World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 4.223

USE OF WATERLEAF (TALINUM TRIANGULARE) IN REMEDIATION OF SOIL EXPOSED TO HEAVY METALS: A GREEN TECHNOLOGY APPROACH

Anyalogbu Ernest A. A.[1]*, Anadi, Chigozie C.[2], Nweje-Anyalowu Paul C.[3] and Nnoli Matthew C.[4]

[1,2,3]Department of Biotechnology, School of Biological Science, Federal University of Technology, Owerri Imo State, Nigeria.
[4]Department of Biochemistry, Clifford University, Owerrinta. Abia State, Nigeria.

***Corresponding Author: Anyalogbu Ernest A. A.**
Department of Biotechnology, School of Biological Science, Federal University of Technology, Owerri Imo State, Nigeria.

Article Received on 14/09/2017 **Article Revised on 05/10/2017** **Article Accepted on 26/10/2017**

ABSTRACT

The major environmental concern due to the disposal of urban and industrial waste generated by anthropogenic activities is the contamination of soil with heavy metals. The potential of waterleaf (*Talinum triangulare*) to abstract heavy metals from contaminated composite soil sample and consequently remediate it was investigated. Plants and soil samples were analysed for their heavy metal contents before and after graded periods of growth using Atomic absorption spectrophotometer. The concentrations (in µg/g) of Cd, Cr, Pb, Hg and As in the composite soil sample decreased as the period of growth was increased given the range; 0.42±0.23 - 2.26±0.83, 0.56±0.21- 1.49±0.39, 9.82±0.16 - 12.34±0.40, 0.64±0.78 - 3.65±1.10 and 6.75±0.80 - 10.02±2.62 respectively. While the metals contents of the plant sample correspondingly increased given the ranges (in µg/g): 0.45±0.19 - 0.80±0.47, 0.30±0.09 - 0.54±0.43, 0.28±0.06 - 0.33±0.40, 0.27±0.14 - 0.49±0.39 and 0.89±1.14 - 1.18±0.90. This amounted to reduction in the concentrations of these metals in the soil by between 4.59% and 82.47% and corresponding increase in the plant biomass by 3.5% to 81.48%. The transfer factor for each of the metals assayed increased steadily. This demonstrated the ability of *Talinum triangulare* to abstract heavy metals and consequently remediates the contaminated soil sample.

KEYWORDS: Heavy metal; transfer factor; remediation; plant biomass; composite.

INTRODUCTION

Increasing consumption and exploitation of fossil fuel and mineral coupled with the exponential population growth that stepped up anthropogenic activities over the past years have resulted in environmental degradation.[1] Very little attention is paid to the treatment of industrial effluents and has led to the buildup of waste product of which metals are of great concern.[2] A major environmental concern due to the disposal of urban and industrial waste generated by human activities is the contamination of soil with heavy metals.[3,4] The term heavy metal refers to any metallic element (including metalloids) that has a relatively high density compared to water and is toxic or poisonous at low concentration,[5] examples include; mercury (Hg), cadmium (Cd), arsenic (As), chromium (Cr), lead (Pb), thallium (Ti). As trace element, some heavy metal such as copper, zinc, selenium are essential for human metabolic processes but become poisonous at higher concentrations possibly leading to poisoning.[6] This may reflect a basic principle of toxicology enunciated by Paracelsus (1493-1541) as stated by Borzelleca,[7] "The Dose Makes the Poison". i.e. "All substances are poisons; there is none which is not a poison. The right dose differentiates a poison.

