PHYTOACCUMULATION OF HEAVY METALS BY *TALINUM TRIANGULARE* (WATER LEAF) CULTIVATED IN CHARCOAL PRODUCTION SOIL IN DELTA STATE, NIGERIA.

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Abstract

Phytoremediation, also known as green solution, is an environmentally supportive and cost effective method for removing heavy metals contaminants from an environment. This study investigated the phytoaccumulation of heavy metals by *Talinum triangulare* cultivated in charcoal production soil. *Talinum triangulare* was cultivated for a period of seven weeks in a charcoal production area in Amukpe, Delta State, Nigeria. Samples of soil and *T. triangulare* were collected from three different points (1, 2 & 3) within the charcoal production environment, and prepared for analysis of Cd, Cr, Zn, Mn, Pb, Ni, Cu, Co, V and As using Flame atomic absorption spectrophotometer. Phytoremediation quotients for the heavy metals were also determined. Results indicated that the concentration of Cd in soil from sampling points 1 and 2 were higher than the reference value of the National Environmental Standard and Regulation Enforcement Agency (NESREA). The concentrations of Pb, Ni, Cu and Co in soil from all sampling points were below their respective NESREA permissible limits. In the root and shoot of *T. triangulare*, Zn, Mn, Cu and Ni were present. The results also showed that *T. triangulare* had biological concentration factor greater than 1 for Zn, Mg and Cu in the three sampling points. Translocation factor was also greater than 1 for Mn in site 3. The order of mobility for the three heavy metals was as follows: Cu > Mn > Zn. *Talinum triangulare* therefore exhibits phytoremediation ability for these metals.

Key words: Phytoremediation, heavy metals, *Talinum triangulare*, biological concentration factor, translocation factor.

Introduction

Metals having a density greater than 5g cm^{-3} which are toxic, persistent and can easily bio-accumulate in the ecosystem are termed heavy metals (Jarup, 2003; Srivastava and Majunder, 2008; Hezbullah *et al*., 2016). Anthropogenic activities release these heavy metals into the environment, with soils being the principal sinks for these metals. Unlike several organic contaminants which undergo microbial degradation to CO_2, most heavy metals in the environment are resistant to microbial and chemical breakdown, and as such, they tend to remain in soils for prolong periods after their release into the environment. One of the alternative technologies to clean up soils contaminated with heavy metals is phytoaccumulation or phyremediation or green solution (Butcher, 2009; Sukyankij *et al*., 2016). This technology entails the use of plants for removal of soil pollutants or contaminants (Salt and Kramer, 1999). It is also environmental friendly, cost effective and aesthetically pleasing (Ghosh and Singh, 2005).

Plants are perceptive to environmental conditions during accumulation of materials. Accumulation of heavy metals can occur in the harvestable parts of plants (i.e. the roots via uptake from soil, atmospheric deposition on the leaves, and foliar adsorption), and the general elemental composition of the plant depends on the magnitude of the accumulation process (Vtorova, 1991). Additionally, some plants may not only accumulate heavy metals in the root, but also translocate these heavy metals to the shoots and leaves, thereby reducing the metals concentration in soil (Jamali *et al.*, 2009; Ogunkunle *et al*., 2016).

Charcoal production can influence soil structure and increases soil pH. It can also reduces soil surface temperature and bulk density (Chidumayo and Gumbo, 2013). Although, Ugwu (2019) reported high concentrations of toxic metals in charcoal obtained from plant biomass, Adio *et al*. (2022) observed that the use of traditional earth kilns for producing charcoal improved the physical and chemical properties of soils for agricultural activities, but did not necessarily influence the concentration of heavy metals in such soils.

Talinum triangulare, locally called water leaf, has been reported to accumulate heavy metals from contaminated soils and dump sites. Several researchers proposed that *Talinum triangulare* could be used for environmental friendly phytoremediation and phytoextraction processes in soil (Arise *et al*., 2015; Osioma *et al*., 2018; Oguh *et al*., 2019). Therefore, this present study focused on the phytoaccumulation of heavy metals by *Talinum triangulare* cultivated in charcoal production soil.

