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PHYTOREMEDIATION OF CHROMIUM AND NICKEL CONTAMINATED SOIL BY *Tridax Procumbens, Spigelia Anthelmia* AND *Ocimum Gratissimum*

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ABSTRACT

The extent of soil pollution by heavy metals is currently alarming because of their toxicity which lead to adverse effects on human and ecosystem health. The effectiveness of three plants regarded as invasive in Nigeria (Tridax procumbens, Spigelia anthelmia and Ocimum. gratissimum) on remediation of soil contaminated with Cr and Ni in the screenhouse of Plant Biology of Bayero University, Kano was assessed. The three selected species have established, spread and threatens ecosystems which potentially causes economic and/or environmental damage. Heavy metals pollution was artificially induced in 12 pots containing 2kg of soil and the plants were raised for 9 weeks on the polluted soil. At the end of the experiment, the plants were harvested, dried and crushed into fine powder in preparation for elemental analysis using PerkinElmer Atomic Absorption Spectrophotometer. Measurements were made on bioaccumulation of Cr and Ni in the three selected invasive plant species and data obtained were subjected to ANOVA and means separated using Pitchers' LSD at P≤0.05. The results obtained from the soil and plant tissues indicated that T. procumbens and O. gratissimum are good candidates to be used in phytoextraction of Cr and Ni in contaminated environment as they both recorded Bioconcentration factor (BCF) and Transfer Factor (TF) values greater than the unity value.

Keywords: Phytoremediation, phytoextraction, bioconcentration, heavy metals.

INTRODUCTION

Tridax procumbens, commonly known as coat buttons or tridax daisy is a species of flowering plant belonging to the family Asteraceae (Daisy family) and is the most potent species among 30 species (Mir *et al.,* 2017). The plant bears white or yellow flowers with three toothed ray florets. The leaves are toothed and generally anchor shaped. Its fruit is hard achene covered with stiff hairs and having a feathery, plume like white pappus at one end. Calyx is represented by scales or reduced to pappus (Choudhari and Maheshwari, 2009).

Spigelia is genus of flowering plants in the family Loganiaceae containing around 60 species. *Spigelia anthemia* is an annual weed also known as pinkroot It is about 60 cm high with a scarcely branched stem and short-stalked, feather-like lobed leaves sets in whorls of four. The spikes with small purple or bright red flowers come out of the whorl. The fruit is a two-lobed capsule with warty seeds (Nathaniel and Addison 2015).

Ocimum gratissimum is commonly known as Wild basil (English), Daddoya (Hausa), Effirin-nla (Yoruba) and Ahuji (Igbo). It is an aromatic herbaceous perennial shrub/subshrub in the order Lamiales and family Lamiaceae. The plant grows up to 1-3m tall, the leaves measure up to 10 x 5 cm, and are ovate to ovate-lanceolate, sub-acuminate to acuminate at apex, cuneate and decurrent at base with a coarsely crenate, serrate margin, pubscent and dotted on both the sides. The leaves show the presence of covering and glandular trichomes (Prabhu *et al.,* 2009).

Lasat (2000), defined heavy metals as elements with metallic properties (ductility, conductivity, stability as cations and ligand specificity), and having atomic numbers greater than 20. Environmental pollution amongst others is one of the consequences of Global development especially in the field of environmental protection and conservation (Bennett *et al.,* 2003). Since the beginning of the industrial revolution, soil pollution by toxic metals has accelerated dramatically. Nearly every government around the world advocates for free environment from harmful contamination because economic, agricultural and industrial development are often linked to pollution of environment. However, the demand for such development outweighs the demand for a safe, pure and natural environment (Ikhuoria and Okieimen, 2000). This affects the ecosystems' surface, subsurface, ground water, food quality and health (Heinaru *et al.,* 2005; Ayoub *et al.*, 2010).

In plants heavy metals in particular, affect the processes of photosynthesis by interfering with electron chain, water relations, biochemical and enzymatic activities (Ghori *et al.,* 2016). River Challawa which is situated along the industrial area of Kano state provides fresh irrigation water to local farmers. However, studies by Dan-Azumi and Bichi (2010) have reported the water samples from the river to contain elevated levels of Pb, Cr, Cu, Pb and Fe higher than the recommended WHO and FEPA guidelines, the study explained that the elevated levels of heavy metals results in the pollution of soil in the farmlands been irrigated with the water from the river and also indicates that human absorption through food chain and occupational exposure is very possible. Other heavy metals such as cadmium, chromium, nickel and zinc were reported to be associated with irritation of the eyes and respiratory passages, damage to brain, liver, bones and kidney, bronchitis, dermatitis, emphysema, hypertension, rickets and asthma. Sonayei *et al.* (2009) also reported that chronic exposure to heavy metals leads to serious kidney malfunction, anaemia, hematological and brain damage.

