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Bioaccumulation of Cadmium in Siam (*Chromolaena odorata*) and Node (*Synedrella nodiflora*) Weeds: Impact of Ethylene Diamine Tetraacetic Acid (EDTA) on Uptake

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Abstract

The translocation and accumulation of Cd by *Synedrella nodiflora* and *Chromolaena odorata* plants growing in artificially Cd-contaminated soils amended with EDTA or without amendment had been studied to assess the phytoremediation potential of both species. Results showed that roots of *S. nodiflora* had the capacity of taking up a maximum 86.2 mg/kg of Cd from the contaminated soil while concentration in shoots amounts to a maximum 73.8 mg/kg. *C. odorata* was able to accumulate 42.8 mg/kg and 33.8 mg/kg in its roots and shoots respectively. The mobility of soil cadmium and the concentration of Cd in plants were both increased by EDTA amendment. EDTA application facilitated the translocation of Cd since the concentration in the roots and shoots of *S. nodiflora* increased to 104.9 mg Cd/kg and 77.0 mg Cd/kg respectively although the amendment effect was more pronounced on *C. odorata* increasing from 42.8 mg Cd/kg in roots from non-amended soils to 83.7 mg Cd/kg in the roots from amended soils. Despite the improved uptake by *C. odorata*, *S. nodiflora* appears to be more suitable for phytoremediation of Cd contaminated soils.

Keywords: *S. nodiflora*, *C. odorata*, Cadmium (Cd), Ethylene diamine tetraacetic acid (EDTA)

1. Introduction

Heavy metal pollution of soil is a widespread global problem that threatens the safety of ecosystems and human health (Evangelou et al., 2007). Industrial activities has brought attention about a dramatic acceleration of heavy metal contamination in soil (Nriagu, 1979) through practices such as mining and smelting of metalliferous ores, electroplating, gas exhaust, energy and fuel production, fertilizer and pesticide application and generation of municipal waste (Kabata-Pendias & Pendias, 1989). Amongst the various heavy metals in the environment, Cd, is of great concern because of its toxicity to plants, animals and humans.

In plants, symptoms of Cd toxicity include for example, lack of root lignification, reduction in chlorophyll *a* concentration and severe mortality (Reboredo, 2001), reduction of shoot and root weight and chlorophyll's concentration (Chen et al., 2011) with decline in the net photosynthetic rate and stomatal conductance, and finally increase of MDA (malondialdehyde) as the final product of peroxidation of membrane lipids and increase of the non-protein thiols phytochelatins and glutathione in both roots and leaves of rice (Yu et al., 2013). In animals Cd may be responsible for damage to the lungs and nasal cavity when inhalation occurs, or anemia, liver disease, nerve or brain damage when eating or drinking Cd (ATSDR, 2012).

Heavy metals can enter human diet and accumulate gradually in the human body (Zhu et al., 2003; Rubio et al., 2006) resulting in a number of adverse health effects, such as nephrotoxicity and osteotoxicity (WHO, 1992; Li et al., 2006). Thus, there is an urgent and imperative need to develop efficient techniques for cadmium removal from the environment.

Phytoremediation of heavy metals that are usually persistent in the environment is a low-cost and environmentally friendly alternative to the chemical methods and therefore has attracted much interest over the years (Baker & Brooks, 1989; Blaylock, 1997). It involves the use of plants to accumulate and remove pollutants from the environment. However, a major constraint of this technique is the poor availability of metals in soil

solution. In the case of Cd, the pH is the most important soil factor that influences its availability for possible uptake by plants. Lowering the pH will favour the availability of the metal.

Complexation of metals by organic complexing agents plays an important role in controlling metal solubility (Reuter & Purdue, 1977; Naidu & Harter, 1998). Ethylene diaminetetraacetic acid (EDTA) has been reported as a chelating agent highly efficient at increasing the solubility of heavy metals in soil solutions from the solid phase (Blaylock et al., 1997; Huang et al., 1997; Ebbs et al., 1997; Wu et al., 1999).

