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Assessment of Phytoextraction Potentials of Three Indigenous Plant Species in Soil Contaminated with Spent Engine Oil

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Abstract
This study was carried out to evaluate the tolerance capacity of three plant species and to ascertain their phytoremediation potentials by pot culture experimentation. An equal volume of pre-sieved soil (20kg) was filled in Polythene bags and arranged at 0.5m spacing between polybags and 1m between replications and perforated at the base to avoid water logging. Concentrations of spent engine oil spiked included 0, 4%, 8%, 12% and 16% (v/w) which was allowed to stabilize for two (2) weeks to simulate the condition of the natural spill. The untreated soil served as a control. Twelve weeks after exposure, the plants were harvested and assayed for selected heavy metals using AAS. Results obtained showed that metal accumulation patterns were in the order *C. odorat>A.africana>A.compressus*. The results further showed that C. *odorata* and *A. africana* exhibited characteristics typical of a phytoextractor while *A. compresses* could be applied as a phytostabiliser of spent engine oil-polluted soils. It is recommended that the three-plant species be tried for phytoremediation of spent engine oil-contaminated soils.

Keywords: Phytoextraction, Plant Species Contamination, Spent Engine oil.

Introduction

Remediation of contaminated soils can be achieved through four ways viz: chemical, physical, thermal or biological techniques (phytoremediation and bioremediation). Most of the remediation techniques focus on exploiting or altering soil chemistry to either remove contaminants from the soil or reduce their solubility and bioavailability (Jidere & Akamigbo, 2009). Chemical remediation is based on chemical oxidation that eliminates harmful compounds from the contaminant. This technique is relatively fast but may negatively impact the surrounding ecosystem. The physical method (which includes excavation and washing) involves the transportation of contaminated soil from the source to an area for disposal, while in the second part (washing), contaminated soil is washed with organic solvents which eventually removes the contaminants (Anukwa et al., 2021). Again, the high cost of chemicals and the threat to flora and fauna makes this method less applicable. Thermal technique entails desorption and incineration which is very expensive with its attendant environmental pollution potential (Singh & Jain, 2003).

Given this, a plant-based remediation technique known as phytoremediation is by far considered the most optimal remediation technique. Phytoremediation, also known as green-remediation is defined as the cleanup of contaminated sites using a unique diversity of plants (Igwe et al., 2016). This is a remediation technique that uses plants to detoxify contaminated soils. It presents an efficient, "green clean," environmental, low-cost, and eco-friendly technology that uses plants to reduce or remove inorganic and organic pollutants from the environment (Pilon-Smits, 2005; Lee et al., 2007). The term phytoremediation consists of the Greek prefix “phyto” which means “plant” and the Latin suffix *“remedium*” which means “renew, able to cure or restore”. Of all the remediation techniques currently in use, phytoremediation has proven to be a more economical and eco-friendly process (Azadeh et al., 2007). The ability of plants to remediate polluted sites is often attributed to the microorganisms resident in the rhizosphere under the influence of the roots (Jones et al., 2004). Before any phytoremediation study, an exhaustive plant selection from the local population must be made to screen plants for potential application. However, research reports on plants that have phytoremediation potentials are scanty and fairly undocumented in tropical areas; particularly in Nigeria (Pulchérie et al., 2018). Therefore, screening plant species to determine those with the capacity to grow on hydrocarbon-polluted soils remains an essential step in phytoremediation technology (Messou et al., 2013). Selection of plants from the populations of the metal-polluted sites for their recovery is now the method (Ariyakanon & Winaipanich, 2006), as they are adapted to soil and climatic conditions in the zone, which should make phytoremediation a much easier task (Anoliefo et al., 2003). One of the strategies that can be adopted when working in phytoremediation is the use of native hyperaccumulator plants of high biomass, mainly those adapted to the climatic and soil conditions of the polluted site.

