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RESEARCH ARTICLE

UPTAKE OF LEAD, NICKEL AND COPPER BY THREE *Mucuna* SPECIES

[1]Omosun, G.*, [1]Edeoga, H. O., [2]Markson, A. A. and [2]Madunagu, B. E

[1]Department of Biological Sciences, Michael Okpara University of Agriculture, Umudike Nigeria
[2]Department of Botany, University of Calabar, Calabar, Nigeria

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ABSTRACT

Agricultural soils of oil producing states in Nigeria have been severely affected by oil exploration activities and there is need to restore such soils to a manageable level. Phytoremediation uses plants whose rhizospheres are able to accumulate heavy metals from the soil. In order to know the capacity of *Mucuna* species to grow in soils polluted with crude oil and assess their capability to accumulate heavy metals which are normal components of crude oil, a field experiment was carried out. This experiment was a factorial (3 x 5) arrangement in a completely randomized design. Factors and levels were three species of *Mucuna* (*M. veracruz, M. jaspodea* and *M. ghana*) and crude oil concentrations (volume/weight) in the soil (0 %, 1 %, 2 %, 3 %, and 4 %). Heavy metals (nickel, lead, copper and vanadium) concentrations of crude oil provoked soils were determined prior to planting and then 12 WAP. The three *Mucuna* species accumulated heavy metals in their vegetative parts. Nickel accumulated more in the roots, than in the leaves, while copper and lead were more concentrated in leaves of the three *Mucuna* species, the concentration of copper was higher (highest value of 87 mg/g). However, the highest percentage of heavy metals reduction in the contaminated soils was that of lead with a percentage of 47.37 % reduction in the soil. The uptake of these three heavy metals by the vegetative parts of these *Mucuna* species in response to oil pollution was discussed as a possible use in phytoremediation.

INTRODUCTION

Crude oil is a complex mixture of thousands of hydrocarbons and non-hydrocarbon compounds including heavy metals. Although the toxicity of each individual component is known, the toxicity of complex mixtures such as crude and refined products is extremely difficult to assess (Overton *et al*., 1994). Excess concentrations of some heavy metals in soils such as cadmium, chromium, copper, nickel and zinc have caused the disruption of natural aquatic and terrestrial ecosystems (Meagher, 2000). Some heavy metals at low doses are essential micronutrients for plants but in higher doses they may cause metabolic disorders and growth inhibition for most of the plant species (Claire *et a*l, 1991).

Onuoha *et al*., (2003) reported that beyond 3% concentration in an environment, crude oil becomes increasingly deleterious to soil biota and crop growth. Some workers have observed that some plants can survive metalliferous soils and can tolerate greater than usual amounts of heavy metals or other toxic compounds (Raskin and Ensley, 2000). Several studies have been conducted in order to evaluate the effects of different heavy metal concentrations on live plants.

***Corresponding author:** *omosun@yahoo.co.uk*

Most of these studies have been conducted using seedlings or adult plants (Pichtel *et al*., 2000; Chartterjee and Chartterjee, 2000; Reeves and Baker, 2000; Raskin and Ensley, 2000). In some studies, the seeds have been exposed to the contaminants (Claire *et al*., 1991; Vojtechova and Leblova, 1991; Xiong, 1998).

Alternative agriculture which expands the use of plants well beyond food and fiber is beginning to change plant biology. Biotechnology offers value added use of plants which include phytoremediation, the use of plants to remove pollutants from the environment or render them harmless. With the exception of root crops, plant roots are less utilized and studied than the shoots. However this situation may be changing because of the emerging biotechnology that exploits the ability of plants to transport valuable molecules into and out of their roots. This root based technology is called phytoextraction, a subset of phytoremediation, which uses plants to remove toxic heavy metals from the soil. Chelate assisted phytoextraction (Gleba *et al*., 1999) has been successfully used to remove heavy metals from contaminated soils using specially selected varieties of plants (Dushenov *et al*., 1997; Raskin *et al.,* 1997).

The idea of using plants to remove metals from soils came from the discovery of different wild plants often endemic to naturally mineralized soils that accumulate high concentrations of metals in their foliage (Baker and Brooks, 1989; Raskin *et al*., 1997). It is unfortunate that the enormous costs associated with the removal of pollutants from soils by means of traditional physico – chemical methods have been encouraging companies to ignore the problem. As reported by Raskin *et al*., (1997), the state of the art technology for the remediation of metal polluted soils involves the excavation and burial of the soil at hazardous waste sites at an average cost of $1,000,000 per acre.

