Assessment of Heavy Metal Bioaccumulation Capacity of *Calopogonium muconoides* and *Senna obtusifolia* as Potential Bioremediation Agents

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Abstract
A wide range of inorganic and organic compounds such as combustibles, and putrescible substances, hazardous waste, explosives, petroleum products and heavy metals (HM) can cause contamination. In addition, the non-biodegradability of heavy metals further exacerbates environmental pollution with its attendant health consequences on the biotic components of the ecosystem including humans. The use of living organisms such as plants and microbes is increasingly becoming acceptable practice of sustainable environmental sanitation. However, identification of potential bioremediation agents is still challenging. This study was carried out to bridge this gap by assessing heavy metal bioaccumulation properties of *Calopogoniun Muconoides* and *Senna obtusifolia* plants at contaminated site of mechanic workshop (site 1) in comparative to a physically non-contaminated site (site 2) within Makurdi metropolis of Benue State Nigeria. The selected plants were obtained from both sites and evaluated for their bioaccumulation capacities using standard procedures. The results revealed the sample plants accumulated high levels of heavy metals particularly in the leaves and roots, suggesting the utilization of phytoextraction, phytostabilization and phytovolatilization mechanisms of remediation. The plants and HM generally presented an order of concentration and bioaccumulation as: *Senna obtusifolia* > *Calopogonium Muconoides*; Zn > Fe > Pb > Cu > Cd > Cr > Ni. These findings suggest that these novel plants, especially *Senna obtusifolia* are good agents of bioremediation of heavy metals. Studies involving isotopic labeling to determine the exact mechanism of remediation as well molecular techniques such as transcriptomics and proteomics to identify genes/molecules that confer phytoremediation potential on the plants would be the next focus of our research in this emerging field of environmental biochemistry.

Keywords: Phytoremediation, bioaccumulation, heavy metals, bioremediation, *Senna obtusifolia*, *Calopogonium Muconoides.*

Introduction

Bioremediation by plants also called phytoremediation refers to the use of green plants to remove, contain or render pollutants harmless (Hussein *et al*., 2013). A wide range of inorganic and organic pollutants such as combustibles, and putrescible substances, hazardous waste, explosives, petroleum product and heavy metals (HM) can cause contamination (Wuana and Okieimen, 2011). A major component of contaminants in the ecosystem is HM, which present a different challenge than other organic contaminants owing to their non-biodegradability (Jan, *et al.*, 2015). In Humans, adverse health consequences are inevitable due to accumulation of HM overload in the body from continued exposure. The most commonly encountered toxic metals are Arsenic (As), Lead (Pb), Aluminum (Al), Mercury (Hg), Cadmium (Cd), and Iron (Fe) (Navas-Acien *et al.,* 2007). These elements may enter the body through inhalation, ingestion, or dermal absorption resulting in different levels of toxicities and health outcomes (Tchounwou *et al.*, 2012). Exposure to environmental contamination with HM is a growing problem throughout the world having risen in the last five decades in the wake of industrial revolution accompanied by numerous industrial processes (Edao *et al*., 2017). Intriguingly, exposure to HM particles, even at levels below what is previously known to be nontoxic, can have serious health effects as a result of the dynamic interaction between these species and many cellular processes that define life. For instance, virtually all aspects of animal and human immune system functions are compromised by HM particulates exposure that has bioaccumulated in the system of the organism (Valco, *et al.,* 2005). A sustainable effort at curtailing incidences of exposure and outcomes of environmental pollution by heavy metals is the use of living organisms such as microorganisms and plants, which have been shown to have the capacity for heavy metal load reduction in the environment through bioaccumulation (which refers to the increase in the level of xenobiotics in an organism overtime when compared to the level of the xenobiotic in the environment (Gupta *et al*., 2013).

Calopogonium Muconoides and *Senna obtusifolia* are both tropical leguminous plants belonging to the *Fabieceae* family, and common to Africa, Asia and the Pacific. Both have been reported for their nutritional and medicinal benefits (Ingweye *et al*., 2010; Sudi *et al*., 2011). Although phytoremediation capacities of these plants have not received any significant attention, they have ability to grow in harsh soil and climatic conditions (Solomon *et al.*, 2014; Udiba *et al*., 2020). Thus, determining their heavy metal bioaccumulation factor would be a plausible step in exploring their benefits in bioremediation. This study is aimed at investigating the properties of *Calopogoniun Muconoides and Senna obtusifolia* plants that have enabled them to survive the contaminated site as possible to remedy to mitigating HM contamination.