The potential of *S. nodiflora* and *C. odorata* for the phytoremediation of Pb-contaminated soil in Nigeria had been reported by Aiyesanmi et al. (2012) showing that *S. nodiflora* has a higher potential for phytoremediation . However, plants are metal specific in their hyper-accumulation ability (Baker & Brooks, 1989). Hence, this research work aims at investigating the tolerance and accumulation capability of these widely spread weeds in Nigeria to Cd. Also, the effect of EDTA as an enhancement for the phytoremediation potential of these plants had been investigated. The capability of these plants to co-exist without suppressing each other prompted their study under similar experimental parameters.

2. Materials and Methods

2.1 Soil sampling and Characterization

Soil samples used in these experiments were obtained from a Cd-uncontaminated area within the premises of Federal University of Technology, Akure, Nigeria. Upon collection, the soil was air-dried for three days followed by sieving through a 2 mm mesh sieve. 0.1 g of the sieved soil was weighed and digested using concentrated aqua regia. The background Cd concentration was determined using Atomic Absorption Spectrophotometer (Buck scientific, 210 VGP). The soil pH was determined in a mixture of soil and deionized water (1:2, w/v) with a glass electrode (McLean, 1982). Total nitrogen was determined using the Kjeldhal method (Bremner, 1982). Total organic carbon content by Walkey-Black wet oxidation approach (Nelson, 1982). Cation exchange capacity (CEC) was determined using the ammonium acetate method (Rhoades, 1982) while amounts of exchangeable Ca and Mg were determined by EDTA titration. Total Phosphorus was determined colorimetrically and the soil particle size was measured using the hydrometer method. All reagents used were of analytical grade (BDH Laboratory supplies, Poole, England).

2.2 Pot Experiments

Seedlings *C. odorata* and *S. nodiflora* were transplanted into a total of 85 pots containing soil sample which had been treated with 50 mg/kg NPK fertilizers and allowed to stabilize for about 2 weeks after which they were thinned to one plant per pot. Five out of these pots were used as the control treatment; 40 pots for Cd treatment alone; and 40 pots for Cd + EDTA treatment. The concentrations of 50, 100, 200, 500 and 1000 ppm for both cadmium and EDTA were used. The source of the cadmium was cadmium nitrate. The pots with the plant samples were placed in a screen-house where they are exposed to approximately 12 hours of daylight. Experiments were conducted for a period of 4 weeks.

2.3 Plant Harvest and Analysis

Plants on treated and untreated contaminated soil were harvested in duplicates on a weekly basis. Prior to analysis, the plant shoot cutted at the soil surface was harvested, then the soil was broken up and the roots were carefully removed by hand. The shoots and roots were then washed with deionized water, oven dried at 70 ^{o}C for 24 hours, weighed and ground to fine powder. Aliquots of plant powder (0.5 g) were digested overnight in 5 ml 69% HNO_3 and 10 ml 30% H_2O_2 and later heated at 120 ^{o}C for 2hours (Mench et al., 1994). The digested solutions were filtered using Whatman No. 1 filter paper and diluted to 50ml with deionized water. The concentrations of cadmium in the digested solutions were determined using Atomic Absorption Spectrophotometer (Buck Scientific, 210 VGP). Replicates of data obtained were expressed as means with standard deviation and subjected to one-way ANOVA at $p \leq 0.05$ using SPSS 17 software.

3. Results and Discussion

3.1 Soil Physicochemical Properties

The result from the analysis of the soil used in this study is as shown in Table 1.

Table 1. Soil physicochemical property

pH	Texture (%)			OM	CEC	P	N	Ca/Mg	EA	Cd
	Sand	Clay	Loam	(%)	(cmol/kg)	(%)	(%)		(%)	(mg/kg)
6.38	62.24	21.76	14.0	5.41	14.20	0.07	9.08	5.5	2.08	1.43

According to Barančíková et al. (2004), pH and organic matter are two of the most important soil factors that control Cd bioavailability, although Reboredo (1992) has showed that neither the organic matter nor the finest fraction of the soil constituted important binding sites for Cd, thus the bioavailability is not dependent of these factors. Studies conducted by Kuo et al. (2004); Sappin-Didier et al. (2005); Tsadilas et al. (2005) showed that as pH decreases (acidity increases), the amount of Cd in their study plants increases due to increased bioavailability of the metal. Furthermore a linear trend between soil pH and Cd uptake by plants was reported (Tudoreanu and Phillips, 2004). The pH value of the study soil will not favour the bioavailability of Cd in soil solution for possible uptake by plants. The value of organic matter (5.04%), nitrogen (9.08%) and phosphorus (0.07%) recorded indicate that the soil used is fertile for planting. The soil particle size obtained was 62.24% sand, 21.8% clay and 14% silt. The classification on the textural triangle is Sandy-Clay-Loam. The Cation Exchange Capacity (CEC) value of 14.2 cmol/kg is relatively low due to the high proportion of sand in the soil matrix.