The extent of soil pollution by heavy metals is currently alarming because of their toxicity which lead to adverse effects on human and ecosystem health (Voet *et al.,* 2013). Therefore, it is important to monitor some of the heavy metals pollutants in soil and find a possible way of removing them from the soil or at least bring their concentration down to a safe level. One of such ways is through phytoremediation; a green technology that uses plants' absorption mechanism to remove toxic substances from the environment/soil. The aim of this study is to evaluate the ability of *Tridax procumbens*, *Spigelia anthelmia* and *Ocimum gratissimum* to decontaminate soil polluted with Cr and Ni.

MATERIALS AND METHODS

Experimental Site and Soil Preparation

The experiment was conducted in the screenhouse of Department of Plant Biology Botanic Garden, Bayero University, Kano, Nigeria during wet season. Kano lies between latitude 11^{o}N58″50′ and 11^{o}98″18′, longitude 8^{o}28″46E and 8^{o}48″01′ and altitude 486.5m.

Top soil of 0-15cm depth was obtained at random from Bayero University, Kano. The soil was air dried for 3 days, crushed sieved to remove dirt and mixed thoroughly with organic fertilizer (cow dung). Mixed soil weighed 2kg was used to fill 12 experimental pots and the experiment replicated three times was laid out in Complete Randomized Design (Oseni *et al.,* 2018).

Soil pollution and Planting of Seeds

Heavy metal contamination of the soil was achieved following the procedure of Mani *et al*. (2015) where Chromium and Nickel weighed 150 and 80mg/kg respectively were dissolved in 1L of distilled water and the prepared experimental pots with their replications were initially treated with the solution and normal irrigation with distilled water was followed at regular interval until it reached equilibrium for about a week. In order to ensure the sustenance of the introduced heavy metals and aeration, bottom of experimental pots were perforated and drained water collected into plastic plates which was later reintroduced into the experimental pots.

The seeds of the plants were sown directly onto the soil by broadcasting method. About 2 weeks after sowing, all the plants have germinated and grown visibly above the soil surface, they were thinned to 5 plants of uniform height in each pot (Juliana *et al.,* 2019). At 9 weeks plants were harvested and biomass prepared for laboratory testing.

Preparation of Harvesting Plant and Soil Samples

Plants harvested were carefully rinsed under running tap water first and then with distilled water to remove dust and clogged soil particles. The above ground shoot was separated from the roots and the samples were then air dried and later crushed into fine powder in preparation for elemental analysis. Soil samples from each pot on another hand were also obtained (before planting and after harvesting) and air dried for 3 days, crushed and sieved through 2.00mm mesh to obtain a representative sample (Oseni *et al.,* 2018).

Heavy Metals Analysis

The powdered samples of the harvested biomass were subjected to acid digestion using Subhashini and Swamy (2014) procedure. The soil samples were digested based on the procedure described by Yusuf *et al*. (2015) and the resulting filtrates were analyzed for the metal contents using Atomic Absorption Spectrometry (PerkinElmer PinAAcle 900H) in Central laboratory of Bayero University, Kano – Nigeria.

Assessment of Phytoremediation Potential

The ability of the three selected invasive plant species to undergo remediation of the two heavy metals (Cr and Ni) was assessed based on what other studies (Subhashini and Swamy 2014; Mani *et al*., 2015; Oseni *et al.,* 2018; Subhashini *et al*., 2018; Njoku *et al.,* 2020) determined through the following relations:

Percentage remediation potential $= \frac{Metal\ lost\ due\ to\ plant\ growth}{Final\ Metal\ conc.\ in\ soil\ without\ plant} x\,100$

Metal lost due to plant growth $=$ $Final\ metal\ concentration\ in\ soil\ without\ plant - Final\ metal\ concentration\ in\ soil\ with\ plant$

Bioconcentration factor $= \frac{Metal\ in\ plant\ tissue(mg/kg)}{Metal\ in\ soil(mg/kg)}$

Transfer Factor $= \frac{Metal\ in\ shoot(mg/kg)}{Metal\ in\ root(mg/kg)}$

Data Analysis

Data obtained were subjected to one way analysis of variance (ANOVA) and means were separated using Fisher's least significant difference test at $p \leq 0.05$ using Microsoft excel.

