in 70 mg/kg As Contaminated Soil.

DISCUSSION

Bioaccumulation of As in Waterleaf (*Talinumtriangulare*)

The result presented has shown that waterleaf can abstract arsenic into its matrix at varying degrees, over a graded period of growth, depending on the concentration of arsenic in the biocide used to contaminate the soil. This observation is in accord with the work of Anyalobu *et al.* (2017) and Blum *et al.* (2017), who did a similar work had found that waterleaf can abstract As from the soil. The result also depicted that the arsenic concentration in the leaf exceeded the prescribed permissible limit of 0.10mg/kg – 0.5mg/kg (Lombi & Nokan, 2005). The arsenic content in the plant could be attributed to the presence of water-soluble arsenic fraction in the soil, and its mobility and subsequently sequester it into other parts of the plant (Beesley & Marmiroli, 2011; Radulescu *et al*., 2013). Again, the pH (6.3) of the soil could be implicated, which falls within the optimum pH (6.1 to 7.8) for most vegetable growth like waterleaf. Furthermore, the texture of the soil on which the plant was grown could also account for the observed result.This soil was typically sandy loam, which is quite different from clay and silt which are characterized by a large surface area which can increase sorption into the plant matrix. In addition, the speciation of arsenic at the suitable pH (6.3) into the thermodynamically stable form that is soluble and easily transported could be implicated.

The result of bioaccumulation between the frequency and time of harvest revealed a statistical difference. The observed difference could be attributed to the difference in bioaccumulation over the graded period of growth and the effect of allowing the plant enough time to fallow before

harvesting. This result could also be attributed to the concentration of As used to contaminate the soil and the ability of the leaf to abstract more of the soil As into the plant matrix differently.

Another comparison at 50 mg/kg (frequency of harvest and time of harvest) and 70 mg/kg (frequency of harvest and time of harvest) contaminated soil was carried out, and the results revealed that a significant difference occurs between 3 weeks (0.861 ± 0.006, $p = 0.000$), 6 weeks (0.990 ± 0.058, $p = 0.000$), and 9 Weeks (1.125 ± 0.004, $p = 0.000$) respectively. This means that bioaccumulation was significant over the graded period of growth. However, no such significance was observed at 70 mg/kg contaminated soil over the graded period of growth which could be attributed to the rate at which the plant abstracts arsenic over the graded period of growth.

Determination of Phytoremediation Quotient of waterleaf *Talinum triangulare*

The result of the bio-accumulation factor in Figures 1 and 2 depict that the BAF values increased as the graded period of growth increased at both treatment levels of 50 mg/kg and 70 mg/kg contaminated soil. This steady increase could be attributed to the presence of and bio-availability of a large amount of soluble and mobile soil arsenic that was transferred into the undisturbed plant matrix. Furthermore, the BAF was generally higher than one (BAF > 1) for time of harvest than the frequency of harvest (BAF < 1) in 50 mg/kg and 70 mg/kg contaminated soil over the graded period of growth. However, these low BAF values for frequency of harvest were observed to be less than the maximum permissible limit of arsenic (0.1mg/kg) prescribed by FAO and WHO (1983). The BAF values were generally less than one (BAF < 1) except in 9 Weeks for frequency of harvest at both treatment levels.

The BAF values obtained for this study were found to be much higher than those reported earlier by Shu *et al*. (2000) and Warren *et al*. (2003) for crops (0.0007 – 0.032 mg/kg), Cao and Ma (2004) for vegetables like carrot and lettuce (0.1 and 1.6 mg/kg) grown on CCA – contaminated soil and mining area of Zarshuran (Karimi *et al.*, 2010). This idea was strongly supported by Malik *et al*. (2010), who also confirmed that some plant species have been reported to have low values of BAF. Generally, the BAF results depicted that waterleaf cannot be classified as a hyper-accumulator of arsenic since all accumulations were lower than 1000 mg/kg which is the critical baseline for such classification.

Again, the result of biological or bio-concentration revealed that all the BCF were greater than one (BCF > 1), and increased over the graded period of growth for both frequency of harvest and time of harvest (Figures 3 and 4). This is in accord with the report of several other researchers who reported BCFs > 1 (Cui *et al*., 2007; Li *et al*., 2007, and Malik *et al*., 2010). For frequency of harvest and time of harvest at 70 mg/kg contaminated soil, the BCF obtained were 0.967, 1.031, 1.056 and 0.967, 1.793 and 2.227 mg/kg for 3 weeks, 6 weeks, and 9 weeks respectively. The result revealed that all the BCFs over the graded period were greater than one, except that of 3 Weeks both in the frequency of harvest and time of harvest.

The BCF >1 could be attributed to the high amount of As present in the root in the form of iron or manganese plaque. The plague formed has the potential to strongly adsorb arsenic to form insoluble complexes or precipitates which prevents arsenic from being available to the aerial part of the plant (Ghosh & Singh, 2005). They, therefore, concluded by classifying plants with BCF > 1 to be suitable for phytostabilization. Thus, Waterleaf could be very useful as a phytostabilizer over the graded period of growth.