Materials and Methods

The study was carried out at the Teaching and Research Laboratory of the Department of Biology, the Federal University of Technology Owerri, Imo state located at latitude 5.3866° N, and longitude 6.9916° E respectively. Phytoremediation potentials of the selected hyperaccumulator species from the various auto mechanic workshops were evaluated *in vivo* by conducting pot experiments in the screen house. Three plant species (*Chromolaena odorata, Axpnopus compressus and Aspillia africana*) were selected based on phase one of this study (Azorji et al., 2021) as well as their dominance at mechanic workshops. Soil samples with no history of oil pollution were collected from a depth of 0-20cm within the Federal University of Technology Owerri using an auger of approximately 7.5cm diameter and taken to the laboratory for pre-planting soil analysis. The soil samples were air-dried and pre-sieved with <2mm sieve and used for physicochemical determination. Vegetative parts of the plant species (stem cuttings of *Chromolaena odorata, Axonopus compressus* and *Aspillia africana*) were collected from an area with no trace of spent oil pollution and stabilized in the nursery for two (2) weeks by which time an average of four (4) fully expanded leaves per plant had been developed before been used for the study.

An equal volume of pre-sieved soil (20kg) was filled in Polythene bags and arranged at 0.5m spacing between polybags and 1m between replications and perforated at the base to avoid water logging. Concentrations of spent engine oil spiked included 0, 4%, 8%, 12% and 16% (v/w) and allowed to stabilize for two (2) weeks to simulate the condition of the natural spill. For the treatment, the control soil was not spiked with spent engine oil. Each treatment was replicated thrice and arranged in a completely randomized design. Two weeks after spiking with different concentrations of spent engine oil, about 100g of soil samples were collected at the surface from each replicate, homogenized and sent to the laboratory for soil analysis before planting (Tóth & Montanarela, 2013). This was done to track the initial level of pollutants of concern in spent engine oil before transplanting (Njoku et al., 2016). Plants were carefully harvested after 12 weeks of treatment and washed with distilled water to rid them of soil particles. The three replicates from each treatment were pooled together to give a composite. Post-analysis of soil and plant tissues was carried out to track the phytoremediation potentials of *Chromolaena odorata* and *Aspillia africana* at the various treatment levels.

Each subsample was oven-dried at 70°C for 24 hours. The acid digestion method of Yusuf et al. (2003) was used for the digestion of grounded plant samples. 1 g each of these was weighed into a 50mL capacity beaker, followed by the addition of a 10mL mixture of analytical grade acids: HNO3; H_2SO_4; $HClO_4$ in the ratio 1: 1 : 1. The beakers containing the samples were covered with watch glasses and left overnight. The digestion was carried out at a temperature of 70°C until about 4mL was left in the beaker. Then, a further 10mL of the mixture of acids was added. This mixture was allowed to evaporate to a volume of about 4mL. After cooling, the solution was filtered to remove small quantities of waxy solids and made up to a final volume of 50 mL with distilled water. Quantitatively, the digested samples were transferred into a 125ml plastic container, filtrated with 50ml de-ionized water and metal analysis performed with Buck scientific Atomic Absorption Spectrophotometer (Model GFA-EX7i, Shimadzu Corporation, Japan) using an air acetylene flame with a digitalized readout system (Sabate et al., 2006). Results were expressed in mg/kg.

Each plant per treatment was examined to determine the percentage removal of contaminants as well as the total accumulation of the contaminants in the plants. The Biological Accumulation Coefficient (BAC), and Transfer Factor were estimated and the results were used to assess the phytoextraction, phytostabilization and hyperaccumulation potentials of the test plants. The biological accumulation coefficient (BAC) is the concentration of contaminant in the plant shoots divided by the metal concentration in the soil (Raymond & Harrison, 2018):

(a) **Bioaccumulation Coefficient (BAC)** $= \frac{metalcontentinshoot}{metalcontentinsoil}$

BAC factor > 1 indicates that the plant species can store contaminants from the soil into the shoots.
The biological Transfer Factor is the ratio of the contaminant concentration in the plant shoot divided by that of the plant root. BTC values > 1 indicate that the species has the potential to accumulate contaminants from the root to the aerial part of the plants.

