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Potentials of *Synedrella nodiflora* and *Sida fallax* growing naturally around an abandoned railway station for phytoextraction of heavy metals

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Phytoextraction is a non-destructive, cost-effective and safe alternative to conventional cleanup techniques of contaminated soils. In this study, the ability of two plants species *Synedrella nodiflora* and *Sida fallax* growing naturally around the abandoned Asafo Railway Station in Kumasi for selective extraction and accumulation of four heavy metals (Pb, Cu, Fe and Zn) were studied using Atomic Absorption Spectroscopy. The degree of translocation of these metals by the plants was also calculated. High translocation indices greater than one in the case of Fe and Zn were recorded in *S. nodiflora* and *S. fallax*, respectively. However Cu and Pb examined in this study were not effectively extracted nor transported within the plants, hence these plants are excluders of Cu and Pb. The soil plant transfer coefficient for both plant species were calculated for each metal. The highest translocation coefficient value in this study for both plants was recorded for Zn. The translocation coefficient values for Pb and Cu were not significant in both plants, hence these plants can be described as Pb and Cu excluders but potential candidates for Fe bioaccumulation using phytoextraction.

Key words: Contaminated soil, heavy metals, phytoextraction, transfer coefficient, translocation index.

INTRODUCTION

There is a great worldwide interest surrounding issues of soil contamination by a large range of pollutants including heavy metals (Body et al., 1991). Heavy metals from natural and anthropogenic sources are continuously being released into the environment, and they are a serious threat because of their toxicity, long persistence, bioaccumulation, and biomagnification in food chain (Papagiannis et al., 2004). Because soil lies at the confluence of many natural systems, soil pollution can easily be spread to other parts of the environment. Water that percolates through the soil can carry the soil pollutants into streams, rivers, wells and groundwater. Plants growing on polluted soil may absorb and

accumulate these pollutants into their tissues and can pass these pollutants on to animals and humans that depend directly or indirectly on these plants for food. Dust blown from polluted soil can also be inhaled directly by passersby. Again contaminated soils especially in areas of human settlements have been identified as a significant source of metal exposure to children (McKone and Daniels, 1991; Sheppard, 1995; Lanphear and Roughmann, 1997; Mielke and Reagan, 1998; Mielke et al., 1999; 2005). Children are known to be more susceptible to the potential negative health effects of metals in soil than adults, both for physiological and behavioral reasons (Schütz et al., 1997; ATSDR, 2000). Soil ingestion has also been recognized as an equally important exposure route for contaminants in children as water and food ingestion (McKone and Daniels, 1991), especially for children up to the age of six due to their hand-to-mouth behavior (Mielke and Reagan, 1998). It

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is therefore particularly important that soil contents of potentially harmful substances are kept low especially in areas frequented by children.

In response to these negative effects, there has been ongoing development of a variety of technologies to remediate contaminated soils (Conservation Currents, 2004). The clean-up of metal-polluted soils is thus of great interest economically as well as for the protection of human and environmental health. Current methods of remediating heavy metal contaminated soils which include "dig-and-dump" or encapsulation (Bauddh and Singh, 2012; Mai et al., 2003) and washing of soil with chemicals, are very expensive and have adverse effect on soil properties such as soil fertility and texture (Salt et al., 1995). The development of cost-effective technologies for cleanup or stabilization of metal-contaminated soils to replace the traditional but expensive technologies such as excavation and soil washing is now of great interest (Chaney et al., 1997; Raskin et al., 1994). This has resulted in the development of a plant-based technology (phytoremediation) which is relatively cheaper and environmentally friendly, to remediate heavy metal contaminated soils.

Phytoremediation is a term used to describe the technologies that use plants to clean up contaminated sites. Some of the techniques and applications represented under phytoremediation are phytodegradation, phytoextraction and phytovolatilization. This report describes the technique of phytoextraction, which employs the ability of certain plants to absorb heavy metals from soil by their roots and transport them to other parts where they are permanently stored (Barbafieri et al., 2011; Chaney et al., 1997; Raskin et al., 1994). The success of a phytoextraction process depends on the identification of suitable species that hyperaccumulate trace elements and produce large amount of biomass using established crop production and management practices (Jadia and Fulekar, 2009; Das and Maiti, 2007; Raskin et al., 1994). There are many evidences that plants grown on metal enriched soils can accumulate high amount of metals in their tissues. Examples of such plants are: *Thlaspi caerulescens* (Maxted et al., 2007; Zhao et al., 2003), *Salix viminalis* (Rosseli et al., 2003) *Vetiveria zizanioides*, (Yang et al., 2003), *Silene armeria* (Dinelli and Lombini, 1996).