Heavy metals are dangerous due to their unchangeable nature and in contrast to many organic pollutants which are biodegradable; they can remain in the environment for a long time.[8] Though majority of heavy metals are phytotoxic even at low concentrations,[9] earlier researches recognized that certain species of plants could accumulate high levels of heavy metals from the soil while continuing to grow and proliferate normally.[10,11] This ability of some plants to survive in contaminated environments has been exploited to remove contaminants from the soil.[12] Phytoextraction, a type of phytoremediation which is the use of vegetative species for *in-situ* treatment of areas polluted by variety of hazardous substances relies upon the plant's natural ability to take certain substances such as heavy metals from the environment and sequester them in their cell until the plant can be harvested.[13] This is the major trust of green technology which is the mitigation or reversal of the effects of human activity on the environment.[14] It has been shown that some plants like Alfalfa, sunflower and millet can take up lead, copper, cadmium, iron and mercury from soil exposed to such metals.[15]

Water leaf (*Talinum triangulare*), a *Portulaceae*, is an erect, glabrous, perennial herb of tropical Africa decent though widely grown in West Africa, Asia, and South America.[16] It is known locally in Nigeria as 'Gborondi', 'Nte-oka' or 'Inene' (Igbo), 'Momoiko' (Ibibio), 'Gbure' (Yoruba), 'Alenyruw-a' (Hausa). Ibeawuchi *et al*.[17] reported that the plant (leaves and young stem) is used in large quantities as vegetable, as "softener" when cooking fibrous vegetables or thickener in sauce in the Southern part of Nigeria. According to Enete and Okon[16] the plant is a short duration crop and could be harvested within 35-45 days of planting. This work is therefore intended to evaluate the ability of waterleaf plant to abstract and accumulate heavy metals from soil exposed to anthropogenic activities.

MATERIALS AND METHODS

Soil sample: Soil samples were collected from Federal University of Technology, Eziobodo, Ihiagwa, Naze junction -all in Owerri zone (Imo State, Nigeria) and well known for their profound anthropogenic activities. The sampling site was first cleared of debris and top soil (15cm depth) collected using soil auger.[18] The samples were pooled together and homogenized to obtain a composite sample.[19]

Plant sample: Waterleaf (*Talinum triangulare*) stands of about 21day old were collected from nursery bed in Emeabiam –a farming community in Owerri zone not common with much anthropogenic activities.

Experimental Design: A 4kg of the composite soil sample was weighed into each of 12 polyethylene bags. The bags were placed in 4 groups of 3 (A, B, C) each in an improvised green house and labelled A2wks, B2wks, C2wks; A4wks, B4wks, C4wks; A6wks, B6wks, C6wks and A8wks, B8wks, C8wks respectively. Five stands (sticks) of the waterleaf sample were planted in each of the polyethylene bags and watered with 50ml of water at 8hr interval as described by Yee *et al*. [20]. At interval of 2 weeks starting with the 1st group (A2wks, B2wks, C 2 wks), 10g of the soil and the plants of each of the three members of the group were harvested, pretreated and analyzed for their heavy metal concentrations. The last group (A8wks, B8wks, C8 wks) was harvested at 8th week.

Pretreatment: Five stands (sticks) of waterleaf from the nursery bed were washed first under tap water and then in two changes of distilled water and air-dried. Ten grammes of the composite soil sample and the air-dried plant sample were each dried for 24 hr in an air-circulatory oven (80^{o}C), ground in a mill and the powder passed through a screen of 0.25mm openings (60 mesh size). The sieved samples were stored in plastic bags labelled 0wkss and 0wksp respectively for soil and plant samples and stored in a refrigerator until analysed to obtain the basal heavy metal contents. The harvested soil and plants of each member of the groups were subjected to this pretreatment and the powdered samples assayed for their heavy metal contents.

Digestion of Samples: The powdered samples (soil and plant) were digested as described by Kudirat and funmilayo.[21] Two grammes each of powdered samples was weighed in an analytical balance and placed in appropriatly labelled 250ml digestion flask. A 30ml of aqua regia which constitutes of hydrochloric acid and nitric acid in the ratio 3:1 was added to each flask and placed upright for 13 minutes.The mixture was heated in a waterbath at approximately 95^{o}C until the whole sample dissolved and a yellow tint of the solution appeared as the digest. The solution was then allowed to cool to room temperature, filtered using a Whatman No. 42 filter paper into centrifuge tubes and made up to 50ml mark with deionized water. It was transferred into sample vials for analysis. Reagent blank was also prepared.