(b) **Biological transfer coefficient**$= \frac{metalcontentinshoot}{metalcontentinroot}$

Data collected was presented in charts and tables. Mean separation was done using the Duncan Multiple Range Test. Probit analysis was carried out to determine the EC50 and LC50 of each organism used in acute toxicity tests. All statistical analysis was run using the SAS package, 20 version.

Results

Percentage reduction of heavy metals by *C. odorata, A. africana* and *A. compressus*

Percentage biodegradation of spent engine oil in the soil by *C. odorataA. africana* and *A. compressus*is are presented in Figures 1, 2, and 3. There was a significant percentage reduction in heavy metal content by *C. odorata* compared with the 0% concentration (left under natural attenuation). At 4%, 8%, 12% and 16% treatment level, *C. odorata* showed a concentration-dependent percentage phytodegradation (between 77.0 and 96.8%) for Fe, Cd, Co, As, Mn, Pb, Cr, Ni, Cu and Zn after 12 weeks. It was also observed that the highest percentage reduction value was recorded in Fe at 16% (96.06%) treatment levels compared with other treatments. *A. compressus* followed a similar pattern in percentage heavy metal biodegradation with the highest and least percentage reduction values of 85% for Zn and 3.01% for Pb. *A. africana* was observed to have biodegraded between 68.6% and 90.71% at the highest level of treatment with increase in concentration level (16%). Generally, the plant species (*C. odorata*, *A.africana* and *A. compressus*) significantly reduced concentrations of heavy metals after twelve weeks of growth in spent engine oil polluted soil.

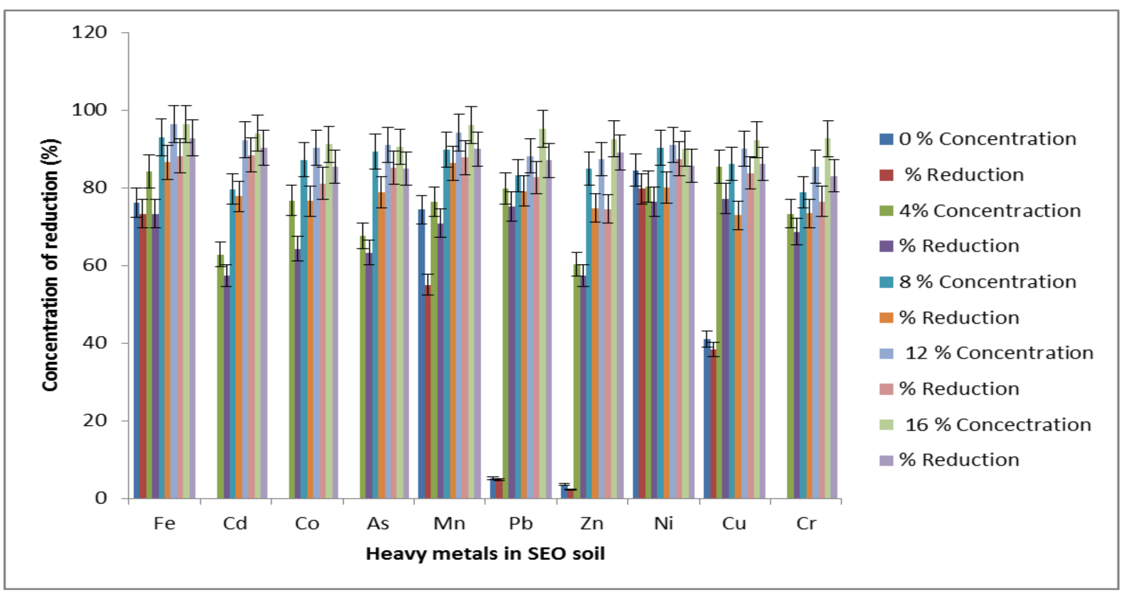

Figure 1: Percentage reduction of heavy metals in *C. odorata* exposed to SEO-polluted and unpolluted soil

