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Assessment of the phytoremediation potential of *Panicum maximum* (guinea grass) for selected heavy metal removal from contaminated soils

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Non-vascular plants have potential for rapid uptake of metals, but are rarely used for phytoremediation because of their short life cycle. This property can however be advantageously used in a number of metal removal cycles within a short time. The selection of promising plants is critical to success of phytoremediation. The potential for heavy metal uptake by *Panicum maximum* a non-vascular plant was investigated using pot plant experiments. Seventy-two (72) pots of 7 L capacity were each filled with 5 kg of mixed soil collected from the Fadama (fertile soil) and College of Animal Sciences and Livestock Production farms (less fertile) of the University of Agriculture Abeokuta, Ogun State Nigeria. The pots were divided into six groups of twelve pots each. Each of the group of 12 pots was further divided into three groups of four pots each, in which *P. maximum* were planted. Five of the six groups were treated with 20, 50, 75, 100 and 120 ppm each of Pb^{2+}, Cr^{3+} and Cd^{2+} and the sixth served as control. Levels of Pb^{2+} decreased from 1.40 to 1.05 µg/g and 1.57 to 1.30 µg/g in soils treated with 20 and 120 ppm of Pb^{2+}, respectively. Generally there was increase in Pb^{2+} uptake by the different tissues of *P. maximum* ranging between 0.21 to 0.38 µg/g, 0.18 to 0.30 µg/g, and 0.09 to 0.18 µg/g in root, stem and foliage, respectively. Corresponding bioaccumulation factors (BAF) ranged between 0.21 to 0.45, 0.17 to 0.35 and 0.08 to 0.21. Metal uptake followed similar trends for Cr^{3+} and Cd^{2+} in plant tissues. The BAF values for Cr^{3+} ranged between 0.31 to 0.69 in root, 0.17 to 0.52 in stem and 0.07 to 0.34 in foliage. Similar values for Cd^{2+} were 0.14 to 0.53, 0.10 to 0.44 and 0.05 to 0.37. Accumulation of heavy metals in *P. maximum* ranged from 13 to 45%, Pb^{2+}; 13 to 65%, Cr^{3+} and 11 to 52%, Cd^{2+} of the soil concentration level with tissue abundance decreasing in the order $Cr^{3+} > Pb^{2+} > Cd^{2+}$. Furthermore, the concentration of metals in *P. maximum* tissues decreased in the order root > stem > foliage. The phytoremediation of Pb^{2+}, Cr^{3+} and Cd^{2+}contaminated soils with *P. maximum* seems to be promising under the conditions of the experiment. Obvious signs of phyto-toxicity however appeared in plants exposed to 120 ppm Pb^{2+} and Cd^{2+} at day twenty-three, suggesting that *P. maximum* may be a moderate metal accumulator.

Key words: phytoremediation, heavy metals, uptake, tissues, accumulator.

INTRODUCTION

Phytoremediation is a biological technique used in removing contaminants from contaminated soil, water or air using green plants, except for phytostabilization. The contaminants may include metals, pesticides, solvents, explosives, crude oil and its derivatives. The technique is relatively cost-effective compared to other techniques such as metal electro-osmosis and excavation/reburial and incineration (Davies et al., 2002; Li et al., 2004). It is also environmentally friendly with significant aesthetic improvement on contaminated soils (Chen et al., 2002; Lyubun et al., 2002; Fayiaga et al., 2004) with less external input from man.

The use of phytoremediation to restore contaminated soils of abandoned metal-mine working and to reduce the impact of polychlorinated biphenyls (PCBs) from dumps and mitigation of contamination in on-going coal mine discharges has been reported (Li, 2005; Azevedo et al., 2005; Sizova et al., 2006). The efficiency and time to effect clean up by phytoremediation is a function of the plant type and population on contaminated site, concentration of pollutants and extent of pollution, soil class and prevailing soil condition which varies with locations. Soil pH, dissolved organic carbon (DOC) and the spatial distribution of electrical conductivity (EC) may however suggest ways to manage any metal loaded field area (Hattab et al., 2013). The United State Environmental Protection Agency (EPA) (2000) reported that, 'in the process of phytoremediation, plants may have to be replaced if they are destroyed by bad weather or animals, which add time to the cleanup'. Often it takes many years to clean up a site using phytoremediation procedures.