This study was designed to evaluate the ability of bioaccumulation of some metals (Pb, Cu, Fe, and Zn) by two plants species; *Synedrella nodiflora* and *Sida fallax* that naturally grow around the abandoned Asafo Railway Station in Kumasi, Ghana. Thus the concentrations of Pb, Cu, Zn and Fe in the shoot and root tissues of these plants were examined. The main objectives of our study were; to identify plant species that grow naturally on metal contaminated soils and determine the ability of these plants to accumulate and tolerate these metals in their tissues. The extent of metal translocation from underground tissues to aboveground tissues in the plants

for each metal was also examined. The research findings could be helpful to assess the magnitude of metals transferred by naturally growing vegetation, the selection of species for in situ bioremediation of contaminated site and also evaluate the associated threats related to its consumption by ruminants.

MATERIALS AND METHOD

The study area and plant species

The area chosen for this study is the abandoned railway station at Asafo in Kumasi. The railroad transportation system in Ghana has shrunk, as a result most of the rail lines and stations have been abandoned which the railway station at Asafo in Kumasi is an example. This station covers a total land area of about 6 Km² and is mainly over grown with weeds. To a lesser extent, this area is used for other unauthorized purposes including habitation, trading and farming. Ruminants also freely graze on the vegetation. The dominant species among the naturally occurring vegetation that did not show any visible toxicity symptoms are *Sida fallax* and *Synedrella nodiflora*. These plants were therefore selected for this study. They were identified and authenticated by Mr. A. B. Arkoh a plant taxonomist in the Department of Theoretical and Applied Biology, Kwame Nkrumah University of Science and Technology.

Sampling and sample preparation

Soil samples were obtained by collecting about 1 kg of surface soil (0 to 20 cm) by hand digging at 5 sampling sites within a maximum area of 500 m² where the plant samples were obtained. These were put together as a bulk sample. A total of fifteen (15) soil samples were collected. The samples were stored in plastic bags for laboratory analysis. Soil samples for metal analysis were air-dried, ground to a fine powder using a pestle and mortar and then sieved through a 2 mm sieve. Soil pH and electrical conductivity (EC) were measured using 1:2.5 (w/v) soil/deionised water suspension by a pH meter with EC meter. Organic matter (OM) content was measured by loss on ignition following heating at 550°C in a muffle furnace. Composition of soil (soil texture) was analyzed by the sedimentation (hydrometer) method. Total metal content was determined by digesting soil sample using aqua regia. Here fine powder soil sample (2 g) were put in 250 ml conical flasks and subjected for acid digestion with aqua regia for 2 h on hot plate. The flask was cooled and the residue dissolved in 10 ml of dilute nitric acid and filtered. The filtrate volume was made to 50 ml with distilled water (Anilava and Das, 1999).

The plants were carefully uprooted and put into polythene bags. The harvested plants were carefully washed with tap water and rinsed with deionized water to remove adhered soil particles, and were separated into the root and shoot portions. Each portion was further cut into small pieces, air dried for 2 days and finally oven-dried at a temperature of 80°C for 24 h. The dried samples were ground to powder and passed through a 2 mm sieve. About 2 g of the fine-powdered samples of each plant were put in 250 ml conical flasks and subjected to acid digestion with 10 ml of concentration HNO₃ and HClO₄ in a ratio of 5:1 v/v on a hot plate. The flasks were then cooled to room temperature, and the residues were dissolved in 10 ml of dilute nitric acid and filtered. The filtrate was diluted to 50 ml with distilled water (Lark et al., 2002).

Sample analysis

Analysis of the heavy metal content of the samples was performed

Table 1. Physico-chemical properties of soil from the study area (n=15).