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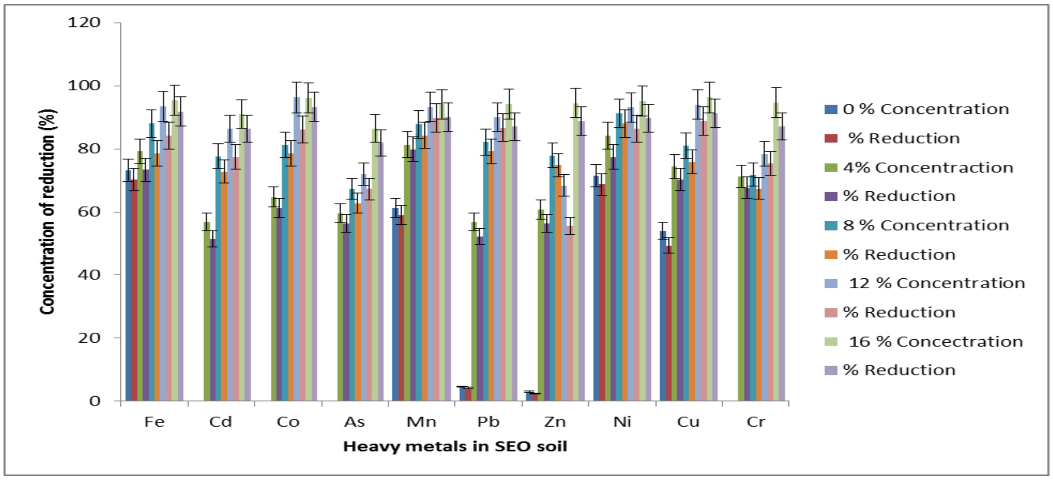

Figure 2: Percentage reduction of heavy metals in *A. compressus* exposed to SEO polluted and unpolluted soil.

