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Full Length Research Paper

Phytoextracting cadmium and copper using *Mucuna pruriens*

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Mucuna pruriens **var pruriens commonly known as Mucuna, a fast-growing, high-biomass-accumulating plant was investigated to underscore its suitability for metal (Cd and Cu) extraction in an oil spill condition. Process enhancement was made using NPK, UREA and Poultry manure fertilizers. These amendments facilitated metals availability in the order PM>NPK>UREA for Cd extraction and PM>UREA >NPK for Cu extraction for both root and shoot samples. Mucuna accumulated appreciable quantities of Cd (up to 32 mg/kg) and Cu (up to 62 mg/kg) metals in their tissue regardless of the concentration of metal in the soil and achieve > 50% contaminant removal for both Cu and Cd. Extraction was maximal with increased metal bioavailability evident in PM treated groups. Below 10% (w/v) pollution, non-assisted Mucuna competed favourably with those of amendment-assisted groups for Cd extraction into tissues. However, there was a marked variation between non-assisted and assisted groups for Cu extraction into tissues.**

Key words: Mucuna, extraction, amendment, metals, soil.

INTRODUCTION

Heavy metal contamination of the biosphere has increased sharply since 1900 (Nriagu, 1979) and poses major environmental and human health problems worldwide (Ensley, 2000). Unlike many organic contaminants, most metals and radionuclides cannot be eliminated from the environment by chemical or biological transformation (Cunningham and Ow, 1996). Although it may be possible to reduce the toxicity of certain metals by influencing their speciation, they do not degrade and are persistent in the environment (NRC, 1999; Cunningham et al., 1997). The various conventional remediation technologies that are used to clean heavy metal polluted environments are soil *in situ* nitrification, soil incineration, excavation and landfill, soil washing, soil flushing, solidification and stabilization electrokinetic systems. Remediation of sites contaminated with toxic metals is particularly challenging. Unlike organic compounds, metals cannot be degraded, and the cleanup usually requires their removal. However, this energy-intensive approach can be prohibitively expensive. In addition, the metal removing process often employs stringent physicochemical agents which can dramatically inhibit soil fertility with subsequent negative impacts on the ecosystem. Phytoremediation has been proposed as a cost-effective, environmental-friendly alternative technology (Lasat, 2002; Johnson et al, 2009). Phytoremediative technologies which are soil-focused are suitable for large areas that have been contaminated with low to moderate levels of contaminants. Each of the conventional remediation technology has specific benefits and limitations. The phytoextraction process involves the use of plants to facilitate the removal of metal contaminants from a soil matrix. It is suggested that the phytoextraction process is enhanced when metal availability to plant roots is facilitated through the addition of acidifying agents to the soil (Gupta and Sinha, 2007). Chemicals that are suggested for this purpose include various acidifying agents, fertilizer salts and chelating materials (Majeti and Helena, 2003). Mucuna is a widespread fodder plant in the tropics and is used in the countries of Benin and Vietnam as a biological control for problematic *Imperata cylindrica* grass (Amin et al., 1996). In many parts of the world Mucuna is used as an important forage, fallow and green manure crop (Giuliano and Allard, 2001). As a member of the legume family (peas and beans), and with the help of nitrogen fixing bacteria,

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Table 1. Mean level of Cd (mg/kg) found in soil, S.

TRMT	CON	NPK	UREA	PM	PRE-P
S20	202j ± 6	168m ± 3	187o ± 12	131d ± 14	342p ± 4
S40	256k ± 6	250e ± 6	243n ± 5	168 m ± 24	348p ± 4
S60	262k ± 5	250e ± 6	256n ± 4	162m ± 23	348p ± 4
S80	275k ± 4	250e ± 4	268n ± 5	237n ± 4	381q ± 5
S100	281f ± 5	275k ± 5	275k ± 2	268n ± 3	387q ± 5

CTRL = 56a ± 6, Values are means ± SE for triplicate determinations (n = 3). Means in the same column having the same letters are not significantly different at $p \leq 0.005$. NPK = Treatment (TRMT) group amended with NPK; PM = Treatment group amended with Poultry manure; UREA = Treatment group amended with Urea; 20, 40, 60, 80, and 100 represent different spill volumes simulated; CON = contaminated treatment with no nutrient amendments; S = Soil sample; PRE-P = Soil after 2 weeks of contamination and watering and before biostimulation and even planting; Root = Root samples; L = Leaf samples; CTRL = control experiment; WAG = Weeks after germination.

Mucuna takes nitrogen gas from the air and combines it with other chemical compounds producing fertilizer and improving the soil (Manyam et al., 2004). Many works have been done on phytoextraction, however, Mucuna has not been investigated in this regard. This study seeks to evaluate the metal (Cd and Cu) extraction properties of Mucuna from an oil spill site and the effects of modifications with various biostimulants.