Materials and Methods

Study area

This study was carried out at Makurdi Benue State Nigeria between December, 2020 and February, 2021. Two sites designated Site 1 for auto mechanic workshop and Site 2, which is an open field within a residential area of Commissioners' village. Both sites are located within the same geographical axis of Makurdi-Otukpo road.

Collection and preparation of samples

Plant samples collected from sites 1 and 2 were identified and authenticated as *Calopogonium Muconoides* and *Senna obtusifolia* at the Department of Botany, University of Agriculture, Makurdi with voucher number (FUAM/BOT/0047) deposited at the University herbarium. The samples were thoroughly washed under running tap water to remove adhered soil before being separated into leaves, roots, and stem. The samples were separately dried in an oven for 48 h at 80 ^{0}C. The dried sample was ground using a mortar and pestle, and a sieved on a 2 mm mesh before being transferred to polyethylene bags and stored until further analyses. Surface soil (0 – 15 cm depth) was sampled by hand auger (2.5 cm diameter) from both sites. The soil sample comprised of a composite of three sub samples obtained from a 1 x 1 meter square distance were thereafter taken to the lab for further analysis.

Determination of heavy metals in plant species and soil

An aqua regia wet method of digestion described by Ang and Lee (2005) was used to digest soil samples. To 1 g of sample, 18 ml of a fresh mixture of hydrochloric acid and nitric acid in the ratio of 3:2 was added and the mixture was boiled over a water bath (95 ^{0}C) for 1 h. After complete digestion, the residue was made up to 50 ml with distilled water. The concentration of HM in the soil samples was determined at the national institute for chemical research technology, Zaria using Atomic Absorption Spectrophotometer (AAS).

Determination of bioaccumulation factor (BAF)

Bioaccumulation factor, the ratio of the concentration of a particular chemical in the organism or tissue of an organism to the concentration in the environment was determined using the following equation according to Jezierska and witesta, 2001:

$$BAF = \frac{Concentration\ of\ Chemical\ in\ Organism\ or\ Tissue}{Concnetration\ of\ the\ Chemical\ in\ the\ environment} \text{(eqn 1)}$$

Statistical Analysis

Data generated were subjected to analysis using one way analysis of variance (ANOVA) followed by Dunnett's test. Heavy metal concentration was reported as mean ± standard deviation (Mean ± SD). Values were considered statistically significant at $P \leq 0.05$.

Results

Mean concentration of heavy metal in leaf, stem, root, whole plant and soil and bioaccumulation factor of *Calopogoniun muconoides* in sample site 1

The results of HM concentration (in mg/kg) in leaf, stem, root and whole plant of *Calopogonium muconoides* as well as in soil sample in site 1 is presented in table 1. The results indicate that Fe in comparison with other metals and organs had the highest concentration (33.37 ± 0.218) in the root, while nickel was the least concentrated in the same part. Similarly, Zn recorded the highest concentration in the whole plant (41.065 ± 0.119), while Pb was the least concentrated (1.279 ± 0.043). Soil sample on the other hand recorded the highest amount of Zn (27.280 ± 0.256) with Pb presented the least concentration (1.567 ± 0.013). Thus the order of heavy metals concentrations in various tissue of the plant as follows: whole plants > leaf > stem > roots.

In the same vein, result of bioaccumulation of heavy metals in samples within site 1 presented in figure 1. The whole plants contained the highest accumulation of all the detected heavy metals ranging from 0.821 to 2.542 for Cr and Pb respectively. The leaf bioaccumulated metals in the range of 0.328 (Ni) to 1.516 for Cr. The results also show that the stem bioaccumulated more metals than the root.