Heavy metals analyses: Heavy metal concentrations of all preparations were determined spectrophotometrically using 240FS Varian Atomic absorption spectrophotometer.[22] Appropriate absorption wavelength, detection tube and other parameters for each metal type, and other operating conditions of the 240FS Varian AAS were strictly followed as described by the manufacturer. All determinations were done in triplicates and standard analytical reagents used.

Determination of the transfer factor (TF): Heavy metal transfer factor (TF), also called accumulation factor AF[23] or bioconcentration factor BCF[24] describes the transfer of the metal from soil to the plant body. This was calculated as stated by Rashid et al.[25] by dividing the concentration of the heavy metal in the plant (C_{plant}) by the corresponding concentration in the soil (C_{soil}).

i.e. $TF = \frac{C_{plant}}{C_{soil}}$

Determination of the Remediation/Metal uptake Rate: The rate at which the plant sample remediated or abstracted/accumulated heavy metal from the polluted soil was calculated as: R = Amount of metal lost by the soil or gained by the plant expressed in percentage of initial amount. i.e.

$$Rate\ (\%) = \frac{C^{nth\ week} - C^{0th\ week}}{C^{0th\ week}}\ X\ \frac{100}{1}$$

Where, $C^{nth\ week}$ and $C^{0th\ week}$ are concentrations of the heavy metals in the soil or plant sample at time (weeks) = 'n' and '0' respectively.

Data analysis

Data were analyzed by analysis of variance (ANOVA) and means compared by the PostHoc-Least Significance

Difference - Duncan's multiple range test.[26] Significance was accepted as 5% level ($p < 0.05$) or 95% confidence limit.

RESULTS AND DISCUSSION

Concentrations of heavy metal in the soil sample: The concentrations of heavy metal in the soil sample at the inception of growth and after graded periods of growth of *Talinum triangulare* are presented in Table 1 while Table 2 contains the world average heavy metal contents in agricultural soils and plant (vegetable) and, maximum permissible limits by different standards/guidelines.

Table 1: Concentration of heavy metal in soil sample after periods (weeks) of growth of *Talinum triangulare* (waterleaf).

Heavy metals	Concentration (µg/g)*				
	0wk	2wks	4wks	6wks	8wks
Cadmium (Cd)	2.26±0.83[a]	2.19±0.28[a]	2.07±0.44[a]	1.82±0.31[a]	1.42±0.23[a]
Chromium (Cr)	1.49±0.39[a]	1.41±0.47[a]	1.30±0.34[a]	1.26±0.25[a]	0.92±0.21[a]
Lead (Pb)	2.34±0.40[a]	2.05±0.30[a]	1.17±0.36[b]	1.09±0.29[b]	0.82±0.16[b]
Mercury (Hg)	3.65±1.10[a]	2.99±0.72[ab]	2.53±0.23[ab]	1.93±0.61[b]	1.51±0.78[c]
Arsenic (As)	10.02±2.62[a]	9.86±2.15[a]	9.53±1.72[a]	8.75±0.91[a]	7.75±0.80[a]

*Values are means ± standard deviations of triplicate determinations. Values in the same row bearing the same superscript letters are not significantly different at the 5% level ($p > 0.05$).

Table 2: World Average heavy metal contents in agricultural soils and Plant (Vegetable) and, Maximum Permissible Limits by different standards/Guidelines.