Materials and Methods

Study Area

The study area, Amukpe, is located in Sapele Local Government Area of Delta State. The area is used by the Natives for charcoal production through pyrolysis for commercial purposes. Figure 1 is a map of Delta State showing the study area, while Plate 1 gives a pictorial representation of the charcoal production area.

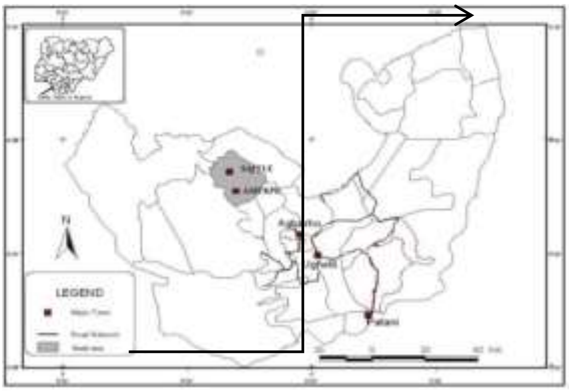

Figure 1: Map of Delta state showing the study area

a. b. c.

Plate 1 (a) starting materials (wood) for charcoal production (b) earth kiln for pyrolysis of wood (c) final product (charcoal)

Cultivation of Talinum triangulare

Talinum triangulare was cultivated in three different sites (80 meters apart) around the charcoal production area. The sites were designated as sites 1, 2 and 3. *Talinum triangulare* was cultivated for a period of seven weeks before harvest.

Collection of soil and plant samples

Soil samples were collected from the three sites around the charcoal production area. Three (3) soil samples were collected from each sampling site at depth of 0-15 cm using a soil auger and pooled together. Approximately, 0.50 kg of soil was obtained from each sampling site, packed in pre-cleaned and well labelled polyethylene bags and taken to the laboratory where they were air-dried, mechanically ground with agate mortar and pestle and sieved with a 2 mm mesh size.

Talinum triangulare (water leaf) was also collected from the various soil sampling sites using a stainless steel sampler (this helped in uprooting the plant from the soil), and was authenticated in the Herbarium unit (No. UPH/P/136), Department of Plant Science and Biotechnology, University of Port Harcourt, Nigeria. The plant samples were also packed in pre-cleaned and well-labelled polyethylene bags and taken to the laboratory. Adhered soils were removed from the plants by rinsing with tap water and distilled water. The plants were then separated into the roots and shoots, dried in an electro-thermal oven at 105 ^{O}C, pulverized, and stored in a desiccator. The dried samples were size-reduced using agate mortar and pestle and passed through a 2 mm mesh sieve.

Digestion of soil and plant samples

Exactly 1.00 g of pulverized soil sample was measured in a 250 mL conical flask. Then, 5 mL of nitric-perchloric acid (3:2) mixture was added, covered with a small glass funnel and left to stand overnight. The flask containing the mixture was then kept in a digestion block and initially heated for 1h at 150 ^{o}C, then, at 235 ^{o}C until dense white fumes were given off. The mixture was removed from the block, cooled, followed by the addition of 10 mL (1:1) HCl and reheated until the solution became colourless. This solution was allowed to cool, filtered through a Whatman No. 40 filter paper into a 25 mL volumetric flask, and made up to mark with distilled water.

For plant samples, each of the separated parts (shoot and root) were placed in different porcelain crucibles and placed in a muffle furnace. The temperature of the furnace was slowly increased from ambient temperature to 450 ^{o}C for 1 h. Ashing of the samples at this temperature was done for 4 h, which formed white/grey ash residues. Approximately, 0.25 g of the residue was weighed, dissolved with nitric acid, filtered into a 25 mL volumetric flask and made to mark using deionized water (Radojevic and Bashkins, 1999)

The filtrates from both digestion processes were analyzed using a Flame Atomic Absorption spectrophotometer (Thermo Jarrell Ash A.A. 12E) for the following metals: Cd, Cr, Zn, Mn, Pb, Ni, Cu, Co, V and As.