Table1: Mean concentration (in mg/kg) of heavy metal in leaf, stem, root, whole plant and soil of *Calopogoniun muconoides* in sample site 1

Sample	Cd	Cr	Cu	Fe	Ni	Pb	Zn
Leaf	0.8843 ± 0.007 ^{a}	2.630 ± 0.033 ^{c}	14.821 ± 0.173 ^{c}	8.554 ± 0.187 ^{b}	0.7790 ± 0.002 ^{b}	1.102 ± 0.096 ^{b}	16.646 ± 0.232 ^{c}
Stem	0.8860 ± 0.004 ^{a}	1.746 ± 0.023 ^{b}	9.761 ± 0.094 ^{b}	5.230 ± 0.230 $^{`a}$	0.7733 ± 0.008 ^{b}	0.5147 ± 0.005 ^{a}	10.963 ± 0.067 ^{b}
Root	1.752 ± 0.046 ^{b}	0.9590 ± 0.075 ^{a}	4.945 ± 0.0060 ^{a}	33.368 ± 0.218 ^{e}	0.001033 ±0.001 ^{a}	0.5147 ± 0.005 ^{a}	5.472 ± 0.141 ^{a}
Whole Plant	4.450 ± 0.006 ^{d}	4.408 ± 0.036 ^{d}	19.574 ± 0.201 ^{e}	23.527 ± 0.195 ^{d}	1.576 ± 0.042 ^{c}	1.279 ± 0.041 ^{b}	41.065 ± 0.119 ^{e}
Soil	2.682 ± 0.024 ^{c}	1.734 ± 0.030 ^{b}	17.265 ± 0.048 ^{d}	10.170 ± 0.164 ^{c}	2.374 ± 0.050 ^{d}	1.557 ± .013 ^{b}	27.280 ± 0.256 ^{d}

Values are presented as Mean ± SD) for triplicate determinations. Values with different superscripts down the column are statistically significant at $P \geq 0.05$.

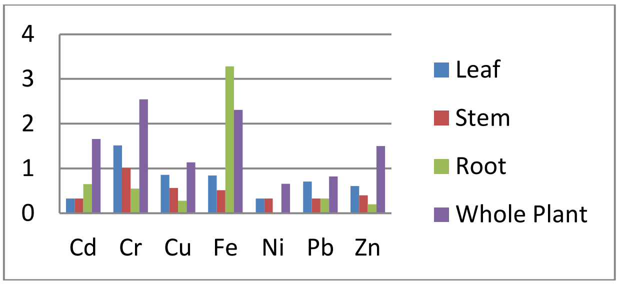

Figure 1: Bioaccumulation factors of heavy metal in leaf, stem, root and whole plant of *calopogoniun muconoides* in sample site 1

Mean concentration of heavy metal in leaf, stem, root, whole plant and soil and bioaccumulation factor of *Calopogoniun muconoides* in sample site 2

Results of heavy metal concentration (in mg/kg) in plant organs of *Calopogoniun Muconoides* as well as soil sample and bioaccumulation factors in these samples in control site 2 are presented in Table 2 and figure 2 respectively. The results show that the whole plant has the highest metal concentration in the range of 0.261 for Pb to 8.202 for Zn. Interestingly, whole plant also recorded the highest accumulation of all the metals as indicated by the bioaccumulation factors

Table 2: Mean concentration of heavy metal in leaves, stem, root, whole plant and soil of *Calopogoniun muconoides* in control site 2

Sample	Cd	Cr	Cu	Fe	Ni	Pb	Zn
Leaf	0.1770 ± 0.001 a	0.4197 ± 0.182 b	2.923 ± 0.030 c	1.648 ± 0.042 b	0.1557 ± 0.002 b	0.2067 ± 0.0012 b	3.220 ± 0.010 c
Stem	0.1683 ± 0.016 a	0.3573 ± 0.007 b	1.975 ± 0.010 b	1.015 ± 0.022 b	0.1553 ± 0.001 b	0.1040 ± 0.001 a	2.195 ± 0.005 b
Root	0.3527 ± 0.007 b	0.1757 ± 0.001 a	0.9550 ± 0.032 a	0.6697 ± 0.003 a	0.02467 ± 0.005 a	0.1037 ± 0.006 a	1.096 ± 0.006 a
Whole Plant	0.8910 ± 0.010 d	0.8840 ± 0.011 c	3.917 ± 0 .022 d	2.667 ± 0.014 c	0.3123 ± 0.003 c	0.2610 ± 0.001 b	8.202 ± 0.069 e
Soil	0.5397 ± 0.102 c	0.3543 ± 0.004 b	2.644 ± 0.001 c	2.007 ± 0.0035 c	0.4673 ± 0.002 c	0.3130 ± 0.001 c	5.454 ± 0.036 d

Data are presented as Mean ± SD for triplicate determinations. Values with different superscripts down the column are statistically significant at $P \geq 0.05$.