Considering the growing number of commercially successful applications and the lack of serious environmental concerns both technologies of phytoremediation and phytoindication are gaining acceptance from the scientific community, the general public and regulators. Due to the problems of pollution in agricultural lands of oil producing states of Nigeria, this is an attempt to validate the technology of phytoremediation to solve these problems. This work therefore will be a major contribution to these innovative technologies which could economically remediate polluted soils by providing a cheap and simple means of restoring crude oil contaminated soils and possibly other soil pollution types.

Most of the plants that have been reportedly used for phytoremediation such as *Arabidopsis thaliana, Thlaspi caerulescens* (Baker *et al.*, 1994); *Helianthus annus*, *Brasicca juncea* (Sridhar and Diehl, 2005) are mostly exotic. There is therefore need to search for a novel phytoextracting plant adapted to our particular ecosystem and climate. In this case, *Mucuna* has been chosen as the plant of choice considering its earlier reported use for phytoremediation (Santos *et al*, 2004). As stated by Garbisu and Alkorta, (2001), a plant to be used for phytoremediation must have a rapid growth rate, possess the ability to produce a high biomass in the field and have a profuse root system. *Mucuna* spp. have these quality stated above, hence its choice for this study. The objectives of this study therefore are to investigate the reduction in heavy metal content of the polluted soil at different levels and also look at the possible accumulation of these heavy metals in the vegetative parts of these *Mucuna* species.

MATERIALS AND METHOD

Study Area

The experiment was conducted in an experimental farm at Michael Okpara University of Agriculture, Umudike. Umudike is located approximately at latitude 05^0 29^1 N, longitudes 07^0 32^1 E. Umudike falls within the rainforest zone of southeastern Nigeria with a mean altitude of 123m. Daily mean temperature ranges from 23^0 C and 32^0 C. The field work was carried out between the months of August to November, 2008.

Design of the Experiment

A factorial arrangement (5 x 3) was used in a completely random experimental design. The factors were crude oil concentration (volume/weight) in the soil (0%, 1%, 2%, 3%, and 4%) and three *Mucuna* species (*M. veracruz, M. jaspodea and M. ghana*), a total of 15 treatments with 5 replications.

Collection of Samples

The soil used in this study had no previous history of crude oil contamination. The soil sample was obtained from the top soil (0 – 15 cm) collected within the Campus of Michael Okpara University of Agriculture, Umudike. The seeds of the *Mucuna* ascensions used were obtained from the Seed Bank of International Institute of Tropical Agriculture, Ibadan. The crude oil was a Nigerian Bonny light blend obtained from Shell Petroleum Development Company (SPDC) Limited Port-Harcourt, Nigeria.

Soil Treatment

Soil samples collected were homogenized and sieved. The contamination with the crude oil was done by thoroughly mixing with the soil in their respective plastic buckets. Soil of 4 kg was treated with 40 ml, 80 ml, 120 ml and 160 ml of crude oil to obtain 1, 2, 3, and 4% v/w (volume/weight) crude oil contamination. Each treatment including the control (0% v/w) was replicated five times.

Determination of Heavy Metals in Soil and Plant Samples

Soil, root and leaf samples were analyzed for presence and quantification of heavy metals. Heavy metals determined were Nickel, Lead and Copper which are normal constituents' of crude oil (Osuji and Onojake, 2004).

Soil Samples

The soil samples at different levels of crude oil contamination and the control (uncontaminated soil sample) were air dried. The dried soil samples were crushed and sieved using a 2 mm sieve. 4 g each of the air dried and finely ground soil samples were weighed and added into crucibles. 4 ml $HClO_4$, 5.0 ml of HNO_3 and 0.5 ml of H_2SO_4 was added into crucibles. The crucibles were swirled gently and slowly digested (heated on a hot plate until white fumes appears. The crucibles were set aside to cool. The samples were transferred to a 50 ml volumetric flask and the content transferred to a 50 ml volumetric flask and the content was diluted to 40 ml volume. The solutions were filtered through Whatman No. 42 filter paper.

Plant Samples

Root and leaf samples of the three *Mucuna* species were oven dried at 30^0 C. These samples were later ground and sieved. 5 g of each ground tissue sample were weighed and placed in crucibles. 20 ml 1:1 HNO_3 / $HClO_4$ acid mixture added into the crucibles, these crucibles were swirled gently, slowly digested and heated to dryness. The cooled residue were then dissolved in 5ml concentrated HCl. This solution was then made up to 50ml using distilled water and filtered using Whatman No. 42 filter paper. Heavy metals (lead, copper, nickel and vanadium) concentrations of the digested soil and plant samples were determined using Atomic Absorption Spectrophotometer (UNICAM SOLAAR 32 Model).