Heavy metal	Soil			Plant (Vegetable)		
	World average conc. (µg/g)	Maximum permissible limit		World average conc. (µg/g)	Maximum permissible limit	
		Conc. (µg/g)	Reference		Conc. (µg/g)	Reference
Cd	0.05 - 1.0	0.01 - 0.80	FEPA, 1991; WHO, 1996	0.05 – 0.5	0.10	WHO, 1996: FAO/WHO, 1999
Cr	10 - 50	0.10 - 0.15	FAO, 1992; WHO, 1993; EC-EU,2002	0.1 – 0.5	0.1 – 0.2	FAO/WHO, 1999
Pb	10 - 30	85.0	WHO, 1996;	0.1 – 0.5	0.2 - 0.3	FAO/WHO, 1999, 2001
Hg	0.05 - 0.5	1.0 - 1.50	EC - EU, 1986	0.005 – 0.05	0.03	FAO/WHO, 2007
As	1.0 - 10	20.0	EC - EU, 2002	0.1 – 0.5	0.10	FAO/WHO, 1999

Sources for World Average Conc.: Adriano (2001) and Kabata-Pendias (2000) as compiled by Blum *et al.*[27]

The concentrations (in µg/g) of all the heavy metal assayed in the soil sample decreased as the period of growth was increased given the range; 0.42±0.23 – 2.26±0.83, 0.56±0.21- 1.49±0.39, 9.82±0.16 - 12.34±0.40, 0.64±0.78 - 3.65±1.10 and 6.75±0.80 - 10.02±2.62 for Cd, Cr, Pb, Hg and As respectively. The decrease was statistically significant ($p < 0.05$) only for Pb from 4th week and Hg from 2nd week of growth. From Table 2, Cd, Hg and As concentrations in the soil at the inception of the growth regimen (week 0) were above the world average for agricultural soils[27] while Cr and Pb were within the range. On consideration of the maximum permissible limits (Table 3) given by FEPA (1999), WHO (1993, 1996), FAO (1992) and EC- EU (1986, 2002) as stated by Kabiru *et al.*[28] and Tamene and Seyoum,[29] the soil sample *in principio* was contaminated/polluted with Cd, Cr, and Hg, while Pb and As were within permissible range on the stated scales.

Heavy metal concentrations in the plant sample

The metal concentrations in the plant sample are given in Table 3.

Table 3: Concentration of heavy metal in *Talinum triangulare* plants after periods (weeks) of growth on the soil sample.

Heavy metals	Concentration (µg/g)*				
	0wk	**2wks**	**4wks**	**6wks**	**8wks**
Cadmium (Cd)	0.75 ± 0.19^{a}	0.84 ± 0.29^{a}	0.98 ± 0.29^{a}	1.21 ± 0.31^{ab}	1.63 ± 0.47^{b}
Chromium (Cr)	0.30 ± 0.09^{a}	0.39 ± 0.16^{a}	0.51 ± 0.17^{a}	0.57 ± 0.14^{a}	0.91 ± 0.43^{b}
Lead (Pb)	0.28 ± 0.06^{a}	0.58 ± 0.15^{a}	1.43 ± 0.59^{b}	1.54 ± 0.44^{b}	1.79 ± 0.40^{b}
Mercury (Hg)	0.97 ± 0.14^{a}	1.43 ± 0.35^{ab}	1.84 ± 0.40^{bc}	2.44 ± 0.37^{cd}	2.91 ± 0.39^{d}
Arsenic (As)	4.89 ± 1.14^{a}	5.12 ± 0.73^{ab}	5.40 ± 0.71^{ab}	6.21 ± 1.53^{ab}	7.11 ± 0.90^{b}

*Values are means ± standard deviations of triplicate determinations. Values in the same row bearing the same superscript letters are not significantly different at the 5% level ($p > 0.05$).