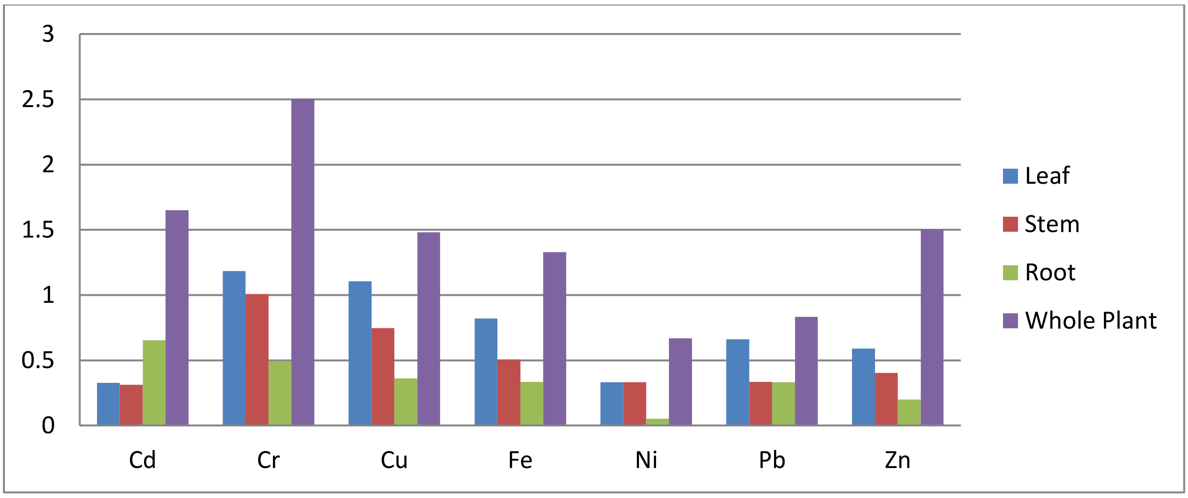

Figure 2: Bioaccumulation factors of heavy metals in leaves, stem, roots and whole plant of *Calopogoniun muconoides* in control site 2

Table 3: Mean concentration of heavy metal in leaves, stem, root, whole plant and soil of *Senna obtusifolia* in sample site 1

Sample	Cd	Cr	Cu	Fe	Ni	Pb	Zn
Leaf	3.577± 0.031 ^{d}	5.266 ± 0.149 ^{d}	17.322 ± 0.086 ^{d}	18.339 ± 0.095 ^{d}	2.363 ± 0.051 ^{c}	2.072 ± 0.058 ^{c}	30.159 ± 0.053 ^{d}
Stem	3.556 ± 0.056 ^{d}	0.8997 ± 0.028 ^{a}	2.534 ± 0.068 ^{a}	5.054 ± 0.060 ^{a}	0.000 ± 0.000 ^{a}	0.2773 ± 0.032 ^{a}	3.256 ± 0.051 ^{a}
Root	2.789 ± 0.173 ^{c}	3.539 ± 0.062 ^{c}	12.314 ± 0.057 ^{c}	13.337 ± 0.110 ^{c}	3.184 ± 0.005 ^{d}	1.575 ± 0.013 ^{b}	16.309 ± 0.181 ^{b}
Whole plant	1.775 ± 0.008 ^{b}	4.643 ± 0.063 ^{d}	19.820 ± 0.039 ^{e}	20.067 ± 0.055 ^{e}	4.529 ± 0.104 ^{e}	2.449 ± 0.053 ^{c}	39.287 ± 0.112 ^{e}
Soil	0.8983 ± 0.010 ^{a}	1.768 ± 0.037 ^{b}	7.417 ± 0.031 ^{b}	10.714 ± 0.044 ^{b}	1.783 ± 0.021 ^{b}	1.253 ± 0.053 ^{b}	25.301 ± 0.057 ^{c}

Data are presented as Mean ± SD for triplicate determinations. Values indicated by different superscripts down the column are statistically significant at $P \leq 0.05$.