Parameter	Min	Max	Mean ± STD
pH	7.84	8.26	8.14±1.03
EC (µS)	36.70	94.50	67.60±6.53
Organic matter (%)	25.50	28.16	26.90±0.78
Moisture content (%)	7.70	8.16	7.93±0.32
Sand (%)	67.60	79.6	69.6± 4.07
Clay (%)	7.00	17.00	15.0±3.12
Silt (%)	13.40	15.40	15.4 ±1.82
Texture Class		Sandy loam	

with a flame atomic absorption spectrophotometer (AAS model, UNICAN 969), using acetylene gas, as fuel, and air as an oxidizer. Calibration curves were prepared separately for all the metals being analysed using different concentrations of standard solutions. The instrument was set to zero concentration for all types of sample, using a reagent blank. Digested samples were aspirated into the fuel-rich air-acetylene flame and the metal concentrations were determined from the calibration curves. Each determination was based on the average values of three replicate samples. The precision of the analytical procedures were expressed as relative standard deviation and ranged from 5 to 10%. All acids and other chemicals were of analytical grade (Darmstadt, Germany). Deionised water was used throughout the study. All the plastics and glassware were cleaned by soaking them overnight in 10% (w/v) nitric acid solution and then rinsed with deionised water.

Enrichment factor (EF) was used to assess the level of heavy metals contaminations and their possible anthropogenic impact in soils samples. In this study iron was used as a conservative tracer to differentiate natural from anthropogenic components. According to Ergin et al. (1991) and Rubio et al., (2000) the metal enrichment factor (EF) is defined as follows:

$$EF = \frac{\frac{M}{Fe(sample)}}{\frac{M}{Fe(background)}}$$

Where EF is the enrichment factor, $(M/Fe)_{sample}$ is the ratio of metal and Fe concentration of the sample and $(M/Fe)_{background}$ is the ratio of metals and Fe concentration of a background. The background concentrations of metals were obtained from soils from an undisturbed area (soils from botanical garden at KNUST in Kumasi).

The geoaccumulation index (I_{geo}) introduced by Muller (1969) was also used to assess metal pollution in soils. It is expressed as:

$$I_{geo} = \frac{\log_2 C_n}{1.5B_n}$$

Where C_n is the measured concentration of the examined metal in the soil and B_n is the geochemical background concentration of the same metal. Factor 1.5 is the background matrix correction factor due to lithogenic effect. The index of geoaccumulation (I_{geo}) includes seven grades (0 to 6) ranging from unpolluted to very highly polluted.

Statistical parameters (maximum, minimum, mean and standard deviation) were calculated for the selected heavy metals in soil and plant samples. Transfer coefficient (TC) or bioconcentration factor

(BCF) for the metals into the plants were also determined using the expression C_p/C_s . Where C_p is the concentration of metals in plants and C_s is the concentration of the metals in corresponding soil. TC or BCF greater than 1 indicated high level of accumulation of metals in the plant. To show metal translocation properties from roots to shoots, the translocation index (TI) for each metal within the plants was calculated using the expression $[metal]_{shoot}/[metal]_{root}$.

RESULTS AND DISCUSSION

Physicochemical properties such as pH, EC, OM content and soil texture affect metal mobility in soil (Tessier et al., 1999). The results of physico-chemical properties of soil from the railway station at Asafo in Kumasi are shown in Table 1. The pH of the soil ranged between 7.84 and 8.29, thus the soil samples were alkaline. The pH values display relatively equal distributions in the entire study area. The soluble salt content of soil is measured by an assessment of EC of water extract of the soil. EC of the soil samples ranged from 36.7 to 94.5 µS. In the investigated soils, the OM content ranges from 25.5 to 28.2% with a mean value of 26.9±0.78%. OM binds mineral particles into granules and is largely responsible for their loose. Again OM content determines the amount of water a soil can hold. The soil in the study area can be classified as loamy soil based on the result from the sedimentation method. The soil has high OM content and relatively high percentage of clay and silt.

Enrichment factor (EF) was used to assess the level of metal contamination and the possible anthropogenic impact of metals in soils samples. According to Zhang and Liu (2002), EF values greater than 1.5 suggest that the sources are more likely to be anthropogenic. The results of the present study show that Pb and Cu were significantly enriched (Table 2). The values of I_{geo} as recorded in Table 2 reveal that the soil of the study area is moderately to highly polluted with respect to Pb and Cu and unpolluted to slightly polluted with respect to Zn and Fe.