The metals contents of the plant sample, *Talinum triangulare*, except Pb, increased steadily through the period of growth given the ranges (in µg/g): 0.45±0.19 – 0.80±0.47, 0.30±0.09 - 0.54±0.43, 0.28±0.06 – 0.33±0.40, 0.27±0.14 – 0.49±0.39 and 0.89±1.14 - 1.18±0.90 for Cd, Cr, Pb, Hg, and As respectively. The increase became statistically significant ($p < 0.05$) for mercury and arsenic from the 2nd week of growth, and for lead and cadmium from 4th and 5th weeks of growth respectively. At the beginning of the growth period, Cd, Cr and Pb contents of the plant sample (0.45±0.19, 0.30±0.09 and 0.28±0.06 µg/g respectively) were all within the world average for the metals while Hg and As (0.27±0.14 and 0.89±1.14 µg/g respectively) were above the range (Table 2) as stated by Blum *et al*.[27] Earlier, Adewuyi *et al*.[18] detected 0.11±0.04 µg/g of Cd and 0.75±0.13 µg/g of Pb in *Talinum triangulare* sample obtained from a control site in Ibadan, Nigeria. Relative to the referenced maximum permissible limits (Table 2), all the metals except Pb, were above the range. With the observed increase with growth, Pb content remained within the world average (0.1 – 0.5µg/g) throughout the growth period but exceeded the maximum permissible limit (0.2 – 0.3µg/g) by the 4th week while Cr content exceeded the world average (0.1 – 0.5µg/g) by the 8th week.

Variation of the transfer factor (TF) with period of growth

The variation of the transfer factor (TF) with period of growth is represented in Figure 1.

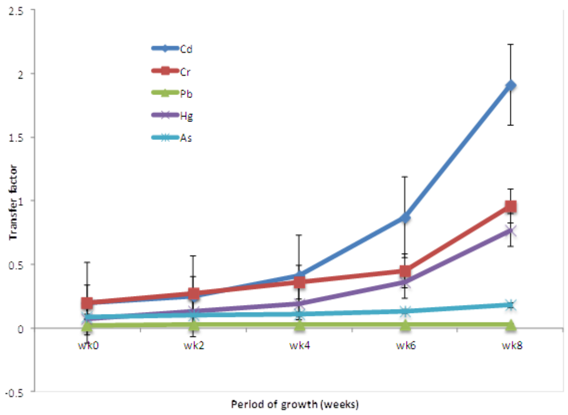

Figure 2: Variation of Heavy metal transfer factor of *Talinum triangulare* with period of growth.

The transfer factor for each of the metals assayed except for Pb increased steadily throughout the growth period given the ranges: 0.20 – 1.91, 0.20 – 0.96, 0.02 – 0.03, 0.07 – 0.77 and 0.09 – 0.18 for Cd, Cr, Pb, Hg and As respectively. According to Balkhair and Ashraf[23] the TF values varied for heavy metals in various food crops. At the first two weeks of growth Cr recorded the highest TF (0.27) followed by Cd (0.25) and then Hg (0.13) with As and Pb having the least (0.10 and 0.03 respectively). For the rest of the growth period the trend in increasing order was Pb < As < Hg < Cr < Cd. Transfer factor (TF), also called accumulation factor AF[23] or bioconcentration factor BCF[24] describes the transfer/migration of metal from soil to the plant body. TF could therefore be considered an index of the potential/ability of plant species to remediate contaminated soil of a specific metal. Fitz and Wenzel[30] stated that plants with TF about one or more are suitable for phytoextraction. With the observation that soil-to-plant transfer factors for radiocaesium varied widely over several orders of magnitude, Guillén *et al*.[31] concluded that the transfer is a complex processes and is affected by many variables other than the concentration of the metal in soil. The authors stated that TF varies with stage of growth due to the different nutritional requirements in each stage of development. With the observed transfer factor being above one or close to one by the eight weeks of growth, the plant sample could be adjudged good for short term bioremediation of soil contaminated with Cd, Cr and Hg.[30] Abstraction of Pb and As would require longer growth period.

Comparison of the *Talinum triangulare* plant sample potentials to remediate - accumulate heavy-metals

The pictorial comparison of the heavy-metal remediation/abstraction (A) and the corresponding uptake/accumulation (B) by *Talinum triangulare* sample (Figure 2) obtained as the amount of metal lost by the soil or gained by the plant respectively, expressed as percentage of initial amount showed the same pattern but differed in order of magnitude.