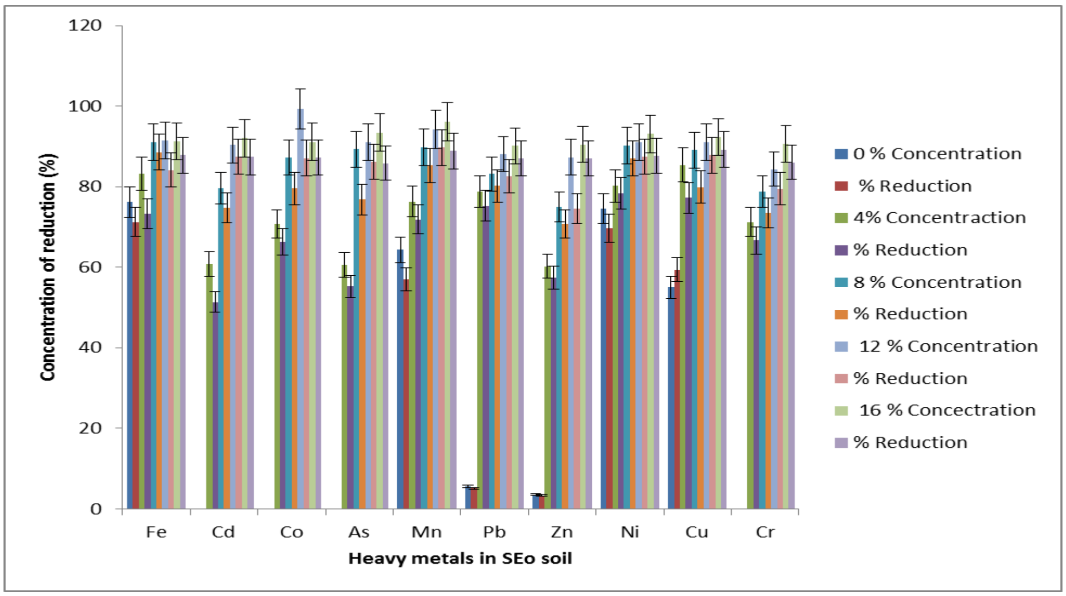

Figure 3: Percentage reduction of heavy metals in *A. africana* exposed to SEO-polluted and unpolluted soil

Bioaccumulation Factors and Transfer Factors in Plants exposed to spent engine oil polluted soil.

Bioaccumulation and Transfer Factors in Plants *C. odorata, A. africana* and *A. compressus*) exposed to different concentrations of spent engine oil is presented in Table 1. while the heavy metal accumulation pattern is presented in Table 2. Results obtained showed that within the below-ground soil) and above ground (shoot) part of the plants, bioaccumulation factor ranged from 1.17 to 0.01 for Arsenic, 1.90 to 0.01 for Cadmium, 1.98 to 0.08 for Chromium, 2.71 to 0.03 for Cobalt, 1.60 to 0.10 for Copper, 8.50 to 0.03 for Iron, 2.91 to 0.50 for Manganese, 1.69 to 0.01 for Nickel, 0.19 to 0.01 for Lead and 5.21 to 0.01 for Zinc respectively. The maximum bioaccumulation factor was noted in *C. odorata* (6.40 mg/kg) for Copper while the minimum (0.01 mg/kg) was observed in *A. africana* for Lead. It was also observed that heavy metal bioaccumulation was higher in the spent engine oil oil-treated plants than in the control.

The ability of the plant species to transfer heavy metals from root to shoot (transfer factor) ranged from 1.12 to 0.01 for Arsenic, 2.65 to 0.01 for Cadmium, 1.08 to 0.01 for Chromium, 3.11 to 0.11 for Cobalt, 1.69 to 0.60 for Copper, 5.47 to 1.40 for Iron, 2.59 to 0.11 for Manganese, 1.90 to 0.01 for Nickel, 0.13 to 0.01 for Lead, and 6.81 to 3.61 for Zinc. The highest transfer factor was observed in *C. odorata* for Zinc (6.81 mg/kg) while the minimum transfer factor was recorded in *A. compressus* for Cadmium (0.01 mg/kg). Bioaccumulation Factor for *A. africana A. compressus* and *C. odorata* were in the order:Zn> Cu> Fe> Mn> Ni> Co> Cr> Cd> As >Pb; Fe> Co> Mn> Ni> Zn> Cu> Cr>

Cd> As> Pb andCu> Fe> Zn> Co> Mn> Ni> As> Cd> Cr> Pb while the Transfer Factor followed: Fe> Zn> Mn> Cd> Cu> Ni> Co> Cr> As>Pb; Fe> Zn> Mn> Ni> Co> Cd> As> Mn> Cr>Pb and Zn> Fe> Cd> Ni> Cu> Mn> As> Cd >Cr>Pb respectively.

Table 1: BCF and TF of heavy metals in above and belowground parts of *A. africana, A. compressus* and *C. odorata* exposed to different concentrations of spent engine oil-contaminated soil