Quality control measures

All glass ware used in the laboratory were washed with detergent and tap water, then soaked in 5% nitric acid for 24 h. They were then washed and rinsed with deionized water. Samples were prepared in duplicate and analyzed in triplicates to check for precision of the results obtained. Reagents blanks and spiked samples were also included in the analysis for validation of the chosen analytical method.

Determination of phytoremediation quotients

Evaluation of biological accumulation coefficient (BAC)

The BAC was calculated as a ratio of metal level in shoots to the metal level in soil (Yoon *et al.,* 2006). It was evaluated using the expression

$$BAC = \frac{[Metal]_{shoot}}{[Metal]_{soil}}$$

Evaluation of biological concentration factor (BCF)

The BCF was calculated as a ratio of metal level in plant roots to the metal level in soil (Salah and Barrington, 2004). |The following expression was used for calculating the BCF:

$$BCF = \frac{[Metal]_{root}}{[Metal]_{soil}}$$

Evaluation of translocation factor (TF)

The TF was calculated as the ratio of metal level in plant shoot to plant root (APHA, 1998):

$$TF = \frac{[Metal]_{shoot}}{[Metal]_{root}}$$

Statistical Analysis

Values obtained in the experiment were expressed as Mean±SD for triplicate determinations. Simple ratio calculations were employed in the determination of phytoremediation coefficients.

Results

The heavy metals concentration and phytoremediation coefficient of *Talinum triangulare* harvested from charcoal production sites are shown in Tables 1 and 2 respectively.

Table 1: Concentration of selected heavy metals in *Talinum triangulare* harvested from charcoal production sites

Metals	Concentration (mg kg^{-1})			
	Soil	Shoot	Root	NESREA (2009)
Site 1	18.37 ± 0.01	ND	ND	3
Cd	8.14 ± 0.01	ND	4.93± 0.04	100
Cr	1.06 ± 0.01	0.02 ± 0.003	3.81± 0.004	421
Zn	49.30 ± 0.21	1.84± 0.03	3.46 ± 0.01	NS
Mn	11.34±0.02	ND	ND	164
Pb	7.35 ±0.01	ND	4.45 ±0.01	70
Ni	47.10 ± 0.01	0.55±0.001	2.10 ± 0.01	100
Cu	4.86 ± 0.01	ND	9.56 ± 0.01	NS
Co	ND	ND	ND	NS
V	ND	ND	ND	NS
As	49.30 ± 0.21	1.84± 0.03	3.46 ± 0.01	NS
Site 2				
Cd	4.53±0.01	ND	ND	3
Cr	13.56±0.05	ND	ND	100
Zn	8.47±0.01	ND	0.151±0.005	421
Mn	10.02 ± 0.01	0.25±0.01	4.72 ± 0.008	NS
Pb	22.51±0.05	ND	ND	164
Ni	5.89 ±0.01	ND	0.15±0.01	70
Cu	22.90 ±0.01	1.95±0.01	2.45±0.01	100
Co	9.56 ± 0.01	ND	0.20±0.008	NS
V	ND	ND	ND	NS
Site 3				
Cd	2.57±0.01	ND	ND	3
Cr	12.75±0.11	ND	0.19±0.008	100
Zn	2.18±0.01	ND	2.08±0.007	421
Mn	18.41±0.01	3.05±0.01	0.106±0.005	NS
Pb	12.78±0.01	ND	ND	164
Ni	10.06±0.01	2.76±0.01	4.35±0.01	70
Cu	37.46±0.05	0.50±0.001	2.70±0.01	100
Co	8.81±0.01	ND	0.60± 0.008	NS
V	ND	ND	ND	NS

ND = not detected; NS = not specified

Results in Table 1 indicated that the concentration of Cd in soil of sites 1 and 2 were higher than the NESREA permissible limit, while its concentration in soil from site 3 was lower than the NESREA value. The concentration of Cr and Zn in soil from sites 1, 2 and 3 were lower than the NESREA standard value. Soil from site 1 contained Mn in high concentrations, but there is no reference standard value for Mn in soil. The results in Table 1 also showed that the mean concentrations of Pb, Ni, Cu and Co from all sites under investigation were below the permissible limit of NESREA.