Furthermore,the translocation result revealed that the frequency of harvest disrupted the translocation of arsenic from the root to the leaf, while the time of harvest effected a higher translocation of arsenic from the root to the leaf (Figures 5 and 6). Although poor translocation was observed for the frequency of harvest, the concentration of arsenic abstracted by the leaf was observed to be above the maximum permissible limit of 0.1 mg/kg recommended by FAO/WHO (1999). Based on the classification by Ghosh and Singh (2005), as regards plants with TF < 1, this plant can be classified as a phytostabilizer. Again, the plant can be classified as either good or bad bio-accumulators of arsenicbased on the TF. In this classification, TF < 1 is termed bad accumulators while those with TF > 1 are termed good bio-accumulators. In this present study, the result revealed that depending on the frequency and time of harvest, Waterleaf could be presented as both good and bad bio-accumulators of Arsenic. This is in agreement with the result obtained from the frequency of harvest with a TF <1, and thus classifies Waterleaf as a bad bio-accumulator of arsenic. On the other hand, when Waterleaf is not frequently harvested, the TF becomes greater than one (i.e. TF > 1) and thus classifies waterleaf as good bio-accumulators of arsenic in this study. This implies that Waterleaf can be conditioned to either become a bad or good bio-accumulator of arsenic in contaminated soil for phytoremediation.

The low TF (TF < 1) observed by the frequency of harvest could be attributed to the formation of complexes or precipitates of Iron (Fe) and Manganese (Mn) with arsenic in the root of the plant. When this happens, a high amount of arsenic becomes present in the root to cause the observed TF < 1. This view is in good agreement with the findings of several researchers Abedin *et al*. (2002); Liu *et al*. (2006); Samal (2005), Rahman *et al*.(2007), and Bhattacharya *et al*. (2010). According to them, the plague formed by Fe $(OH)_2$, Mn and Al can easily be adsorbed or form insoluble precipitates with arsenic species to affect the mobility of the bioavailable arsenic in the soil. This immobile form in the root is the major contributor to the presence of a small amount of arsenic translocated to the edible or aerial parts of the plant and high levels in the root. This view is strongly supported by Liu *et al*. (2004), who claimed that Iron oxide (Iron plague) formation around the root of rice plants binds the arsenic and reduced its translocation to the above-ground tissues (Leaf, stem, husk, and grain) of the plant.

The high TF (TF > 1) observed by the time of harvest may be attributed strongly to the presence of enzymes that can function effectively at a high level of arsenic ions and metal exclusion strategies of the plant species (Hall, 2002; Cui *et al*., 2007). Again, the presence of organic matter

which is responsible for the increased release of arsenic from the surfaces of oxide onto which arsenic is adsorbed is another contributing factor to the high TF at the time of harvest. However, this high TF from this study is at variance with those of Ghosh and Singh (2005), Lazaro *et al*. (2006), and Malik *et al*. (2010) respectively who had values lower than our present result. Yoon *et al*. (2006), reported that plants with High TF (TF > 1) are considered suitable for phytoextraction because generally, they can translocate heavy metals to easily harvestable parts. Hence, this plant can be used as a good phytoextractant when the plant is not frequently harvested, but allowed time before being harvested. This implies that Waterleaf could be conditioned to be both good and bad phytoextractant. If the plant is allowed enough time to grow over the graded period of growth it can have enormous potential for phytoextraction of arsenic in contaminated soil, while the reverse is the case if frequently harvested. The phytoextraction potential of plants due to their high TF was supported by Del-Rio-Celestino*et al*. (2006); Li *et al*. (2007), and Chehregani and Malayeru (2007). However, the plant can also be used as a phytostabilizer due to its low translocation potential of arsenic to the aerial part of the plant.

CONCLUSION

The findings indicate that throughout the course of the graded period, waterleaf (*Talinum triangulare*) plants were able to bioaccumulate/abstract a sizeable quantity of soil arsenic into their matrix at all treatment levels. The phytoremediation indices (TF, BAF, and BCF) were all greater than unity, indicating that over the graded period for both treatment levels, as the soil arsenic decreased, the arsenic in the plant matrix increased. The plant may be capable of phytoextraction and phytostabilization of soil contaminated with arsenic based on the phytoremediation indicators. However, rather than frequency of harvest, this attribute depends on the time of harvest. The plant's capacity to endure in an environment where there is arsenic contamination can be used to eliminate arsenic from arsenic contaminated soil. Since this vegetable has the innate ability to absorb arsenic from the environment and sequester it in their cell until the plant can be harvested, it can be utilized for in-situ remediation of areas that have been contaminated by arsenic. Therefore, it was recommended that additional research be done to determine whether or not the phytoremediation potential can be maintained with treatment doses greater than 50kg/mg and 70mg/kg. This technique can be applied as a unique strategy to manage arsenic concentration and content while monitoring plant responses. Additionally, research should be taken into account to see how well the plant can remove other heavy metals from contaminated soils.

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