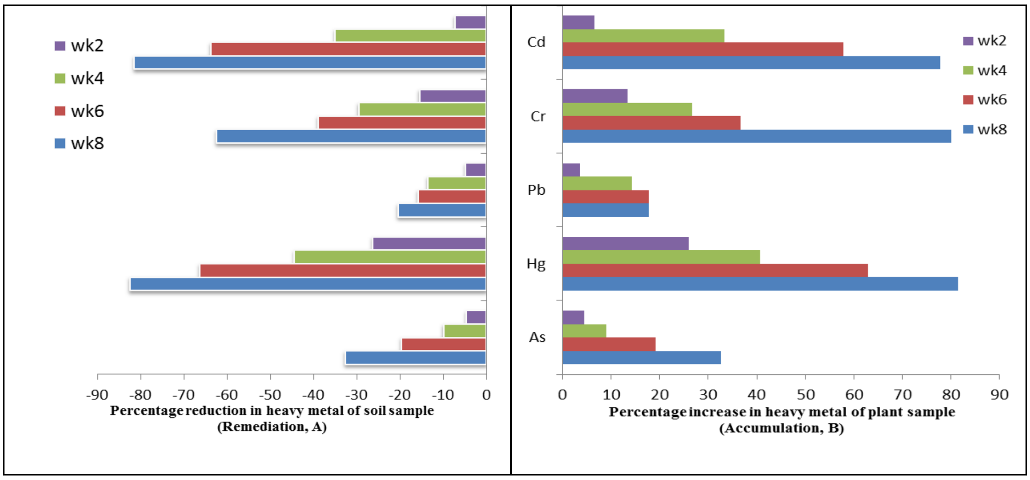

Figure 2: Comparison of heavy-metal remediation (A) and accumulation (B) potentials of *T. triangulare.*

Considering Cd for instance, by the 8th week, 63.72% was lost from the soil while at the same time 57.78% was gained by the plant sample. This is re-emphasizing that metal uptake by plants is not a function of metal concentration in the growth medium only.[31] It is affected by other factors which affect the transfer factor such as soil properties (like pH, cation exchange capacity and distribution of metals in different soil fractions,[32] and nutrient management and climatic conditions which indirectly affect the transfer processes through their control of major variables that affect the plant growth such as temperature, water regime, humidity, etc.[23] These incidental factors could be regimented to optimize the transfer processes enabling effective application of Waterleaf (*Talinum triangulare*) in phytoextration of the assayed heavy metals from polluted soil.

CONCLUSIONS

The composite soil sample, relative to the maximum permissible limits for heavy metal in agricultural soils, was contaminated/polluted with Cd, Cr, and Hg, while Pb and As were within permissible range. The growth of the plant sample variously reduced the concentrations of these heavy metals in the soil by between 4.59% (As, wk2) and 82.47% (Hg, wk8) and accordingly increased the metals concentrations in the plant biomass by 3.57% (Pb, wk2) to 81.48% (Hg, wk8). It could therefore be concluded that by this metal transfer/translocation from soil to plant, the ability of waterleaf (*Talinum triangulare*) to abstract/bio-accumulate heavy metals and consequently remediate the contaminated soil sample to the observed degree in line with green technology principle is demonstrated by this study. Also indicated though tangentially is that consumption of waterleaf planted on heavy metal-polluted soil is of public health concern.