Conc.(%)	Species	As	Cd	Cr	Co	Cu	Fe	Mn	Ni	Pb	Zn	
						Bioconcentration Factor (soil-to-shoot Ratio)						
0			ND	ND	ND	ND	0.10	0.03	ND	ND	ND	0.01
4	***A. africana***		0.17	0.61	0.99	0.09	4.82	2.01	2.91	5.11	0.02	2.19
8			0.02	1.80	1.90	1.70	0.60	2.40	0.51	1.61	0.09	2.33
12			0.20	0.80	0.91	1.71	1.10	4.41	1.51	0.01	0.11	4.01
16			ND	0.01	1.98	2.71	1.60	4.50	1.81	1.69	0.19	4.11
4	***A. compressus***	0.11	ND	0.88	0.03	ND	3.40	0.50	1.06	0.07	1.70	
8			0.12	0.80	1.22	1.71	1.50	2.40	2.51	1.61	0.07	1.61
12			0.42	1.80	1.32	2.21	1.57	3.41	2.54	1.61	0.09	1.71
16			0.45	1.90	1.96	2.51	1.61	5.10	2.51	1.21	0.09	1.71
4	***C. odorata***		0.03	0.81	0.08	0.71	3.00	3.41	1.11	1.01	0.01	1.01
8			1.33	1.82	0.38	1.18	4.30	3.48	1.21	1.21	ND	2.21
12			1.31	0.84	0.18	1.01	4.20	3.12	1.41	1.31	0.03	3.41
16			1.35	1.85	0.58	1.11	6.40	3.20	1.61	1.61	0.09	5.21
						Transfer Factor (Root to shoot Ratio)						
4			ND	0.01	ND	0.11	1.09	2.40	0.11	1.21	ND	3.61
8	***A. africana***		0.12	1.00	1.08	0.70	0.75	4.40	1.05	1.66	0.01	5.71
12			0.02	0.21	0.08	0.77	0.60	4.42	1.51	1.81	0.09	5.31
16			1.12	1.80	0.91	1.53	1.65	5.47	2.04	1.61	0.10	7.01
4	***A. compressus***	0.21	0.01	ND	0.71	1.69	2.40	0.11	1.71	ND	3.71	
8			0.04	1.80	0.98	1.71	1.10	2.30	0.51	1.01	0.01	3.77
12			0.05	1.00	0.91	2.01	1.67	3.40	0.61	1.61	ND	4.71
16			0.19	0.30	0.93	2.71	1.69	3.44	0.53	1.61	0.01	4.79
4	***C. odorata***		0.04	1.90	ND	3.11	0.90	1.40	1.61	1.90	ND	5.71
8			0.10	0.01	0.01	2.80	1.50	1.46	2.50	0.01	0.10	5.10
12			0.01	2.65	ND	2.71	1.20	2.44	0.54	1.32	0.13	6.71
16			0.22	2.22	0.08	2.99	1.10	2.49	2.59	1.11	ND	6.81

LEGEND:CONC. = concentration; *BCF and TF=Bioconcentration Factor* and *Transfer Factor. ND= Not detected.*

Table 2: Heavy Metal Accumulation Pattern for *A. africana, A. compressus* and *C. odorata* exposed to different concentrations of spent engine oil-contaminated soil

Species	Heavy metal bioaccumulation pattern
	BAF
A. africana	Zn>Cu>Fe>Mn>Ni>Co>Cr>Cd>As>Pb
A. compressus	Fe>Co>Mn>Ni>Zn>Cu>Cr>Cd>As>Pb
C. odorata	Cu>Fe>Zn>Co>Mn> Ni>As>Cd>Cr>Pb
	TF
A. africana	Fe>Zn>Mn>Cd>Cu>Ni>Co>Cr>As>Pb
A. compressus	Fe>Zn>Mn>Ni>Co>Cd>As>Mn>Cr>Pb
C. odorata	Zn>Fe>Cd>Ni>Cu>Mn>As>Cd>Cr>Pb

Legend: BAF= Bioaccumulation Factor, TR= Transfer factor.