3.2 Plant Tolerance to Cadmium Contamination and Amendment Treatments

No visible symptoms of Cd toxicity in *S. nodiflora* plants growing in soils contaminated with 1000 ppm Cd were observed during the first week. However, this does not mean that the stress effects were absent. Reboredo (2012) clearly showed that the stress response to Zn, began at the cellular level without immediate translation at the morphophysiological levels, the unusual number of starch grains in the chloroplasts of *Halimione portulacoides* being the first signs of an abnormal metabolic mechanism. Thus, a similar situation may well occur in our study.

At the end of the second week after initial contamination with 1000 ppm Cd plus EDTA, leaves become yellowish which indicates phytotoxicity This may be due to increased bioavailability of Cd in soil due to the EDTA amendment. The plants however were able to survive the stress throughout the study period suggesting that they adopted a means of Cd detoxification which may be by exudation (Lasat, 2000).

At the end of the first week, a yellow color appeared on the leaves of C. odorata plants growing in 1000 ppm Cd-contaminated soil amended with EDTA. The growth of the plant, however, was not hindered and the coloration disappeared by the end of the 3rd week. This observation implies that Cd contamination at the concentration range considered in this study has no significant effect on *C. odorata* but higher concentrations could be harmful to the plant.

Plants (*C. odorata* and *S. nodiflora*) growing on soils not treated with EDTA did not show any sign of Cd phytotoxicity, regardless the different Cd concentration used. This may be due to the lower bioavailabilty of Cd in the soil solution compared to that of EDTA amended soil which is expected to be higher (McBride, 1994).

3.3 Cadmium Uptake Into Plant Parts

Tables 2a and 2b show the concentration of Cd in the roots and shoots of plants from Cd-contaminated soils but without EDTA.

As shown in Table 2a, the uptake of Cd into the roots of plants on untreated contaminated soil increases significantly ($P \leq 0.05$) as contaminant concentration increases. For *C. odorata*, the highest uptake of Cd in the root was 42.8 ± 0.18 mg/kg after the fourth week of contamination while *S. nodiflora* gave an uptake of 86.25 ± 1.91 mg/kg which is 101.5% higher than that of *C. odorata*.

Table 2a. Uptake (mg/kg) of Cd in the roots of plant on untreated contaminated soil

Plant	Concentration of Cd contaminant				
	50 ppm	100 ppm	200 ppm	500 ppm	1000 ppm
Week 1					
S. nodiflora	5.25 ±0.32a	6.73 ±0.43b	9.99 ±0.30c	18.10 ±0.42d	25.25 ±0.64e
C. odorata	2.10±0.41a	7.90 ±0.33b	9.0±0.42b	9.2±0.57b	26.05±1.27c
Week 2					
S. nodiflora	7.23 ±0.28a	9.35 ±0.71ab	15.96±3.53ab	23.4 ±1.56b	43.10 ±12.16c
C. odorata	3.80 ±1.12a	8.82±0.57b	9.30±0.3b	9.00 ±2.83b	28.80 ±0.43c
Week 3					
S. nodiflora	10.79 ±0.64a	21.25 ±0.49b	41.29 ±0.48c	43.60 ±0.28c	77.63 ±1.80d
C. odorata	6.80±3.51a	8.90±0.35a	7.90±0.51a	11.21 ±0.85a	34.40±2.56b
Week 4					
S. nodiflora	12.11 ± 1.53	28.20 ±2.12b	47.75 ±8.2c	46.86 ±1.27c	86.25 ±1.91d
C. odorata	7.21 ±0.01a	7.30±0.52a	10.60±0.61b	18.90 ±0.51c	42.80 ±0.18d

Values with different lowercase letters (a-e) along the rows are significantly different from each other using LSD test ($P \leq 0.05$).