REFERENCES

1. Yusuf, A.J.; Galadima A.; Garba, Z.N. and Nasir I. Determination of some heavy metals in soil sample from Illela Garage in Sokoto State, Nigeria. *Res. J. Chem. Sci*, 2015; 5(2): 8-10.
2. Bernardini, A.; Salvatori, E.; Di Re, S.; Fusaro, L.; Nervo, G. and Manes, F. Natural and commercial Salix clones differ in their ecophysiological response to Zn stress. *Photosynthetica*, 2016; 54 (1): 56-64.
3. Jolly, Y. N.; Islam, A. and Akbar, S. Transfer of metals from soil to vegetables and possible health risk assessment. *Springer Plus*, 2013; 2(1): 385-393.
4. Bamuwamye, M.; Ogwok, P.; Tumuhairwe, V.; Eragu, R.; Nakisozi, H. and Ogwang, P. E. Human health risk assessment of heavy metals in kampala (uganda) drinking water. *J. Food Res*, 2017; 6(4): 6 - 16.
5. Tchounwou, P.B.; Yedjou, C.G.; Patlolla, A.K. and Sutton, D. J. Heavy Metals Toxicity and the Environment. *NIH Public Access*, 2012; 101: 133-164.
6. Ahmed, M.K.; Baki, M.A.; Kundu, G.K.; Islam, M.S.; Islam, M.M. and Hossain, M.M. Human health risks from heavy metals in fish of Buriganga River, Bangladesh. *Springer Plus*, 2016; 5(1): 1697- 1709.
7. Borzelleca, J.F. Paracelsus: Herald of modern toxicology. *Toxicol. Sci*, 2000; 53: 2-4.
8. Adedeji, O.B. and Okocha, R.C. Bioconcentration of heavy metals in prawns and water from Epe Lagoon and Asejire River in South West Nigeria. *J. Appl. Sci. Environ. Sanitation*, 2011; 6(3): 377-384.
9. Ekwumemgbo, P.A.; Eddy, N.O. and Omoniyi, I.K. Decontamination of heavy metals in polluted soil by phytoremediation using *Bryophyllum pinnatum*, E3S Web of Conferences, 2013; 1: 13004. 10.1051/e3sconf/20130113004.
10. Udosen, E.D.; Benson, N.U.; Essien, J.P. and Ebong, G.A. Relation between aqua-regia extractable heavy metals in soil and *Manihot utilissima* within a municipal dumpsite. *Int'l J. Soil Sci*, 2006; 1: 27-32.
11. Bhagure, G.R. and Mirgane, S.R. Heavy metal concentrations in groundwater's and soils of Thane Region of Maharashtra, India. *Environ. Moni. And Assess*, 2010; 173 (1-4): 643-652.
12. Ebah, E.; Tersagh, I. and Okpokwasili, G.C. Studies on Seasonal Variation and Effect of Heavy Metal