In all the soil samples, Fe recorded the highest concentration ranging from 298.65 to 304.90 mg/kg as shown in Table 3. The rails are made from steel and their wearing, scrapping and rusting could be a contribution

Table 2. EF and I_{geo} of metal in soil from the study area.

Metal	EF	I_{geo}
Pb	43.03	2.90
Cu	35.19	3.87
Zn	4.80	0.52

Table 3. Concentration of heavy metals (mg/kg) in plants parts and soil from the study area; n = 15.

Metal		<i>S. fallax</i>		<i>S. nodiflora</i>		Soil
		Root	Shoot	Root	Shoot	
Fe	Range	44.13-50.55	57.85-60.8	78.98-83.03	168.9-158.55	321.23-289.65
	Mean±STD	47.34±4.54	59.32±2.08	81.20±2.89	163.73±7.32	298.55± 12.09
Cu	Range	3.66-4.93	2.45-2.85	4.48-4.63	3.05-3.63	49.09-25.24
	Mean±STD	4.30±0.88	2.65±0.28	4.55±0.11	3.34±0.41	38.85± 4.67
Pb	Range	11.27-14.70	8.83-9.65	14.93-15.85	11.55-12.28	339.98-270.09
	Mean±STD	12.99±2.42	9.24±0.58	15.38±0.65	11.91±0.51	302.13±21.22
Zn	Range	48.6-53.13	52.90-55.53	53.57-63.30	49.18-44.30	80.76-67.34
	Mean±STD	50.86±3.20	54.21±1.85	58.44±6.87	46.738±3.45	70.63±10.11

factor to the high concentration of Fe in the soil. Concentration of Cu in the soil ranged from 38.85 to 76.53 mg/kg. The source of high levels Cu in the soil may be due to the use of Cu cables by the railway company. Pb concentration in the soil ranged from 302.13 to 324.25 mg/kg. The high concentration of Pb in the soil may be as a result of the use of lead containing fuels in automobile engines in the past since the site is at the city centre and therefore experience heavy vehicular movement. Zn has a concentration range from 70.63 to 87.00 mg/kg. The source of Zn may be due to coal which was used as fuel to power the locomotive engines. Generally the concentrations of metals in soil were more than those of the plants. The relatively abundance of the metals measured in the soil was found to be the order of $Pb > Fe > Zn > Cu$ as presented in Table 3.

The concentrations of metals in plants indicate the metal status and also the abilities of plant species to uptake and accumulate metals from the substrate upon which they grow. The levels of metals accumulation in roots and shoots of *S. nodiflora* and *S. fallax* growing naturally at the sampling site are presented in Table 3. This result shows that, metal content in the plant tissues varied widely among the species indicating their different capacities for heavy metal uptake. Generally, root parts of plants accumulated high metal concentrations. Among the species, *S. nodiflora* recorded the highest mean concentration of all metals measured in its root portion. Zn was recorded as 58.44 ± 6.88 mg/Kg, Pb, 15.39 ± 0.65

mg/Kg, Cu, 4.55 ± 0.11 mg/Kg and Fe, 81.01 ± 2.89 mg/Kg accumulation in the root portions of *S. Nodiflora* (Table 3).

Higher concentrations of Cu were observed in root portions of the plants. The highest concentration of Cu was found in the root portion of *S. nodiflora* as 4.55 ± 0.11 mg/kg and lowest in *S. fallax* as 4.30 ± 0.88 mg/kg (Table 3). For shoot parts, highest concentration of Cu was found in *S. nodiflora* (3.34 ± 0.41 mg/kg) and lowest in *S. fallax* (2.65 ± 0.28 mg/kg). Cu is an essential element for plant growth but Alloway, (1990) reported that the critical concentration of Cu in plants ranges between 20 to 100 mg/kg, above which toxicity effects are likely to occur and if the concentration ranges from 5 to 64 mg/kg, it is likely to cause 10 % depression in yield (Reeves, 2002). The concentrations of Cu in both plant species were less than the above mentioned value hence the levels of Cu in these plants can not produced any toxic effects on both plants. The higher concentration of Cu in the root tissues of both plants as compared to the shoot tissues indicate availability of the metal in the soil to the plant but a low mobility of the metal within the plants. However the concentration of Cu in shoot tissue of *S. nodiflora* indicates a better translocation probability within the plant as compared to *S. fallax*. The TCs of Cu as presented in Table 4 for both plants were very low indicating that both plants have low potential of extracting Cu.