The selection of promising plants is an important approach to successful phytoremediation. Plants used for phytoremediation clean up procedures can range from those with natural ability, moderate accumulator to hyper-accumulator or those that degrade or render harmless contaminant in soils, water and air (Hemen, 2007). Various plants have been used differently for different pollutant types. Such hyper-accumulator plants reported include mustard plants, alpine, pennycress, kenaf and pigwood which were used on toxic wastes sites. Others include ladder fern *Pteris vittata* which accumulate arsenic (As) even at trace levels in soils. *P. vittata* is more efficient at As levels below 6 ppm in soils, and increased up to 40% higher than normal when As reaches up to 100 ppm (Wei and Chen, 2007).

In most studies, phytoremediation of contaminated sites has been evaluated using vascular plants, which in most cases may not be native plants. The use of non native plants requires adaptation procedures such as soil adjustment in order to survive the intent of planting, and for which studies revealed less than 60% plant survival (SERG, 2011). Moreover, phytoremediation with vascular plants may require soil amendments to effect clean up. Very little information is available on the use of forbs for phytoremediation procedures, perhaps because of their short life cycle. The short life cycle can be exploited for fast and efficient phyto-procedures if optimized and modified to cash in on a number of cycles for sequential remediation of contaminated sites (SRCSs), once the effective and best performing forbs are identified.

In this study, the potential of *P. maximum* (Guinea grass), a native and non vascular plant common in rain forest edge habitat to savannah grass land was evaluated for phytoaccumulation of Pb, Cr and Cd on moderately fertile contaminated soil.

MATERIALS AND METHODS

Soil collection and the determination of its physico-chemical properties

Soils were collected from the College of Animal Science and Livestock Production farm (less fertile soil) and Fadama farm (fertile soil) at the University of Agriculture Abeokuta, Ogun State, Southwest Nigeria. The soils were air dried, thoroughly mixed, and then subjected to physical-chemical analysis to determine the pH (McLean, 1982), conductivity (IITA, 1979), particle size distribution and mixed soil textural classification (IITA, 1979), exchangeable cation exchange capacity (ECEC) (Stewart, 1989), organic matter (Walkey and Black, 1934), total nitrogen and available phosphorus (Zhao et al., 1994).

Sources and cultivation of *Panicum maximum*

Five kilograms each of the blended soils were introduced into 72 units of 7 L capacity experimental pots which were segregated into six groups of 12 pots each. Each group of 12 pots was further subdivided in a 3x4 matrix (three groups of four pots). To each pot, about one week old juvenile guinea grasses (collected from nearby bush) were quickly transplanted (three stalks per pot) and the grasses nurtured to survival, and growth stabilization for one week.

Determination of phytoremediation potential of *Panicum maximum*

Five of the six groups of segregated experimental pots were exposed to different concentrations of Cd, Pb and Cr (20, 50, 75, 100 and 120 ppm, respectively) with daily wetting. The sixth group had no treatment and was used as experimental control. After the heavy metal treatment, plants were harvested from each group on a weekly interval and the various parts (roots, stem and foliages). The concentrations of Cd, Pd and Cr in roots, stems and foliage tissues of the harvested plants were determined according to the methods of Zhao et al. (1994). The soil resident concentrations of the heavy metals were measured weekly according to the method of Onianwa (2000).

Determination of metals bioaccumulation factors (BAF)

The bioaccumulation factor (BAF) defining the transport of metals from soil to different parts of *P. maximum* tissue were determined by the ratio of the metal concentration in plant tissue part to that in soil ($BAF = [metal]_{plant\ tissue}/[metal]_{soil}$) for each experimental pot.

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Table 1. Result of chemical analysis on soil.