Mean concentration of heavy metal in leaf, stem, root, whole plant and soil and bioaccumulation factor of *Senna obtusifolia* in sample site 1

The concentration of HM in the leaf, stem, root and whole plant of *Senna obtusifolia* as well as soil sample in site1 is presented in table 3 below. Results show that for the plant organs Zn showed the highest amount in leaf with concentration of 30.159 ± 0.053 (mg/kg), while Ni presented the least concentration. Metal concentration in plant organs was in the order: leaf > root > stem. While metal presented order of abundance as follows: Zn > Fe > Cu > Cr > Cd > Ni > Pb. Similarly, bioaccumulation of heavy metals for the *S. obtusifolia* plant in site 1 is presented in Figure 3. As indicated, the whole plant contained the highest amount of all heavy metals detected followed by plant parts in the order: leaf > stem > root. Bioaccumulation of metal follow the order: Cd > Cr > Cu > Ni > Fe > Zn, except that no detectable amount of nickel was found in the stem.

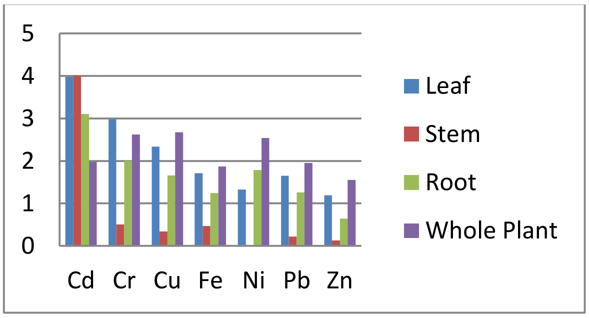

Figure 3: Bioaccumulation factors of heavy metals in leaves, stem, roots and whole plant of *Senna obsitufolia* in sample site 1

Mean concentration of heavy metal in leaf, stem, root, whole plant and soil and bioaccumulation factor of *Senna obtusifolia* in control site 2

Results of mean concentration of HM in the leaf, stem, root, whole plant and soil of *S. otusifolia* in control site 2 is presented in table 4. The results showed Zn to be the highest concentrated metal (6.051 ± 0.012) followed by Fe (3.714 ± 0.065) while Ni was the least in the plant organs. The order of metal abundance in control site differs from that of sample site 1 having the order: leaf > root > stem. For whole plant, Pb was the least in concentration (0.807 ± 0.001) while Zn was the most concentrated. For bioaccumulation result shown in figure 4, the leaf had the highest bioaccumulation factor than any other plant organ of *S. otusifolia*, while the stem recorded the least bioaccumulation factor. Whole plant generally had the most bioaccumulation factor and had Zn as the metal with the highest bioaccumulation factor as represented on the graph.

Table 4: Mean concentration of heavy metal in leaves, stem, root, whole plant and soil of *Senna obtusifolia* in control site 2

Sample	Cd	Cr	Cu	Fe	Ni	Pb	Zn
Leaf	0.7190 ± 0.010^{b}	1.052 ± 0.001^{c}	3.438 ± 0.009^{c}	3.714 ± 0.065^{c}	0.4603 ± 0.009^{b}	0.4603 ± 0.009^{b}	6.051 ± 0.012^{d}
Stem	0.7133 ± 0.002^{b}	0.1747 ± 0.002^{a}	0.4910 ± 0.002^{a}	1.004 ± 0.001^{a}	0.006667 ± 0.003^{a}	0.2097 ± 0.270^{a}	1.645 ± 0.010^{a}
Root	0.5293 ± 0.015^{a}	0.5300 ± 0.015^{b}	2.453 ± 0.015^{b}	2.665 ± 0.002^{b}	0.6277 ± 0.016^{c}	0.2443 ± 0.108^{a}	3.318 ± 0.085^{c}
Whole Plant	0.9583 ± 0.004^{c}	1.527 ± 0.006^{c}	3.962 ± 0.034^{c}	3.802 ± 0.004^{c}	0.9160 ± 0.004^{d}	0.8070 ± 0.001^{c}	7.849 ± 0.005^{e}
Soil	0.8763 ± 0.002^{c}	0.7547 ± 0.003^{b}	1.463 ± 0.013^{b}	1.378 ± 0.001^{a}	0.6623 ± 0.010^{a}	0.9040 ± 0.001^{c}	2.742 ± 0.007^{b}

Data are presented as Mean ± SD for triplicate determinations. Values indicated by different superscripts down the column are statistically significant at $P \leq 0.05$.