Table 2b shows the Cd concentration in the shoots of the plants. *C. odorata* absorbed 33.8 ± 0.79 mg/kg of Cd while *S.nodiflora* had 73.85 ± 2.09 mg/kg Cd absorbtion which is 118% higher than that of *C. odorata* but 26.15 % lower than the Cd uptake of over 100mg/kg that has been recorded by *Thlaspi* genus, the widely known Cd hyperaccumulator (Kirkham, 2006). In both plants, concentration of Cd were considerably higher in roots than in shoots which conform with previous reports on linseed, sunflower amongst others (Bjelková et al., 2011; Elkhatib et al., 2001). This pattern is also similar to that reported by Aiyesanmi et al. (2012) for the phytoremediation of Pb - contaminated soil using *T. triangulare*, *S. nodiflora* and *C. odorata*.

Table 2b. Uptake (mg/kg) of Cd in the shoots of plant on untreated contaminated soil

Plant	Concentration of Cd contaminant				
	50 ppm	100 ppm	200 ppm	500 ppm	1000 ppm
Week 1					
S. nodiflora	3.76 ±0.39a	3.88 ±0.39a	7.56±0.31ab	11.79±2.70ab	15.05 ±6.29b
C. odorata	1.8±0.42a	5.5±1.13b	7.1±0.14bc	8.1±0.57c	17.10±0.85d
Week 2					
S. nodiflora	5.72 ±0.88a	7.87 ±0.79a	9.50 ±1.56a	14.20 ±0.42b	32.28 ±2.58c
C. odorata	2.3±1.36a	6.1±0.37b	8.61±0.64c	8.93±0.20c	21.70 ±0.95d
Week 3					
S. nodiflora	10.39 ±0.57a	10.9 ±0.22a	25.30 6.22ab	29.95 ±0.21b	57.12 ±12.94c
C. odorata	5.31±0.17a	6.8±0.37b	10.9±0.51bc	8.5±0.65c	18.8±0.33d
Week 4					
S. nodiflora	10.36 ±0.71a	17.2 ±0.57b	32.60 ±1.41c	37.32 ±4.10c	73.85 ±2.09d
C. odorata	6.1±0.37a	7.0±0.57ab	12.5±0.92b	16.6±0.49c	33.8±0.79d

Values with different lowercase letters (a-e) along the rows are significantly different from each other using LSD test ($P \leq 0.05$).

In addition, there was a steady increase in the uptake of Cd by the plants regardless the various concentrations used, as the exposure time increases. Although this study was conducted over a period of 4 weeks, there is a possibility of higher uptake of the metal over more weeks since the plant were able to tolerate the stress initially experienced after contamination.

3.4 Uptake by Plants on Cd-Contaminated, EDTA-Treated Soil

According to (Nanda-Kumar et al., 1995), the low solubility of Cd in soil often constitutes a limiting factor for phytoextraction from a contaminated soil. Ethylene diamine tetraacetic acid (EDTA) is probably the chelating agent that is most efficient at increasing the solubility of heavy metals in soil solutions by desorbing the metals from the solid phase and form stable complexes with them (Blaylock, 1997; Huang, 1997; Ebbs, 1997; Wu et al., 1999). Table 3a and 3b show the concentration of Cd in the roots and shoots of plants grown on soils treated with EDTA. There was a significant increase in the uptake of Cd into the plant parts. The highest absorbtion of Cd was recorded on the 1000 ppm contaminanted soil at the fourth week.

Table 3a. Uptake (mg/kg) of Cd in the roots of plant on EDTA treated contaminated soil

Plant	Concentration of Cd contaminant				
	50 ppm	100 ppm	200 ppm	500 ppm	1000 ppm
Week 1					
S. nodiflora	8.18±0.11a	10.01±0.43a	14.68±0.51a	30.53±5.48b	42.98±0.74c
C. odorata	4.21±0.63a	9.8±1.25ab	12.49±3.52ab	18.32±6.11b	38.12±5.09c
Week 2					
S. nodiflora	9.95±0.15a	21.6±8.49a	34.12±0.99b	47.01±4.74c	56.92±3.38c
C. odorata	5.80±0.65a	12.10±3.96ab	16.83±5.09ab	22.06±5.01b	49.11±6.11c
Week 3					
S. nodiflora	12.15±1.27a	24.65±1.63a	48.35±0.78b	51.95±2.33b	89.72±2.99c
C. odorata	6.90±5.09a	15.21±1.30ab	20.11±5.04ab	28.26±5.09b	61.16±8.78c
Week 4					
S. nodiflora	15.08±1.91a	32.04±3.11b	53.6±5.37c	57.3±0.57c	104.9±7.07d
C. odorata	7.9±1.25a	23.1±3.96b	28.6±0.65bc	36.70±8.78c	83.70±5.09d

Values with different lowercase letters (a-e) along the rows are significantly different from each other using LSD test ($P \leq 0.05$).