Parameter	Minimun value	Maximum value	*Ibadan, Nigeria	**Southeast Ethiopia
pH	6.30	6.50	7.24 - 7.26	6.3 - 7.1
Electrical Conductivity (µS/cm)	94	105	-	-
Exchangeable Ca (cmolkg^{-1})	1.07	1.38	3.28 - 3.44	2.41 - 3.50
Exchangeable Mg (cmolkg^{-1})	0.85	1.10	1.20 - 1.27	0.65 - 0.73
Exchangeable K (cmolkg^{-1})	0.12	0.18	1.28 - 1.43	0.30 - 0.41
Exchangeable Na (cmolkg^{-1})	0.10	0.12	1.66 - 2.05	0.14 - 0.21
Organic carbon (%)	1.90	2.00	0.96 – 4.95	0.76 - 1.70
Available phosphorus (P) (µg/g)	5.82	7.50	11.97 - 12.59	-
Total nitrogen (N) (g/kg)	0.95	1.20	0.20 - 0.39	0.10 – 0.12
Soil class	Sandy loam	Sandy loam	Sandy loam	Sandy clay – clay soil

Data from *Eludoyin and Wokocha, 2011; **Belachew and Abera, 2010.

RESULTS AND DISCUSSION

Physico - chemical properties of soils

Physical examination conducted on the soil showed that the soil is porous and well aerated. This is as a result of the soil mixing process, which distorted the surface compaction order at soil collection, thus losing its confined nature; with concomitant effect on some geophysical and engineering properties of the soil. The soils are typically sandy loam based on particle size distribution analysis with 75.5, 17.0 and 7.5% sand, silt and clay, respectively.

Analysis results (Table 1) showed that the soils are slightly acidic with a pH range of 6.30 to 6.50. According to Romkens and De Vries (1995), this pH may allow for fair mobility of metals, though metal mobility and bioavailability also depends on their chemical form in soils (Ma and Rao 1997), and the soil physical properties. The electrical conductivity of the soil is moderate (94 to 105 µS/cm). The level of organic carbon (OC) (1.90 to 2.00%); available phosphorus (P) (5.82 to 7.50 *µ*g/kg) and total nitrogen (N) (0.95 to 1.20 *µ*g/kg) of the soil were moderate when compared to the findings of Eludoyin and Wokocha (2011) and Belachew and Abera (2010) (Table 1). The soil concentrations of the exchangeable cations or basic metals (Na, K, Ca and Mg) are within the normal range available in arable agricultural soils (Brady and Weil 2002).

Phytoremediation potential of *Panicum maximum*

The summary of the concentrations of Pb, Cr and Cd detected in the tissues: root, stem and foliage of *P. maximum* and soils are presented in Table 2. The uptake of Pb, Cr and Cd increased steadily in the root, stem and foliage system of *P. maximum* planted on the soil exposed to different level of metal treatment. Results showed that there was a general decrease in soil concentrations of Pb, Cr and Cd, with attendant increase in the metal concentrations in different part of *P. maximum* over the 28 day study period. The roots of *P. maximum* retained the highest concentration of all metals investigated, with tissue abundance decreasing in the order Cr > Pb > Cd. The concentrations (*µ*g/g) of Pb, Cr and Cd in soils; roots; stems; and foliages of the harvested *P. maximum* in experimental control (that is, plants in soils not spiked with the metals) sampled on the seventh, fourteenth, twenty-first and twenty-eight day were [0.28, 0.30, 0.24]; [0.08, 0.11, 0.06]; [0.06, 0.08, 0.04] and [0.02, 0.05, 0.01], respectively. Hattab et al. (2013) reported that the predication of the concentration of Cr in soil depends on some soil factors such as pH, EC, DOC etc. and the type of treatment or amendments to which the soil is subjected.