Soil particle size analysis

Particle size of soil components was determined using the Gee and Bauder (1986) method. 50 g of air dried soil was weighed into 500 ml dispersing cup. Sodium hexametaphosphate was used as dispersing agent. 50 ml of calgon was added into the dispersing cup containing the soil samples and allowed to soak for 15 minutes. After 15 minutes, the sample was stirred for 10 minutes and poured into 1 litre cylinder and made up to the 1 litre mark with distilled water. The cylinder was covered with the palm and inverted several times and placed on a flat surface and immediately a hydrometer was slowly and carefully dropped into the soil suspension. The hydrometer reading was taken at exactly 40 seconds (H_1). The temperature readings were recorded (T_1) using a thermometer. The suspension was allowed to stand for 2 hours. After 2 hours the second hydrometer and thermometer readings were recorded (H_2 and T_2).

Calculation

% Silt and Clay = $[H_1 + 0.2\ (T_1 - T) - 2.0] \times 100/50$

% Clay = $[H_2 + 0.2\ (T_2 - T_1) - 2.0] \times 100/50$

% Sand = 100 - % (silt and clay)

Where T = constant (68^0 F)

Soil pH

10 g of soil was weighed into a plastic cup. 25 ml of distilled water was added. The mixture was stirred with a glass rod and allowed to stand for 30 minutes. The electrode of the pH meter was inserted into the soil suspension and the reading taken. (Thomas, 1996).

RESULTS AND DISCUSSION

Soil physical and chemical properties and heavy metal bioavailability

The result of the textural class and the pH of the soil are shown in Table 1. From the result, the particle size analyses show the texture of the soil to be sandy clay loam. The pH of the soil is 5.23, this indicates that the soil is acidic in nature. These two physical parameters are very important when considering metal interactions in soils and their bioavailability to plants. Naidu *et al*., (2003) stated that soil characteristics (e.g. soil pH, clay, organic matter content and type, and moisture content) determine availability of metals to plants by controlling the speciation of the elements, temporary binding by particle surfaces (adsorption–desorption processes, precipitation reactions and availability in soil solution.

Table 1. Physical properties of soil studied.

Properties	Values
Ph	5.23± 0.007
% sand	53.4%
% silt	21.8%
% clay	24.7%
Textural class	Sandy clay loam

Fotovat *et al*., (1997) have shown that natural soil of pH values of 5–6 retain less metals when compared to soils of pH 6–7 which retain more metals. On the contrary, soils with pH 5–6 exhibit high metal bioavailability to plants, while soils of 6 – 7 exhibit low bioavailability of metals to plants.

This means that plants growing in different soils with the same total metal concentration may vary in their phytotoxic response due to differences between the soils in their sorptive capacity. The results of particle size analysis shows that the soil is high in clay content (24.7%) and clayey soils are known to have a high, therefore low binding capacity to the soil. Vanadium's low concentration in the soil might have been caused by the metal not binding to the soil and hence lost through leaching.

From Table 2 it can be seen that nickel and copper out of the three heavy metals investigated had higher concentration in the soil. Naidu *et al*., (2003) have listed cadmium, copper, zinc, arsenic, and lead as heavy metal contaminants of major concern. Copper and lead belong to this group.

Table 2. Heavy metal content of the different concentrationof crude oil provoked soils investigated

Treated soils	Heavy metal concentration (mg/g)			Mean pH values
	Ni	Pb	Cu	
0%	98±0.034	7±0.087	95±0.500	5.23±0.007
1%	256±0.043	18±0.009	168±0.765	5.04±0.005
2%	273±1.090	26±0.560	192±0.548	4.94±0.098
3%	301±0.874	34±2.288	212±0.151	4.81±0.001
4%	323±01.776	38±1.124	241±0.009	4.72±0.061

Heavy metals uptake by the *Mucuna* species investigated (phytoextraction)

The result of the determination of the four heavy metals content in the different crude oil polluted soils and of the roots and leaves of the three *Mucuna* species are represented in Fig. 1 – Fig. 3. Fig. 1 shows the concentration of nickel in the soil and roots and leaves of *M. jaspodea, M. vereacruz* and *M. ghana*. The highest concentration of nickel in the soil was (241 mg/g), while the lowest was observed in the leaves of *M. veracruz* (21 mg/g) and *M. jaspodea* (14.2 mg/g). The same trend of high contents of heavy metals in the soil than in the roots and leaves of the three *Mucuna* spp. was recorded for nickel, lead, and copper. However, nickel, lead, and copper accumulated more in the leaves than in the roots of the three *Mucuna* spp. The mean value of copper concentration (51 mg/g) in *Mucuna ghana* was more than that of lead (3 mg/g) (Fig. 2 – Fig. 3).