Results in Table 1 also showed that the shoot of *Talinum triangulare* contained the following heavy metals: site 1 (Zn, Mn and Cu), site 2 (Mn and Cu), and site 3 (Mn, Ni and Cu). The heavy metal detected in the root of *Talinum triangulare* were Cr, Zn, Mn, Ni, Cu and Co. Cd was below detectable limit in the root of water leaf in all three experimental sites while V and As were not detected.

Table 2: Phytoremediation Coefficient of *Talium triangulare* for Heavy Matals

	Site 1			Site 2			Site 3		
Metals	**BAC**	**BCF**	**TF**	**BAC**	**BCF**	**TF**	**BAC**	**BCF**	**TF**
Cd	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Cr	0.00	0.61	0.00	0.00	0.00	0.00	0.00	0.02	0.00
Zn	0.02	3.59	0.01	0.00	0.02	0.00	0.00	0.95	0.00
Mn	0.037	1.88	0.53	0.025	18.71	0.053	0.17	0.04	28.59
Pb	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ni	0.00	0.61	0.00	0.00	0.03	0.00	0.27	0.43	0.63
Cu	0.018	3.78	0.26	0.085	1.25	0.80	0.013	5.38	0.19
Co	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
V	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
As	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

BAC = Biological accumulation factor; **BCF** = Biological concentration factor; **TF** = Translocation factor.

The phytoremediation coefficient of *Talinum triangulare* for heavy metals are expressed in Table 2. Zn, Mn and Cu showed a biological accumulation factor of less than 1 (BAC < 1). Table 2 also revealed that the biological concentration factor (BCF) of Zn, Mg and Cu in the three sites (1,2 and 3) under investigation were greater than 1 (BCF > 1). The translocation factor for Mn in site 3 was also greater than 1 (TF > 1). Results obtained in table 2 also showed that Cd, Cr, Pb, Co, V and As did not have phytoremediation coefficient values.

Discussion

Metals, unlike most organic contaminants, remain in the soil for a long period of time after their release into the environment. One of the alternative technology to remediate the contaminated area is phytoremediation (Sukyankij *et al*., 2016) and the extraction coefficient or biological accumulation coefficient of specific plant of interest can be employed to achieve this process (Ho *et al*., 2008).

The concentrations of Cr, Zn, Pb, Ni, Cu and Co investigated in the soil of the charcoal production sites were below the NESREA permissible limits except Cd concentration in site 1 (18.37±0.01 mg kg^{-1}) and site 2 (4.53±0.001 mg kg^{-1}), which were higher than the NESREA permissible limit (3.0 mg kg^{-1}). Toxic metals such as vanadium (V) and Arsenic (As) where not detected in the soil of the experimental sites.

Generally, the phytoextraction coefficient is used for estimating the potential of a plant for carrying out phytoremediation (Yoon *et al*., 2006; Li *et al*., 2019). Plants exhibit two basic strategies with respect to tolerating enormous quantities of heavy or toxic metals in their vicinities. They act as either excluders (i.e. having high concentration of heavy metals in the roots but with low translocation factor less than 1 (TF < 1) (Boularbah *et al*., 2006), or as accumulators by removing heavy metals from contaminated soils. Accumulators tend to increase the shoot content of heavy metals at both low and high metals concentration in soil (Rotkittikhim *et al*., 2006).

Results from the study indicated that the biological concentration (BCF) value for Cu in sites 1, 2 and 3 were higher than 1 (BCF >1). This means that *Talinum triangulare* accumulated Cu at the experimental sites more than other heavy metals. Mn was accumulated more in site 2, and Zn in site 1. The phytoextraction of Mn by *Talinum triaangulare* in site 3 was also high.

Overall, the order of mobility for the three toxic metals was as follows: Cu > Mn >Zn. This suggest that *Talinum triangulare* possesses the potential for the phytoextraction of these metals.

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