In fact 104.9 ppm Cd was measured in the root of *S. nodiflora* an increase of 21.6% over the concentration recorded for the roots collected from untreated (without EDTA) contaminated soils. Conversely, the concentrations of Cd in the *S. nodiflora* shoots collected from Cd-contaminated soils and Cd-contaminated soils plus EDTA were very similar suggesting that the translocating of Cd from roots to shoots in *S. nodiflora* is poor which may be due to efficient immobilization mechanisms in the below-ground organs, which agrees with Aiyesanmi et al. (2012) who verified that *S. nodiflora* exhibited a lower translocation of Pb when compared to *C. odorata*. Similarly for *C. odorata*, the highest uptake in the root was recorded for the plant on 1000 ppm contaminated soil treated with EDTA. The uptake of Cd into its root increased from 42.8 ± 0.18 mg/kg (untreated soil) to 83.7 ± 5.09 mg/kg representing an increase of 95.6% while in the shoot an increase of 103.9% was recorded. The increase in uptake was due to the ability of EDTA to increase the bioavailability of Cd in soil solution. In addition, the EDTA significantly increased the uptake of Cd more in *C. odorata* than in *S. nodiflora*. This is evident in the yellowing of leaves of *S. nodiflora* on 1000 ppm contaminated soil treated with EDTA after two weeks of contamination while *C. odorata* on the same treatment recovered from the initial stress observed and survived throughout the study period.

Table 3b. Uptake (mg/kg) of Cd in the shoots of plant on EDTA treated contaminated soil

Plant	Concentration of Cd contaminant				
	50 ppm	100 ppm	200 ppm	500 ppm	1000 ppm
Week 1					
S. nodiflora	5.5±0.23a	6.85±0.78ab	8.49±0.79b	19.37±0.60c	24.90±1.27d
C. odorata	3.88±1.30a	9.41±0.79a	11.60±5.09a	16.10±6.11a	35.21±13.98b
Week 2					
S. nodiflora	7.28±1.80a	11.57±2.51a	13.12±1.41a	22.72±3.72b	28.5±6.36b
C. odorata	4.60±0.79a	10.11±6.11a	14.01±2.83a	18.90±6.11a	42.25±8.78b
Week 3					
S. nodiflora	9.83±2.02a	11.80±0.11ab	17.25±0.41b	29.12±1.56c	58.03±4.10d
C. odorata	5.6±3.96a	13.35±5.09ab	18.70±5.02ab	23.98±0.79b	58.71±6.11c
Week 4					
S. nodiflora	11.35±1.27a	24.16±8.86b	34.25±0.91bc	38.47±0.98c	77.02±0.99d
C. odorata	6.11±0.82a	18.72±5.08ab	25.28±8.78b	29.86±0.45b	68.93±6.14c

Values with different lowercase letters (a-e) along the rows are significantly different from each other using LSD test ($P \leq 0.05$).

3.5 Phytoremediation Potential

The success of phytoremediation greatly depends on the ability of a plant to absorb a certain contaminant into its root and subsequent translocation to the above ground parts where they can be easily harvested (Nanda-Kumar et al., 1995). The potentials of these plants for phytoremediation had been investigated using the bioaccumulation factor (BF) and translocation factor (TF). The bioaccumulation factor is the ratio of the metal concentration in the shoot to that in the soil while the translocation factor is the ratio of metal in the shoot to that in the root.