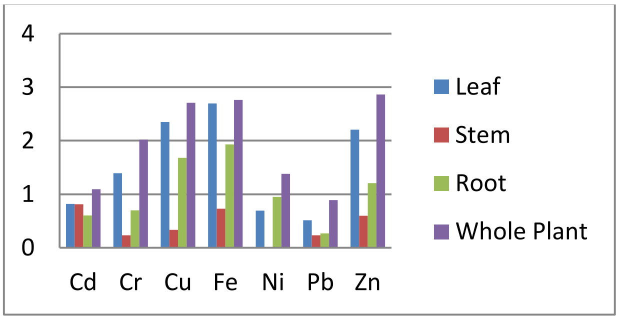

Figure 4: bioaccumulation factors of heavy metals in leaves, root, stem and whole plants in control site 2

Discussion

The results obtained from the atomic absorption spectrometry experiments show that there is a varied metal concentration among the selected sites, plant organs and even among the metals analyzed. The findings revealed that metal concentrations in site 1 was significantly different from site 2, which is not unconnected with the activities carried out in the later relative to the former. Auto-mechanic workshop is usually heavily polluted with engine oils which contain many of these heavy metals as well as metal scraps which have consequently resulted in metal enrichment at this site. These results are in consonance with other reported pollution of auto mechanic sites by heavy metals (Adebayo *et al.*, 2018). Certain factors may influence continued metal availability, including pH, soil texture, soil organic matter, redox potential of metals and root zone as noted by previous findings (Laghlimi *et al.*, 2015). The observation that Zn, Fe, Pb and Cu appeared in higher concentrations regardless of the site be it may be due to the fact that those metals are often used for coating/plating of other metals and used generally due to their ductility (Oguntimehin *et al.*, 2013). The presence of lead in control site may be the result of contamination arising from car exhaust or leaded fuels characteristics of such a residential complexes with many vehicles. Contrary to the findings in this study that Zn was generally noted to be more concentrated in the auto-mechanic workshop site, other report observed that Zn was the least contaminated metal (Abba & Ibrahim, 2017). This difference in concentration from similar sample site may be the result of variations in soil organic matter and redox potential of metals as properties that have been previously suggested to affect levels of metals in soils (Kashem & Singh 2001).

Our investigation revealed that *Calopogoniun moconoides* bioaccumulated heavy metals particularly Cd, Cr and Zn to a significant level in site 1 evidenced by comparatively higher bioaccumulation factor values. Phytoremediating potential of *C moconoides* have previously been documented even with higher values (Kos *et al.*, 2003). The ability of this plant to thrive in the heavily polluted environment attests to the fact that it may be a good metal accumulator. In the plant organs (leaves, stems and roots), leaves were generally observed to be more concentrated with metals more than the other organs with the stem being the least in HM concentration. This is an expected trend for most plants due to the uptake of metal contaminated fluids and nutrients (Matavos-Aramyan, and Moussavi, 2017). In the same vein, our results revealed that *S. obsitufolia* accumulated heavy metals particularly in the "above ground level" parts of the plant. Comparatively, *C. muconoides* accumulated more Fe in the roots than *S. obsitufolia* in the same site 1. Based on the observed patterns of bioaccumulation exhibited by *S. obsitufolia* and *C. muconoides*, coupled with previous suggestions, *S. obsitufolia* may have utilized phytoextraction mechanism while *C. muconoides* may have utilized phytostabilization and/or rhizofiltration as mechanisms of remediating soils of HM contamination. The mechanism of phytoremediation utilised by a plant may also determine longevity of that plant on a soil regardless of the pollutant in the soil (Islam *et al.*, 2012).

Conclusion

Findings from the present study corroborated previous reports that soil from auto-mechanic workshop are enriched with heavy metals of varying concentrations. Additionally, sample plants (*Calopogoniun Muconoides and Senna obtusifolia*) possess the capacity for phytoremediation evidenced by their bioaccumulation of investigated metals at the various organs, possibly through phytoextraction and phytostabilisation mechanisms. Thus, *Calopogoniun Muconoides and Senna obtusifolia* are good candidates for bioremediation. Further studies would be carried out to determine transcriptomics/proteomics of these plants with the view to identifying molecules that bestow in them the quality of being effective phytoremediating agents in this growing practice of bioremediation.

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