RESULTS AND DISCUSSION

Results from the present study revealed that the 3 selected invasive plants can effectively absorb Cr and Ni from the soil and store/accumulate them in different parts of their bodies, a greater proportion of both the metals were stored in the above ground biomass when compared to the concentration found in the plants' root systems indicating low metal restriction and efficient internal translocation of the toxic metals towards the aerial parts of the plants. This finding is consistent with that of Singh *et al.* (2022), the study reported highest concentrations of Cr accumulated in the stem of *J. curcas*. In another study by Smical *et al.* (2008), it was reported that the roots and stems of plants are the most important parts which absorbs and eliminates heavy metals in an excess amount. The result of this study also agrees with the findings of many scholars (Wang and Chen, 2009; Rahman and Hasegawa, 2011; Huang *et al.,* 2013) who recorded the highest concentration of Cr in the stems of some plants.

Remediation potential (RP) is used to indicate the percentage reduction of heavy metals in the soil due to plant growth. At the end of the experiment, the 3 plants were able to cause a significant reduction in concentration of Cr and Ni in soil due to their growth.

In Table 3, the highest reduction of Cr (79.62%) and Ni (76.01%) was caused by the growth of *Ocimum gratissimum* whereas the least reduction in the amount of the two heavy metals (50.04% of Cr and 51.23% of Ni) was caused by the growth of *Spigelia anthelmia* and *Tridax procumbens*. The high metal remediation potential observed in this study may suggest that a good proportion of the metals in the soils is present in the mobile phase, thus readily available for uptake by plants. This agrees with the findings of Njoku *et al.* (2020) where significant reduction in the amount of Cd, Cr, Ni and Pb were attributed to the growth of *Senna alata* plants. Similar observations were also reported by Singh *et al.* (2022) where growth of *Jatropha curcas* and *Cassia occidentalis* resulted in significant reduction of heavy metals in the soil. Considering the concentrations of Cr and Ni left in the soil after harvest in the present study, it further confirms that the growth of these 3 invasive plants can result in a significant reduction of the heavy metals concentrations in soil (Table 2).

Bioconcentration factor (BCF) indicates the efficiency of a plant species in accumulating a metal in its tissues from the surrounding environment (Ladislas *et al.,* 2012). Translocation factor (TF) on the other hand indicates the efficiency of the plant in translocating the accumulated metal from its roots to shoots. Both BCF and TF are important in screening hyperaccumulators for phytoremediation of heavy metals. The evaluation and selection of plants for phytoremediation purposes entirely depend on BCF and TF values (Wu *et al*., 2011). In the present study, the highest BCF value was recorded by *Ocimum gratissimum* (3.75) in Cr whereas the lowest BCF value was recorded by *S. anthelmia* (0.96) in Cr (Table 3). The BCF values recorded by *T. procumbens* and *O. gratissimum* for the two heavy metals were found to be greater than 1. This suggests that the plants are potential candidates for remediation of the heavy metals in contaminated environment, similar observations were reported by Kumari *et al.* (2016) that *Cassia tora* is suitable for revegetation of metal contaminated wastelands and also may be used for decontamination purposes. In addition, *Sida acuta* and *Cassia tora* were reported to be used for the remediation of Mn, Cu, Zn, Fe and Cr from fly ash (coal combustion residuals). In that report, the BCF values of Mn, Cu, Zn, Fe and Cr in *Cassia tora* were greater than 1 under low level of fly ash (50%) amended soil (Panda *et al.,* 2020). Research by Badamasi *et al.* (2017) on phytoremediation of Pb using *Cassia tora* also revealed that the BCF values of Lead in *Cassia tora* were greater than one in all treatments which makes the plant a potential remediator of lead. Studies on *Tridax procumbens* by Kumar *et al.* (2013) also revealed that the plant has enrichment coefficient greater than 1 for Cr, Cu, Ni, Pb and Cd and implies that *T. procumbens* can be used to clean up pollution in areas contaminated with the said heavy metals. On the other hand, the BCF values of *Spigellia anthelmia* for Cr and Ni were less than one. This also indicates that *S. anthelmia* is not a potential candidate for remediation of Cr and Ni in contaminated soil. Similar findings by Petelka *et al.* (2019) have also revealed that the BCF values of Hg, As and Pb in *Spigelia anthelmia* were less than one.