Over the four-week period of sampling, the levels of Pb decreased from 1.40 to 1.05 *µ*g/g and from 1.57 to 1.30 *µ*g/g, in soils, treated with 20 and 120 ppm of Pb, respectively. However, uptake of the Pb by *P. maximum* increased from 0.21 to 0.38 *µ*g/g in the root, 0.18 to 0.30 *µ*g/g in the stem and 0.09 to 0.18 *µ*g/g in the foliage. The metal bioaccumulation factors (BAF) were 0.21 to 0.45 in root, 0.17 to 0.35 in stem and 0.08 to 0.21 in foliages of plants grown on soil treated with 20 ppm Pb, compared with soil levels of Pb measured weekly. Thus *P. maximum* accumulated between 13 to 45% of the soil concentration level. However, the levels detected in different plant tissues increased as soil treatment concentration of Pb increased. For example, soils treated with 120 ppm Pb showed increase in concentration level which ranged between 0.64 to 0.74 *µ*g/g in the roots, 0.53 to 0.63 *µ*g/g in the stem and 0.21 to 0.34 *µ*g/g in the foliages, with corresponding BAF of 0.50 to 2.26 in the roots, 0.41 to 1.94 in stem and 0.03 to 1.00 in the foliages, compared to soil levels of Pb measured weekly.

The concentration of Cr in the treated soils diminished from 1.01 to 0.85 *µ*g/g and 1.28 to 1.20 *µ*g/g in soils

Table 2. Concentration levels (µg/g) of lead, chromium and cadmium in soil and different tissue part of guinea experimental grass .

Parameter	Control			Group 1 20 ppm Treatment			Group 2 50 ppm Treatment			Group 3 75 ppm Treatment			Group 4 100 ppm Treatment			Group 5 120 ppm Treatment		
	Pb (µg/g)	Cr (µg/g)	Cd (µg/g)	Pb (µg/g)	Cr (µg/g)	Cd (µg/g)	Pb (µg/g)	Cr (µg/g)	Cd (µg/g)	Pb (µg/g)	Cr (µg/g)	Cd (µg/g)	Pb (µg/g)	Cr (µg/g)	Cd (µg/g)	Pb (µg/g)	Cr (µg/g)	Cd (µg/g)
Week 1																		
Soil	1.38	0.35	0.28	1.40	1.01	0.90	1.44	1.08	0.92	1.48	1.14	0.94	1.52	1.22	0.97	1.57	1.28	1.00
Root	0.08	0.11	0.06	0.21	0.43	0.13	0.34	0.56	0.17	0.47	0.60	0.21	0.52	0.71	0.22	0.64	0.82	0.25
Stem	0.06	0.08	0.04	0.18	0.24	0.09	0.25	0.31	0.12	0.35	0.42	0.15	0.41	0.56	0.18	0.53	0.63	0.21
Leaf	0.02	0.05	0.01	0.09	0.11	0.05	0.11	0.15	0.07	0.14	0.17	0.09	0.17	0.20	0.11	0.21	0.29	0.15
Week 2																		
Soil	1.36	0.33	0.26	1.12	0.97	0.67	1.15	1.00	0.71	1.20	1.10	0.74	1.30	1.18	0.80	1.33	1.25	0.83
Root	0.10	0.14	0.08	0.28	0.35	0.15	0.30	0.44	0.17	0.43	0.48	0.22	0.56	0.61	0.25	0.10	0.14	0.08
Stem	0.08	0.12	0.05	0.22	0.27	0.10	0.26	0.32	0.12	0.35	0.37	0.15	0.44	0.49	0.21	0.08	0.12	0.05
Leaf	0.04	0.06	0.03	0.13	0.15	0.06	0.15	0.17	0.08	0.18	0.19	0.10	0.20	0.24	0.14	0.04	0.06	0.03
Week 3																		
Soil	0.34	0.31	0.26	1.08	0.95	0.65	1.11	0.98	0.68	1.16	1.07	0.71	1.27	1.16	0.77	0.34	0.31	0.26
Root	0.13	0.15	0.09	0.31	0.38	0.18	0.32	0.41	0.22	0.45	0.54	0.25	0.60	0.63	0.27	0.70	0.66	0.28
Stem	0.11	0.12	0.07	0.27	0.31	0.11	0.29	0.35	0.20	0.38	0.42	0.16	0.48	0.46	0.18	0.60	0.48	0.21
Leaf	0.06	0.37	0.06	0.15	0.20	0.08	0.18	0.24	0.10	0.21	0.26	0.12	0.24	0.28	0.14	0.31	0.35	0.17
Week 4																		
Soil	0.38	0.30	0.24	1.03	0.85	0.62	1.10	0.92	0.73	1.12	1.02	0.75	1.22	1.13	0.79	1.30	1.20	0.81
Root	0.14	0.17	0.12	0.38	0.71	0.33	0.41	0.73	0.35	0.51	0.77	0.37	0.63	0.80	0.41	0.74	0.83	0.43
Stem	0.12	0.14	0.11	0.30	0.53	0.27	0.32	0.56	0.28	0.42	0.59	0.30	0.50	0.62	0.33	0.63	0.64	0.35
Leaf	0.07	0.01	0.09	0.18	0.35	0.23	0.21	0.39	0.24	0.25	0.42	0.27	0.27	0.45	0.28	0.34	0.47	0.31