METHODOLOGY

Soil samples from an agricultural grassland lying fallow in Choba community in the Niger Delta, Rivers State of Nigeria was spiked with Bonnylight crude oil (Table 7 for composition) sourced from Nigerian National Petroleum Corporation in a topdressing manner following pot experiments. There was no history of spill in this area located about 26 km East-West of the city of Port Harcourt in the tropical rain forest of southern Nigeria. Activities around the area include palm wine tapping, farming, and thatch sourcing among others. Soil samples were collected using a stainless steel hand auger in a randomized sampling design with 10 sampling points for effective representation of the whole. Fresh poultry manure, sourced from Zuru poultry farm in Port Harcourt city were sun-dried and characterized (Table 9). NPK and Urea fertilizers ere sourced from Okro market at Iriebe, Rivers State of Nigeria. The bulked, air-dried, gently crushed soils were sieved (2 mm screen), chemical and physical properties of the soils were determined (Table 10) prior to experimentation (IXTER or initial character). Mucuna beans, were seeded into soil groups (that were watered and allowed to equilibrate for 2 weeks) after 2 weeks of contamination using Bonny light crude oil in the concentrations of 20, 40, 60, 80 and 100 ml per 2 kg soil in a 45 x 45 cm polyethylene bag (with 3 - 5 holes for free drain and adequate aeration) after conducting toxicity trials to arrive at these concentrations and after biosstimulation. Set-up was in five groups viz: Control experiment (devoid of oil contamination), oil contaminated with no amendments and oil contaminated with NPK, UREA and poultry manure (PM) respectively. Three replicates per treatment were prepared. The spiked soils were allowed 2weeks equilibrations (PRE-P) after which biostimulants (NPK fertiliser, Urea fertilizer and PM) were added (topdressed) and measured quantity were according to the method of Akobundu (1987). Nutrient amendments were covered with a layer of soil. This 3 month outdoor study was done in the rainy season (April - July) and growth parameters measured were leaf area, plant height and root length and vigour. Plants were supplied with adequate clean tap water and were sheltered, although windy rains still interfered. Apart from the treatment groups with biostimulants, no other external nutrient provision was made and thinned plants were put back to their corresponding pots. At harvest, the dried soil and vegetative samples were digested according to Allen et al. (1984) before the potentially toxic heavy metals were estimated using Atomic Absorption Spectrometer (Perkin-Elmer Model 403). The ratio of shoot mean weight to mean height was calculated as a simple index of plant vigor. The ratio was used to facilitate comparison of results from the experimental and control treatments.

RESULTS AND DISCUSSION

Mean concentrations of Cd in soil at 12WAG showed approximately 300% increases on natural Cd load of the soil stemming from spill (Table 1). However, marked decreases were observed with post remediation assessment for all treatment groups. Harvestable portion gave higher Cd concentrations than the root section (Tables 2 and 3). This offers hope for phytoremediation of oil spill site. PM gave mean values ranging from 18.12 - 30.63 mg/kg; UREA, 11.25 - 18.13 mg/kg; NPK, 16.25 - 18.75 mg/kg; CON, 15.00 - 21.88 mg/kg and control CTRL, 5.13 mg/kg in the root samples. There was a linear relationship between treatment groups. At 10% pollution (w/v), a critical load was observed for both UREA and NPK treatment groups (Figures 1 - 5). Also, the shoot gave non-significant values between UREA and NPK. Theirs was however, marked variation between PM and NPK extractability. Analysis of both root and leaf materials indicated evidence of metal uptake and bio-accumulations into plant tissue. The highest Cu load occurred in the shoot, whereas the lowest occurred in the root tissue (Tables 5 and 6). Soils with higher dissolved Cu and Cd have greater potential to release Cu and Cd into plants. This may be due to the fact that extraction is the closest estimation of the pool of the metals in the soil that is susceptible to loss. The highest load was 6times more than the lowest. Most of metal loss load in soil

Table 2. Mean level of Cd (mg/kg) found in root, R.

TRMT	CON	NPK	UREA	PM
R20	15h ± 4	16h ± 5	11g ± 6	18i ± 5
R40	17i ± 5	16h ± 4	15h ± 5	18i ± 5
R60	18i ± 5	18i ± 5	15h ± 6	19i ± 5
R80	19i ± 4	18i ± 6	18i ± 7	20i ± 5
R100	21i ± 5	18i ± 6	18i ± 7	30l ± 5

CTRL = 5b ± 6.

Table 3. Mean level of Cd (mg/kg) found in leaf, L.

TRMT	CON	NPK	UREA	PM
L20	17h ± 5	17h ± 5	12g ± 7	18i ± 23
L40	18i ± 3	18i ± 5	16h ± 7	20i ± 24
L60	19i ± 5	19i ± 5	16h ± 6	21i ± 6
L80	20i ± 6	20i ± 9	18i ± 6	21i ± 5
L100	24i ± 6	25l ± 7	20i ± 7	32l ± 24

CTRL = 8b ± 6.

Figure 1. Mucuna at 12 WAG in different treatment options of poultry manure (PM) treatment groups.

Figure 2. Mucuna at 12 WAG in different treatment options of CON treatment groups.

Figure 3. Mucuna at 12 WAG in different treatment options of NPK treatment groups.