3.5.1 Bioaccumulation Factor

Of the two study plants, *S. nodiflora* gave a higher BF ranging from 0.52-0.84 across the various contaminant concentration for soil not treated with EDTA as shown in figure 1a while *C. odorata* had a BF ranging from 0.23-0.44. This further establishes the higher potential of S. nodiflora as a phytoremediator of Cd-contaminated soil over *C. odorata*. For EDTA-treated soil (Figure 1b), the BF of *S. nodiflora* was 0.57-0.91 while that of *C. odorata* ranges from 0.45-0.86. This corresponds to an increase of 8.8% in the BF of *S. nodiflora* while that observed in *C. odorata* is 92.6%. This shows that EDTA treatment favours the increased uptake of Cd by *C. odorata* more than *S. nodiflora* which may be due to the higher tolerance of *C. odorata* to the EDTA-metal complex that is formed.

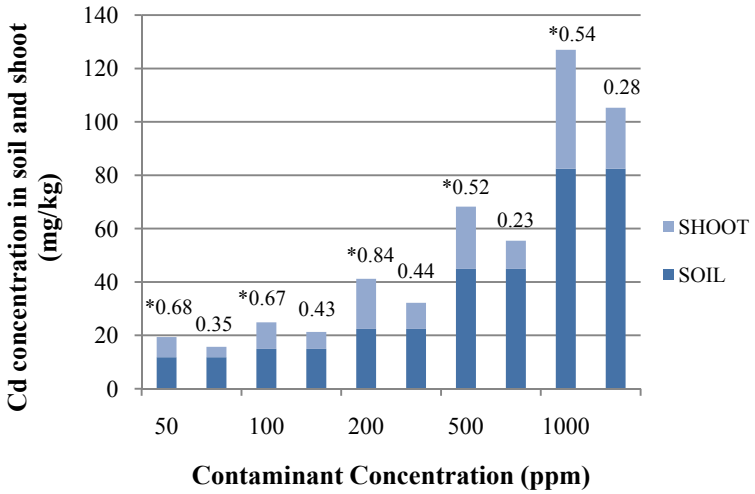

Figure 1a. Bioaccumulation factor (BF) of *S. nodiflora* and *C. odorata* on untreated soil

* The first bar under each contaminant concentration represent the BF of *S. nodiflora* while the second bar represents the BF of *C. odorata*.

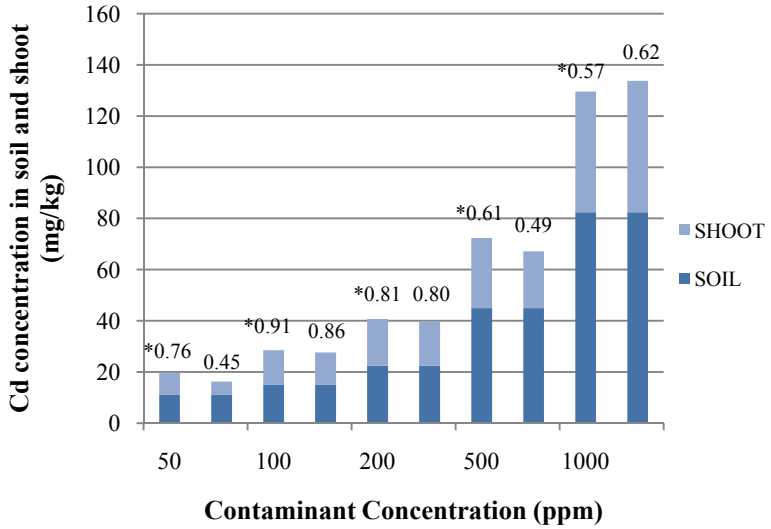

Figure 1b. Bioaccumulation factor (BF) of *S. nodiflora* and *C. odorata* on EDTA-treated soil

* The first bar under each contaminant concentration represent the BF of *S. nodiflora* while the second bar represents the BF of *C. odorata*.

3.5.2 Translocation Factor

S. nodiflora, although gave a higher accumulation of Cd, demonstrated a lower ability to translocate the metal from the root to shoot. Figure 2a shows the TF of plants on contaminated soil not treated with EDTA. *S. nodiflora* gave a TF ranging from 0.61-0.85 while *C. odorata* recorded a higher TF from 0.69-0.94. A hyper-accumulator plant should demonstrate a TF or shoot: root ratio 1 (Baker & Brooks, 1989). The plants in this study, particularly *C. odorata*, have performed fairly close to this standard. The lower TF of *S. nodiflora* compared to *C. odorata* for the phytoremediation of Pb had also been reported (Aiyesanmi et al., 2012).