treated with 20 and 120 ppm of Cr, respectively over the four week study period. The uptake of Cr by *P. maximum* increased from 0.43-0.71 µg/g in root, 0.24-0.53 µg/g in stem and 0.11 to 0.35 µg/g in foliages of plant in soils treated with 20 ppm. The BAF of Cr in different part of *P. maximum* also increased over the study duration and ranged between 0.31 to 0.69 in the root, 0.17 to 0.52 in the stem and 0.07 to 0.34 in the foliages of plant sample grown on 20 ppm Cr - treated soils. This showed that *P. maximum* accumulated between 13 to 69% of the heavy metals. The detected concentration levels of Cr in the different plant tissues increased as soil treatment concentration of Cr increased. The increase in Cr concentration observed in tissues of *P. maximum* in soils treated with 120 ppm Cr which ranged: 0.14 to 0.83 µg/g in root, 0.24 to 0.53 µg/g in stem and 0.63 to 0.64 µg/g in foliages, with corresponding BAF of 0.11 to 1.94 in root, 0.09 to 0.49 in stem and 0.05 to 1.03 in foliage compared with soil levels of Cr measured weekly.

The concentration levels of Cd increased in tissues of *P. maximum* over the study period, while Cd levels in soils decreased. Soil levels of Cd dropped from 0.90 to 0.24 µg/g and from 1.00 to 0.81 µg/g in soils treated with 20 and 120 ppm Cd respectively. The uptake of Cd by *P. maximum* increased from 0.13 to 0.33 µg/g in the roots, 0.09 to 0.27 µg/g and 0.05 to 0.23 µg/g in the foliages. Cadmium BAF in *P. maximum* were 0.14 to 0.53 in the roots, 0.10 to 0.44 in stem and 0.05 to 0.37 in foliages of plant grown in soils treated with 20 ppm Cd compared with soil levels of Cd measured weekly. Cadmium accumulation in *P. maximum* however lies between 9 to 53%. However the levels observed in different plant tissues increased as soil treatment concentration of Cd increased. For instance, soils treated with 120 ppm Cd showed increase in concentration which ranged between 0.25 - 0.43 µg/g in the roots, 0.21 to 0.35 µg/g in the stem and 0.15 to 0.31 µg/g in the foliages, with corresponding BAF of 0.25 to 1.08, 0.21 to 0.82 and 0.04 to 0.65, respectively, compared to soil levels of Cd measured weekly.

The results showed that the rate of metals uptake or absorption by the plant is proportional to the metal concentration in the soils and plant age i.e. duration of exposure.