Pollution on Microbial Load of Marine Sediment. *Am. J. Marine Sci*, 2016; 4(1): 4-10.
13. Bentjen, S. Bioremediation and Phytoremediation Glossary. 1998. Accessed 13 July, 2017. from: http://members.tripod.com/~bioremediation/
14. Aithal, P.S. Opportunities & Challenges for Green Technology in 21st Century. Managema 2016- National Conference on Changing Perspectives: Paper 3. 2016. Accessed 7 Sep, 2017. from: https://www.researchgate.net/publication/303924110_Opportunities_Challenges_for_Green_Technology_in_21st_Century
15. Daniel, V.N.; Nanven, D.N. and Daniang, I. E. The Potentials of Eucalyptus camaldulensis for the Phytoextraction of Six Heavy Metals in Tin – mined Soils of Barkin Ladi L.G.A. of Plateau State, Nigeria. *Int'l J. Eng. Res. and Appl.* (IJERA), 2012; 2(2): 346-349.
16. Enete, A.A. and Okon. U.E. Economics of Waterleaf (*Talinum triangulare*) Production in Akwa Ibom State, Nigeria, Field Actions Science Reports: *The J. Field Actions*, 2010; 4: 1-6.
17. Ibeawuchi, I.I.; Nwufo, M.I.; Oti, N.N; Opara, C.C. and Eshett, E.T. Productivity of Intercropped Green (Amaranthus cruentus)/ Waterleaf (*Talinum triangulare*) with Poultry Manure Rates in Southeastern Nigeria. *J. Plant Sci*, 2007; 2(2): 222-227.
18. Adewuyi, G.O.; Okonkwo, J. and Okoli, C.P. Assessments of some heavy metals in soils and waterleaf (*Talinum Triangulare*) in the vicinity of a major quarry factory in Ibadan metropolis, Nigeria. *Fresenius Environ. Bull*, 2009; 18(12): 2396 - 2401.
19. Kaewtubtim, P.; Meeinkuirt, W.; Seepom, S. and Pichtel, J. Heavy metal phytoremediation potential of plant species in a mangrove ecosystem. *Appl. Ecol. and Environ. Res*, 2016; 14(1): 367-382.
20. Yee, B.C.; Maynard, J.A. and Wood, T. K. Rhizoremediation of trichloroethylene by a recombinant root colonizing *Pseudomonas fluorescemes* strain expressing toluene orthomonoxigenase constitutively. *Appl. and environ. Microbial*, 2013; 64(1): 112-118.
21. Kudirat, L.M. and Fumilayo, D.V. Heavy metal levels in vegetables from selected markets in Lagos, Nigeria. *Afr. J. Food Sci. and Technol*, 2011; 2(1): 018-021.
22. Lawal, N.S.; Agbo, O. and Usman, A. Health Risk Assessment of Heavy Metals in Soil, Irrigation Water and Vegetables Grown around Kubanni River, Nigeria. *J. Physical Sci*, 2017; 28(1): 49-59.
23. Balkhair, K.S. and Ashraf, M.A. Field accumulation risks of heavy metals in soil and vegetable crop irrigated with sewage water in western region of Saudi Arabia. *Saudi J. Biol. Sci*, 2016; 23: S32–S44.
24. Sakizadeh, M.; Sharafabadi, F.M.; Shayegan, E. and Ghorbani, H. Concentrations and Soil-To-Plant Transfer Factor of Selenium in Soil and Plant Species from an Arid Area. IOP Conf. Series: Earth and Environmental Science, 2016; 44.
25. Rashid, H.M.; Fardous, Z.; Chowdhury, M.A.Z.; Alam, M.K.; Bari, M.L.; Moniruzzaman, M. and Gan, S.H. Determination of heavy metals in the soils of tea plantations and in fresh and processed tea leaves: An evaluation of six digestion methods. *Chem. Central J.*, 2016; 10: 7.
26. Duncan B.O. Multiple range and Multiple F-tests. *Biometrics*, 1955; 11: 1-42.
27. Blum, W.E H.; Horak, O.; Mentler, A. and Puschenreiter, M. Trace Elements. *UNESCO-EOLSS Environmental and Ecol. Chem*, 2017; 2: 1-5. Accessed 2 Oct, 2017. from: http://www.eolss.net/Sample-Chapters/C06/E6-13-03-04.pdf.
28. Kabiru, S.; Yakubu, R.; Lukman, A.; Akintola, T. and Alegbemi, M. Heavy Metal Content in Soil in Garki Area Council of Federal Capital Territory, Abuja, Nigeria. *Analytical Biochem.* 2015, 4(4), 197 - 202.
29. Tamene, F.D. and Seyoum, L. Determination of Levels of As, Cd, Cr, Hg and Pb in Soils and Some Vegetables Taken from River Mojo Water Irrigated Farmland at Koka Village, Oromia State, East Ethiopia. *Int'l J. Sci. Basic and Appl. Res.* (IJSBAR), 2015; 21(2): 352-372.
30. Fitz, W.J. and Wenzel, W.W. Arsenic transformation in the soil-rhizosphereplant system, fundamentals and potential application of phytoremediation. *J. Biotechnol,* 2002; 99: 259 - 278.
31. Guillén, J.; Baeza, A.; Salas, A.; Muñoz-Muñoz, J.G. and Muñoz-Serrano A. Factors Influencing the Soil to Plant Transfer of Radiocaesium. In: D.K. Gupta, C. Walther (eds.), Impact of Cesium on Plants and the Environment. Springer International Publishing, Switzerland, 2017; 19-33.
32. Kos, B.; Greman, H.and Lestan, D. Phytoextraction of lead, zinc and cadmium from soil by selected plants. *Plant Soil Environ*, 2003; 49(12): 548–553.