Another important mobility index that should be taken into consideration when assessing the phytoremediation potentials of any plant is the ability of the plant to translocate metals from the roots to the shoots which can be measured using a mobility index refer to as the transfer factor (TF). Translocation factor value greater than 1 indicates the translocation of the metal from root to above-ground part (Jamil *et al.,* 2009). In the present study, all the 3 invasive plants have TF values greater than 1. According to Yoon *et al.* (2006), only plant species with both BCF and TF greater than 1 have the potential to be used for phytoextraction thus, *Tridax procumbens and Ocimum gratissimum* can be used as phytoextractors of the metals. The grass plant, *Perotis indica* was used to remove Pb, Ni, Cd and Cr from an artificially contaminated soil. The results from the study revealed that the plant is a good accumulator of the metals and can be used as a phytoextractor (Subhashini *et al.,* 2018). On the other hand, *S. anthelmia* despite absorbing the metals and having good internal translocation does not fit to be a phytoextractor of the metal at the course of this work (9 weeks). However, the plant may also achieve phytoremediation purposes if the duration of the experiment is extended.

Table 1: Heavy metals concentrations (ppm) in the plant tissues after harvest

	Chromium			Nickel		
Plants	Shoot	Root	Plant	Shoot	Root	Plant
Tridax procumbens	56.37±1.76^{b}	43.30±1.30^{b}	99.67±0.66^{b}	25.08±0.13^{b}	18.82±0.60^{a}	43.90±0.70^{b}
Spigelia anthelmia	56.23±0.50^{b}	15.77±0.10^{c}	72.00±0.45^{c}	21.30±0.05^{c}	15.28±0.16^{b}	36.58±0.21^{c}
Ocimum gratissimum	64.62±0.63^{a}	50.70±0.08^{a}	115.32±0.70^{a}	39.42±0.34^{a}	14.78±0.22^{b}	54.20±0.46^{a}
LSD (5%)	5.64	3.80	3.09	1.08	1.93	2.53

LSD = Least Significant difference. Means with different letters within column are significantly different at P≤0.05 using Fisher's LSD.

Table 2: Heavy metals concentrations (ppm) in soil before and after remediation with the plants

	Chromium		Nickel	
Plants	Initial conc.	Final conc.	Initial conc.	Final conc.
Tridax procumbens	160.17±0.44[a]	48.85±0.35[c]	85.02±0.04[a]	29.82±0.15[c]
Spigelia anthelmia	159.85±0.38[a]	75.33±0.39[b]	84.98±0.28[a]	37.62±0.33[b]
Ocimum gratissimum	159.53±0.83[a]	30.78±0.09[d]	84.53±0.22[a]	18.50±0.66[d]
Control	159.42±0.29[a]	150.78±0.57[a]	85.30±1.66[a]	77.13±0.30[a]
LSD (5%)	NS	1.62	NS	1.88

LSD = Least Significant difference. Means with different letters within column are significantly different at P≤0.05 using Fisher's LSD.

Table 3: Bioconcentration, transfer factors and remediation potential (%) of the plants

Heavy metals	Chromium			Nickel		
Plant species	BCF	TF	RP	BCF	TF	RP
Tridax procumbens	2.04	1.31	67.60	1.47	1.34	61.34
Spigelia anthelmia	0.96	3.57	50.04	0.97	1.39	51.23
Ocimum gratissimum	3.75	1.27	79.62	2.94	2.67	76.01

BCF = Bioconcentration factor, RP = Remediation potential and TF = Transfer factor

CONCLUSION

The TF and BCF values of *Tridax procumbens* and *Ocimum gratissimum* in chromium and nickel were greater than 1 indicating efficient internal translocation and phytoremediation potential, thus they are concluded to be phytoextractors. *Spigelia anthelmia* has recorded BCF values less than the unity value implying non-phytoremediation potential. *Ocimum gratissimum* is a green leafy herb that is often consumed in the society for its medicinal values. However, this plant can bioaccumulate heavy metals and its consumption may in any way predispose people to high risk of metal toxicity especially if it is grown in contaminated area.

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