Performance of *P. maximum* on metal contamination soil

The growth profiles of *P. maximum* were steady with an average rate of height increase of 20 cm per week, and reaching a maximum height range of between 100 and 120 cm over the four-week period. The plants were green and appeared unaffected, except group 5 plants whose foliages experienced leave burning after the third week resulting into yellowing-browning of the foliages. Consequently, the growth pattern of *P. maximum* plants used in this study were considered to be above average, as their growth rate were observed to be consistent with the report of FAO (2003) and Humphrey and Patridge (1995) who noted rapid growth reaching a height of about 1.5m during the first four weeks.

Profile of Pb, Cd and Cr in different plant tissues

The concentrations of Pb, Cr and Cd detected in the different plant parts (root, stem and foliage) increased over the study period, except for Pb and Cd which showed a sudden down surge of concentrations in roots, stems and foliage at week 2 harvest in group 5 plants (120 ppm Pb and Cd treated soils), compared to the detected levels in week 1 harvest. Higher concentrations were measured in roots, stems and foliages of *P. maximum* planted on most of the contaminated soils reaching 100 ppm Pb, Cr and Cd. Thus, the uptake pattern of Pb, Cr and Cd by *P. maximum* appeared to be a function of their concentrations in their respective soil environment. This is consistent with the findings of Page et al. (1981) and Kabata-Pendias et al. (1993), which implies that metals uptake by *P. maximum* is dependent on the degree of metal contamination in soil. On the contrary, soil concentration of Pb, Cr and Cd decreased over the study period. Also the concentration levels of Pb, Cr and Cd available in *P. maximum* decrease in the order root > stem > foliage. The concentrations of Pb, Cr and Cd also increased steadily in the different parts of *P. maximum* with duration (plant experimental age) from day seven today twenty eight during the harvest periods. The highest concentrations were noted in soils spiked each with 120 ppm of Pb, Cr and Cd (Experiment group 5 pots) compared with the other treatment groups.

Expression of metal toxicity in the form of foliage damage or burns (resulting in yellowish-brown foliage) appeared in *P. maximum* planted on soil treated with 120 ppm Pb and Cd (experiment group 5), on day 23 (about week 3). Concentration of Cd and Pb reaching 120 ppm appeared to be phytotoxic to the plant. The phytotoxic point is the concentration level at which the plant begin to manifest obvious toxicity effect from the contaminant. Apparently, there was obvious cytotoxic damage in *P. maximum* leading to loss of control in metal uptake at this treatment concentration. This occurred at some point after day fourteen, leading to excessive levels of Pb in tissues of *P. maximum* which showed up to 226% Pb compared with soil level at day twenty-one. This signifies the maximum holding capacity of *P. maximum* at 56% soil level, in 100 ppm treatment 5 kg soils if other conditions are optimized. This suggest that the concentrations of the toxic metals in soil is not as much an issue in the evaluation of phytoremediation potential of plant species, than the levels at which phytotoxic response of plant appears during remediation process. It is also not absolute to conclude that the foliage burns effect is a response to high concentration levels of Cd and Pb, because the experimental soils used. Metal uptake and accumulation by *P. maximum* also need to be tested on high nutrient quality soils at concentration of 120 ppm and above for of Cd and Pb removal.

Therefore, the potential for uptake of Pb, Cr and Cd by *P. maximum* when planted on heavy metals contaminated soils is high and when contamination is moderate, because high metal concentration may be phytotoxic to the plant at some point.

Conclusion

Phytoremediation of metal contaminated soils with *P. maximum* showed a promising potential under the conditions of experiment. The distribution and accumulation of metals in the plants were variable, with the root tissues accumulating significant concentration of the Cr, Pb and Cd than the stem and the foliar tissues. Obvious signs of phyto-toxic effect appeared in plants treated with 120 ppm Pb and Cd at day 23, which implies that *P. maximum* may not be metal hyper accumulator. It is however important to note that the selection of promising plants is critical to success of phytoremediation. Further work is recommended to evaluate the effect of soil amendments on the response of *P. maximum* to metal uptake and plant performance. Positive outcome will further reduce technical and human input in phytoremediation procedure as well as reduce clean up duration since the cycle of *P. maximum* is perennial.

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