Table 3. Percentage reduction of nickel, lead and copper in soil 12 weeks after planting of the Mucuna species investigated

Mucuna species	Crude oil polluted soil	Percentage reduction in the soil (%)		
		Ni	Pb	Cu
M. veracruz	0%	18.37	14.29	5.26
	1%	32.81	27.78	29.76
	2%	33.00	38.46	35.41
	3%	31.56	47.06	36.79
	4%	25.70	44.74	41.49
M. jaspodea	0%	23.47	14.29	6.32
	1%	31.64	27.78	30.35
	2%	25.64	38.46	34.90
	3%	32.56	44.12	36.32
	4%	26.31	47.37	41.49
M. ghana	0%	26.53	28.57	10.53
	1%	33.20	27.78	31.55
	2%	34.06	42.30	35.42
	3%	32.23	47.06	36.32
	4%	28.39	47.37	42.74

Garbisu and Alkorta (2001) reported that some trace heavy metals such as lead are accumulated in roots, probably due to some physiological barriers against metal transport to aerial parts, while some others are easily transported in plants. This probably accounts for the high concentration of lead and nickel in the roots compared to the low concentration of copper in the roots of the three *Mucuna* species. Raskin *et al*. (1994) stated that most plants can accumulate heavy metals essential for growth and development such as iron, manganese, zinc, copper,

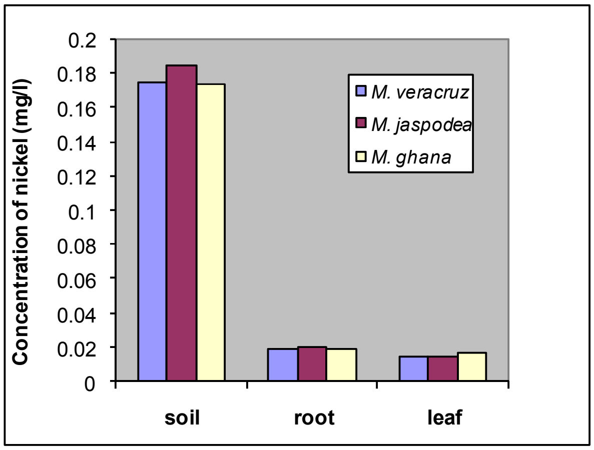

Fig 4.1. Respective concentrations of the nickel in soil, roots and leaves of the *Mucuna* spp. studied.

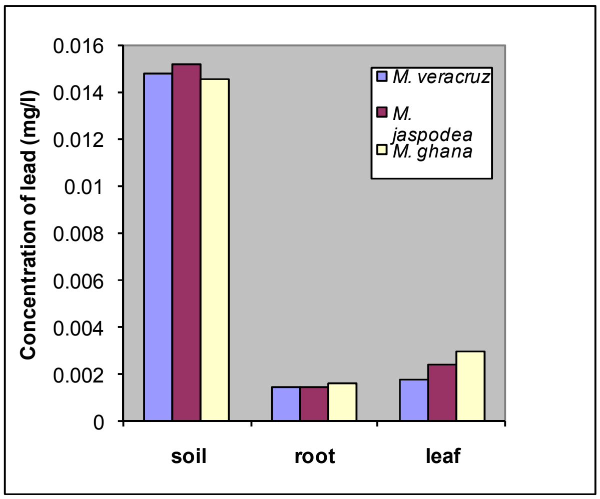

Fig. 4.2. Respective concentrations of lead in soil, roots and leaves of the *Mucuna* spp. studied.