Zn is an essential element to all plants. Plants predominantly absorb Zn as a divalent cation which act

Table 4. Soil-plant transfer coefficient and translocation index for metal in both plants.

Parameters Metal	Plant specie	Transfer coefficient (TC)		Translocation index (TI)	
		<i>S. fallax</i>	<i>S. nodiflora</i>	<i>S. fallax</i>	<i>S. nodiflora</i>
Fe		0.199	0.548	0.975	2.020
Cu		0.068	0.086	0.616	0.734
Pb		0.031	0.039	0.771	0.774
Zn		0.768	0.662	1.066	0.800

either as a metal component of enzymes or as a functional, structural or regulatory co-factor of a large number of enzymes (Alloway, 1990). The concentrations of Zn in the examined plants were within the normal values (10–300 mg/kg) as reported by Reeves and Baker (2002). In this study, highest concentration of Zn was found in the root portions of *S. nodiflora* (58.44±6.87 mg/kg) and lowest in *S. fallax* (54.21±1.85 mg/kg). *S. nodiflora* was found to accumulate more Zn in their root tissue compared to shoot. The TI value for Zn recorded in *S. fallax* (1.07) was higher than that of *S. nodiflora* (0.80) as shown in Table 4. The TI value of Zn in *S. fallax* indicates less resistance in the translocation of Zn from roots toward shoots in this plant. The TC of Zn is comparatively high in both plants though that of *S. fallax* exceeded that for *S. nodiflora*. This indicates that *S. fallax* has a higher Zn extraction and accumulation potential than *S. nodiflora*, hence can accumulate a large amount of this metal in its shoot. From the TC calculated for Zn, it shows clearly that though a larger concentration of Fe was present in *S. fallax* species the level of accumulation of Zn by this plant is higher than that for the accumulation of Fe. This observation can be explained by the fact that while Zn is an essential nutrient (macronutrient) required for plant metabolism Fe is a micronutrient. The TC and TI values of Zn for *S. nodiflora* indicate that the plant has moderate Zn extraction and accumulation potential.

Fe accumulated maximum in *S. nodiflora*, whereas the lowest values of this element corresponded to the roots of *S. fallax* (Table 3). In all of the plant samples, Fe concentrations were within the sufficiency range (50–250 mg/kg), as defined by Kabata-Pendias and Pendias (1992). This indicates that all the plant species do not have the capability to accumulate Fe greater than the normal range.

Pb concentrations in both plants were very low as compared to the concentration of Pb found in the soil. TC values obtained for Pb in both plants, happened to be the least recorded for both plant species. The TC of *S. nodiflora* is higher though but by a very insignificant amount (Table 4). This shows that the potential for Pb extraction is very poor for both plants. This observation can be explained by the fact that the extraction of Pb from soils is very difficult since it precipitates with most common ions such as SO_4^{2-} , PO_4^{2-} , CO_3^{2-} and S^{2-} and

hence has extremely low solubility thus not easily extractable and therefore not bioavailable. Highest concentration of Pb was found in the root portion of *S. nodiflora* (15.38±0.65 mg/kg). In the case of shoot parts, highest concentration of Pb was found in *S. nodiflora* (11.91±0.51 mg/kg) and lowest in *S. fallax* (9.24±0.55 mg/kg) Table 3. Alloway (1990) reported that the critical concentration of Pb in plants ranges between 30–300 mg/kg above which toxicity effects are likely. Normal range of Pb in plants is 0.2–20 mg/kg (Alloway, 1990; Reeves, 2002). It can therefore be deduced from this study that Pb concentration in root and shoot portion of the naturally growing vegetation in the abandoned railway station are within safe limits, however, towards higher side.