Furthermore, treatment with EDTA did not change the order of the TF as *C. odorata* gave a higher value of 0.81-0.89 while *S. nodiflora* had 0.49-0.75 (Figure 2b) which is quite a reduction over that recorded for it on the untreated soil. It thus appears that despite the increased bioavailability of the metal by amending the soil with EDTA, it had a retrogressive effect on the potential of *S. nodiflora* as a phytoremediator of Cd which may be as a result of the toxicity of the EDTA-metal complex to the plant.

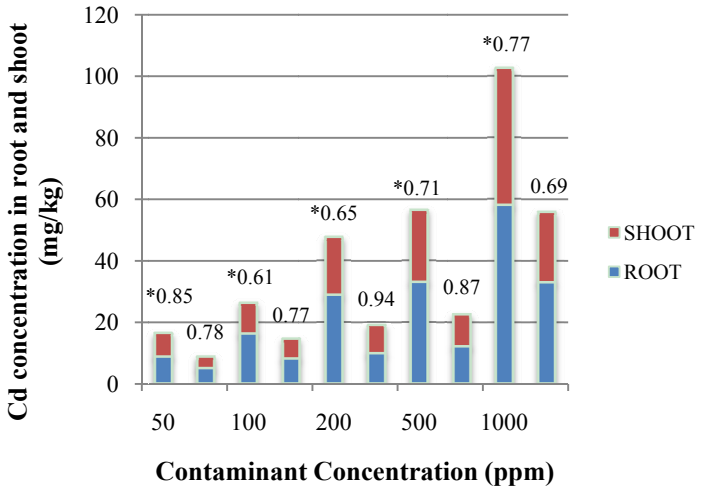

Figure 2a. Transfer factor (TF) of *S. nodifora* and *C. odorata* on untreated soil

* The first bar under each contaminant concentration represent the TF of *S. nodiflora* while the second bar represents the TF of *C. odorata*.

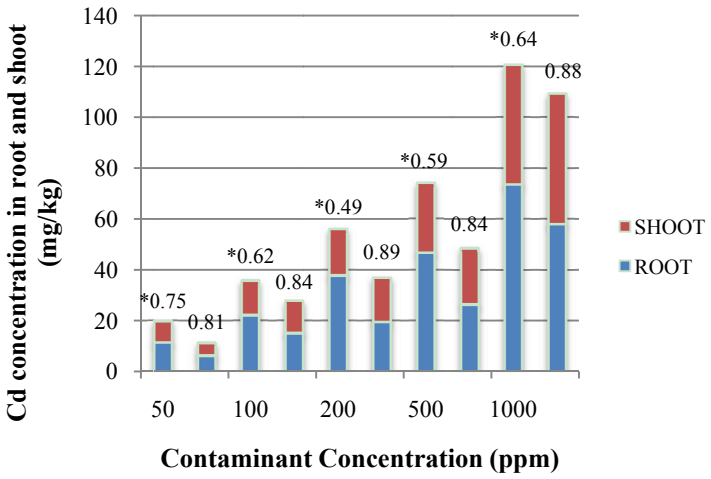

Figure 2b. Transfer factor (TF) of *S. nodiflora* and *C. odorata* on EDTA-treated soil

* The first bar under each contaminant concentration represent the TF of *S. nodiflora* while the second bar represents the TF of *C. odorata*.

4. Conclusion

The plants investigated in this study showed a high potential to bioaccumulate Cd without compromising apparently, a balanced development. In Cd-contaminated soils without EDTA amendment *S. nodiflora* exhibited the highest absorption rates with 86.25 mg Cd/kg in the roots and 73.85 mg Cd/- kg in the shoots while *C. odorata* recorded a lower uptake of 42.8 mg Cd/kg and 33.8 mg Cd/kg in its roots and shoots respectively. Amendment with EDTA gave us an enhanced uptake of 104.9 mg Cd/kg in the roots and 77.0 mg Cd/kg in the shoot of *S. nodiflora* corresponding to an increase of 21.6% and 4.29% in the roots and shoots respectively. EDTA amendment, appears to favour *C. odorata* more since an increase in Cd concentration of approximately 100% in plant organs. However, it seems to us that *S. nodiflora* is the best suitable and a promising plant in the phytoremediation of Cd-contaminated soil since it was able to achieve a high uptake of Cd into its tissues without EDTA amendment.

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