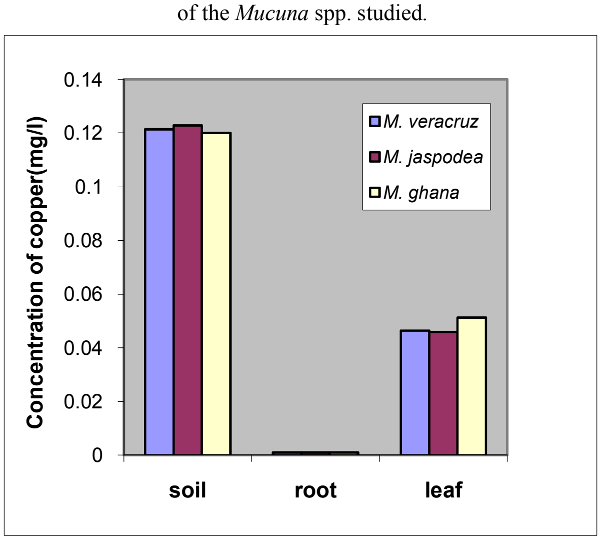

Fig 4.3. Respective concentrations of copper in soil, roots and leaves of the *Mucuna* spp. studied.

magnesium and molybdenum. Copper belongs to this class of essential metal elements needed by plants for growth. This could be an explanation for the high concentration of copper in the leaves of the three *Mucuna* species.

In addition, some plants have the capacity to accumulate heavy metals with no known biological functions such as cadmium, chromium, lead, cobalt, mercury and selenium (Baker and Brooks, 1989). Lead has no known biological functions. Excess lead causes a number of toxicity symptoms in plants e.g. stunted growth, chlorosis and bsslackening of root system. Lead inhibits photosynthesis upsets mineral nutrition and water balance (Xiong, 1998). Highest mean value of 3 mg/g of lead was observed in *M. ghana*. Lead is not usually available for plant uptake in normal range of soil pH (Raskin *et sssal*., 1997). Cunningham *et al*., 1995) have reported vegetation growing in heavily contaminated areas having less than 50 mg/g in shoots. In the present study, the highest value of lead concentration in shots recorded (3 mg/g) was far less than the 50 mg/g mark reported by Cunningham *et al*. (1995).

The low level of nickel and lead in the roots and leaves compared to the relatively high concentration in the soil could be attributed to the heavy metal tolerance of these three *Mucuna* species. Tolerance to heavy metals as explained by Robinson *et al*. (1994), is based on the sequestration of heavy metal ions in vacuoles, on binding them by appropriate ligands like organic acids, proteins and peptides and on the presence of enzymes that can function at high levels of metallic ions. Garbisu and Alkorta (2001) listed among other factors, the tolerance of a plant to high levels of a heavy metal, as a good characteristic of an ideal plant to be used for phyto extraction purposes.

Plant roots can increase metal bioavailability by extruding protons to acidify the soil and mobilize metals. Crowley *et al.* (1991) observed this mechanism for iron (Fe) in some Fe-deficient dicot plants. Studies have shown that lowering the soil pH decreases the adsorption of heavy metals and thus increases their concentration in the soil solution for uptake by plants (Harter, 1983; Salt *et al*., 1995; Garbisu and Alkorta, 2001). Salt *et al.* (1995) further stressed that it may be possible to increase metal availability and hence plant uptake by maintaining a moderately acid pH in the soil through the use of ammonium containing fertilizer or soil acidifiers. *M. jaspodea, M. veracruz* and *M. ghana* are promising hyperaccumulators considering the factors discussed so far. Salt *et al*. (1995) identified the first hyperaccumulators to be characterized to be members of the Brassicaceae and Fabaceae families. *Mucuna* spp. belongs to the Fabaceae family.

Table 4.3 shows the level of the heavy metals in the soil 12 weeks after planting. The highest percentage (47.37%) of ressduction was observed in lead, across the soils of the three species. From the table, the trend is a general reduction of metals in the soil as the crude oil concentration increases. This means that the *Mucuna* species accumulate more heavy metal as the concentration of oil in the soil increases. This increased accumulation or bioavailability of metals could be attributed to lower pH of the more polluted soils (Table 4.2). Fotovat *et al*. (1997) have reported that soils of lower pH exhibit high metal bioavailability to plants, while soils of higher pH exhibit low bioavailability of metals to plants.

CONCLUSION

This study has provided an insight into the value-added use of *Mucuna* species in the novel areas of phytoremediation and phytomonitoring. The information generated can be practically applied for restoring oil polluted soils by using it to remove the heavy metal components of crude oil. The percentage reduction of heavy metals from the soils recorded proves that these *Mucuna* species are good phytoextractors of heavy metals from the soil. Using *Mucuna* for phytoremediation of polluted soils will be innovative because it takes advantage of natural plant processes. This method if adopted requires less equipment and labor than other methods since plants do most of the work. The *Mucuna* plants will also help to revegetate the polluted site since these plants can tolerate oil polluted soils, and as well make the site more attractive. The site can be cleaned up without removing polluted soil or pumping polluted groundwater.

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