In phytoextraction the ability of plants to absorb and accumulate metals in their shoots and other tissues is very essential, hence the soil-plant TC for the plant species were calculated for each metal. The TCs for different heavy metals from soil to plant are a key component of human exposure to the metals via the food chain. The highest TC value in this study for both plants was recorded for Zn (Table 4). Reason being that Zn is highly mobile in the environment than other heavy metals. Overall, the TC values for Zn and Cu were found to be significant, supporting the finding that the accumulation of Ni, Pb and Cr is comparatively less, while that of Cd, Cu and Zn is more in plants (Olaniya et al., 1998). *S. nodiflora* species recorded higher Fe TC values than *S. fallax*. The high TC value in *S. nodiflora* indicates that this plant can extract more Fe than *S. fallax* per kg of dry mass. It can also be said that the root portion of *S. nodiflora* has a good Fe accumulation potential. The TC values recorded for both plant species indicates that the plants have very low to moderate extraction potentials.

The translocation indices (TI) which is the ratio of metal concentration in the aerial part of the plant to the concentration of metal in the roots were also calculated since the ability of plants to translocate metals from their roots their shoot is a very important factor in phytoextraction. The TIs for Fe were high for both plants but that of *S. nodiflora* was more than twice that for *S. fallax*. This result is confirmed by the fact that the concentration of Fe in the shoots of *S. nodiflora* is more than double the concentration in the roots. This shows that *S. nodiflora*

has a better translocation system for Fe than *S. fallax*. The general trend in Table 3 shows that the root tissue of *S. fallax* accumulates significantly greater concentration of metals than shoots indicating high plant availability of the metals as well as its limited mobility once inside the plant.

Phytoavailability of metal from root to shoot part can be assessed by the TI. The data indicate that metals accumulated by the plants were largely retained in roots, as shown by general TI values <1 (Table 4). Exceptions occurred in *S. fallax* for Zn which recorded TI value of 1.07 and that of *S. nodiflora* for Fe was 2.02. Fe and Zn were translocated more in shoot portion from root, thus higher concentration of these metals were observed in shoot portion and the TI was found >1. The rate and extent of the translocation within plants depend on the metal and plant species concerned. The TI values of some metal varied among the plant species grown at the same site. For example, TI values for Fe in *S. nodiflora* and *S. fallax* were found 2.02 and 0.98, respectively. Out of four metals studied, Cu shows the least TI values for all species, thus *S. nodiflora* recorded TI value of 0.73 while *S. fallax* had TI value of 0.62. This means that Cu accumulated in the root portion and lesser amount was transferred to the shoot portions of the plants. *S. nodiflora* and *S. fallax* recorded nearly the same translocation factor of 0.774 and 0.771, respectively for Pb, implying Pb accumulates in the same proportion for root and shoot part of the plants. Pb adhered only to root portion and it does not easily translocate to shoot portion. The possible reason for this observation might be the self-adjusting of plants which, plays an important role on sequestering metals in their root, depriving the metals to translocate to the upper part of the plant. The ability of these plants to translocate essential trace metals such as Fe and Zn to moderate levels and extrude toxic metals like Pb makes them good foliage for pasture.

Conclusion

The study indicated that the soil in the studied area is alkaline and can be classified as loamy soil. The geoaccumulation index values also describe the soil as highly contaminated with Pb and Cu and slightly contaminated with respect to Zn. The surface soils of the study site probably constitute a health hazard to the population since it is contaminated with toxic heavy metals.

The study also indicated that metals accumulated by the plants growing in the study area were largely retained in roots, as shown by translocation index values <1. The rate and extent of the translocation within plants also depended on the metal and plant species concern. High Fe concentration in shoot portion of *S. nodiflora* indicates active transport of this metal by roots. This allows us to treat *S. nodiflora* as a potential candidate for Fe bio-remediation using phytoextraction. The TI value for Fe in

S. fallax is also high indicating that this plant can also be considered as a potential Fe accumulator. Zn with a translocation index > 1.0 in *S. fallax* is an indication that this plant has the potential for remediation of Zn contaminated soils. The TC values for Pb and Cu are not significant (≤ 0.1) and hence both plant species can be considered as Pb and Cu excluders, though their TI shows a moderate ability of translocation.

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