Figure 4. Mucuna at 12 WAG in different treatment options of UREA treatment groups.

Figure 5. Mucuna at 12 WAG in different treatment options of CTRL treatment groups.

(Tables 1 and 4) occurred in a few heavy rain events from May - July because of high rainfall during the period.

Table 4. Mean level of Cu (mg/kg) found in soil, S.

TRMT	CON	NPK	UREA	PM	PRE-P
S20	118c ± 6	125g ± 5	62h ± 6	62h ± 5	312q ± 9
S40	143d ± 5	137g ± 5	68h ± 5	62h ± 5	318q ± 5
S60	187e ± 5	143d ± 5	100i ± 5	62h ± 5	318q ± 6
S80	187e ± 6	162p ± 4	118c ± 6	81m ± 10	312q ± 9
S100	200f ± 4	162p ± 4	125g ± 7	93m ± 8	331r ± 6

CTRL = 48a ± 7, IXTER = 25k ± 5.

Table 5. Mean level of Cu (mg/kg) found in root, R.

TRMT	CON	NPK	UREA	PM
R20	10c ± 4	6n ± 6	12o ± 6	18j ± 6
R40	10c ± 5	18j ± 5	12o ± 6	31k ± 5
R60	10c ± 5	18j ± 5	18j ± 4	43l ± 5
R80	11c ± 5	31k ± 4	37k ± 6	43l ± 5
R100	11c ± 5	31k ± 4	43l ± 4	50l ± 4

CTRL = 37.5b ± 4.09.

Table 6. Mean level of Cu (mg/kg) found in leaf, L.

TRMT	CON	NPK	UREA	PM
L20	16c ± 5	12o ± 5	14o ± 6	25k ± 5
L40	16c ± 4	19j ± 5	18j ± 6	37k ± 6
L60	25g ± 6	25k ± 6	31k ± 6	56k ± 5
L80	25g ± 6	43l ± 5	56l ± 6	62h ± 6
L100	25g ± 6	43l ± 5	62h ± 5	62h ± 6

CTRL = 43b ± 3

Table 7. Composition of the Bonnylight crude oil used in this study.

S/N	Parameter	Amount
1	API gravity at 60F	38.1
2	Specific gravity	0.84
3	Characterization factor	11.75
4	Colour	Brownish-green
5	Acid number	0.39
6	Pour point deg F.	35
7	Salt Content Lbs/1000bbl	77.9
8	Reid water pressure	4.9
9	Sulphur w/w%	0.18
10	Viscosity @ 60F Deg fsu	54.7
11	Viscosity @ 100F Deg fsu	37.8
12	Sediment and Water (bsw) v/v%	Trace
13	Organic Chlorides	8.0
14	Copper strip corrosion	IB
15	Carbon residue w/w%	0.92
16	Iron wt (ppm)	1.0
17	Vanadium wt (ppm)	2.0
18	Nickel wt (ppm)	4.0
19	Crude volume v/v%	32.7
20	Density @ 15℃	0.83

Table 8. Dry Weight (g) of M at Twelve (12 wk) Harvest, DW M.

TRMT	CTRL	CON	NPK	UREA	PM
M20	2.93a±1	1.36b ± 1	1.90b ± 1	2.39b ± 1	6.85a ± 2
M40		0.96c ± 1	1.55b ± 1	2.41b ± 1	6.18a ± 2
M60		0.79c ± 1	1.48b ± 1	3.57b ± 1	5.43a ± 1
M80		0.78c ± 1	1.20b ± 1	1.44b ± 1	4.93a ± 1
M100		0.42d ± 1	0.47c ± 1	0.63c ± 1	2.98b ± 1

Table 9. %Composition of poultry manure used.

Total Nitrogen	1.09
Phosphorus as P_2O_5	1.01
Potassium K_2O	0.55
Calcium	3.62

Table 10. Initial Soil Character prior to experiment.

S/N	Parameter	Amount
1	pH	7.04
2	NO_3 (mg/kg)	4055.00
3	SO_4 (mg/kg)	1725.00
4	PO_4 (mg/kg)	13.90
5	% Total Nitrogen	0.34
6	Cl^- (mg/kg)	70.00
7	% TOM	6.00
8	% TOC	3.49
9	Mg (mg/kg)	215.00
10	Na (mg/kg)	112.00
11	Cd (mg/kg)	103.00
12	Cu (mg/kg)	25.00
13	Fe (mg/kg)	18690.00
14	Conductivity(µs)	12.00
15	K (mg/kg)	5.30
16	Ca	516.00

Generally, results indicate excellent levels of extraction into the plant tissues leaving relatively low residual values in soil in a short reaction period with significant support using poultry manure amendment. Also, following the growth parameters measured - plant height, leaf area, produced biomass and vigour over 12 weeks (data not shown), Mucuna had a radiant growth and survival was approximately 80%. Produced biomass (Table 8), a more reliable growth indices projected poultry manure - assisted phyto-extraction as the best option among the alternatives assessed in this study. Wide range of tolerance was evident and may be exploited by selection in the field of phytoremediation.

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