Discussion

The accumulation of heavy metals in roots shoots and leaves may be an indication that the test plants (*C. odorata, A. africana* and *A. compressus*) possess the potential to remediate sites with low to medium contamination (Olajuyigbe et al., 2019). This was evidenced by the survival and continued, though concentration-dependent reduction in growth of the test plants, in spent engine oil-contaminated soils (Nwoko et al., 2007). The ability of *C. odorata, A. africana* and *A.compressus* to grow in soils contaminated with spent engine oil suggests that the species may have the ability to phytodegrade the toxicants resident in spent engine oil. This result is similar to the findings of Raymond and Harrison (2018). Similar growth responses have been reported for seedlings of *Terminalia ivorensis, Terminalia* *superba* and *Khaya senegalensis* (Olajuyigbe & Aruwajoye, 2014). A significant and positive correlation was recorded between TPH concentration in soil and plant tissue. This result is in agreement with the report of Raymond and Harrison (2018). The reason might be that an increase in TPH content in soil resulted in an increased level of TPH in plants.

Bioconcentration and transfer factors are two major parameters used in evaluating the rate of heavy metal uptake in plant tissues and the potential for phytoremediation (Sharmar & Reddy, 2020). The ratio of metal concentration in plant root to soil (contaminant source) gives the Bioconcentration factor (BCF) and the further ratio of root-to-shoot transfer of contaminant is referred to as Transfer factor (TF) (Zitte et al., 2016). By convention, when both BCF and TF are >1 it means that the plants have the potential for phytoremediation of spent engine oil-polluted soils (Zitte et al., 2016). The BCF of all plant species exposed to different concentrations of spent engine oil was found to be greater than one (>1) for the majority of the heavy metals assayed, this is an indication that the plants were accumulating the metals from soil to root. Although bioaccumulation ratios of the plant species for some of the heavy metals indicated values less than unity (<1) to be classified as hyperaccumulators, the plants were identified as potential species that can successfully carry out phytoextraction of spent engine oil polluted sites. The bioconcentration pattern in the plant species was in the order: *C. odorata>A. africana>A. compressus*. The result of this study showed that the plant species have the potential to be used as phytoaccumulators. Results of this study agree with the report of previous researchers (Sharma, & Reddy, 2020). The variation in heavy metal accumulation patterns might be attributed to differences in the specific physiological characteristics of the test plants based on their genetic makeup (Sharma & Reddy, 2020).

The ability of all plants to transfer metals from root to shoot which is an important parameter of phytoremediation potential also indicated that these tested plants showed TF > 1 for most of the heavy metals assayed (Table 1. The TF pattern was in the order: *C. odorata>A. compressus>A. Africana*. A study by Oseni et al. (2015) reported decontamination of Lead (Pb) contaminated soil with *C. odorata* this is in tandem with the findings in this study. Reginald et al. (2018) reported that *A. compressus* (Poaceae) degraded 47% and 48% in petroleum-impacted soils. According to Ma et al. (2001), plant species with TF>1 are categorized as hyperaccumulators for metal accumulation from roots to shoots. According to Osadolo and Animetu (2018), the root appears to accumulate higher levels of heavy metals compared to other parts of the plants. This is because the root acts as a barrier for metal translocation and possibly protects the stem from metal contamination. This might have led to higher ratios of transfer factor obtained in this study. This is in line with the report of Onwuka et al. (2012). In a related study by Raymond and Harrison (2018), plant species with TF >1 actively mopped up metals to the aerial parts which makes them good phytoremeditors. TF>1 obtained in this study suggested a high metal accumulation property of these plant species for the translocation and metal movement to the aerial parts of plants. This result corroborates the findings of previous authors (Nwoko et al., 2007).

Conclusion

The ability *of O. odorata, A. africana* and *A. compressus* to bioaccumulate and transfer heavy metals in their aboveground tissues (root, shoot and leaf) suggests that these plants could be used to remediate spent engine oil-contaminated sites. Thus, the three plant species have demonstrated the ability to tolerate heavy metal stress, and grow and accumulate biomass in spent engine oil-polluted soil. This underscores the need to further explore the adaptability of these indigenous plant species to heavy metals for their selective exploitation in phytoremediation of spent engine oil polluted sites.

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