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Phytoremediation potential of velvet bean (*Mucuna prurens* L. DC) and maize (*Zea mays* L.) on petroleum product polluted soil

Edache Bernard Ochekwu*, D. I. Anyanwu and Kenechukwu Dike

Department of Plant Science and Biotechnology, University of Port Harcourt, Port Harcourt, Nigeria

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ABSTRACT: Phytoremediation potential of Velvet bean and Maize was carried out at the University of Port Harcourt, Botanic Garden. The seeds where grown in different soil bags of 20kg and polluted with 1000ml each of different treatment of AGO, PMS and DPK. The values of Heavy metal concentration in the soil was assessed and analyzed for Zn, Fe and Cu. Velvet bean and maize in AGO contaminated soil (Fe: 355g/ml., Zn: 240g/ml and Cu: 100g/ml., PMS soil (Fe: 75.0g/ml, Zn: 3.0g/ml, and Cu: 155.5g/ml), DPK soil (Fe: 150g/ml, Zn: 30.0g/ml, Cu 150g/ml). After 12 weeks of planting velvet bean, heavy metal in AGO contaminated soil (Fe 170.5g/ml, Zn 100.5g/ml and Cu 60.4g/ml), PMS soil (Fe 60.5g/ml, Zn 2.0g/ml and Cu 101g/ml), DPK soil, (Fe 103g/ml, Zn 24.8g/ml and Cu 55.0g/ml). For Maize soil, the heavy metals in AGO soil (Fe 350g/ml, Zn 220g/ml and Cu 105g/ml)., PMS soil (Fe 70.0g/ml, Zn 2.3g/ml and Cu 110g/ml)., DPK soil, (Fe 105.5g/ml, Zn 27.2g/ml and Cu 75.5g/ml. At harvest, the result of plant height showed a gradual increase in PMS concentration of velvet bean than AGO and DPK. Differences were not recorded for number of leaves and Leaf Area. Also the different treatment of petroleum product on maize showed reduction in yield after harvest. However unpolluted (control) soil gave the highest total plant height and highest biomass value. Velvet bean had a better phytoremediation potential to maize as AGO, PMS and DKP showed more deleterious effect on maize plant by interfering with the physiology and metabolic activity of the plant.

Key words: Phytoremediation, Petroleum products, velvet bean and maize

Introduction

Velvet bean (*Mucuna pruriens* L. DC) is an annual climbing legume that grows 3-18m in height, the genus mucuna, belonging to the family Fabaceae, the plant grows well in tropical areas of India and Africa (Engelking, 2005).When velvet bean plant are young they are covered with hair which disappears as the plant gets old, the leaves are of various sizes and shapes, and when velvet bean plant are young you can find hairs on both sides of the leaves. Mucuna varieties are herbaceous with an annual life cycle which ranges from 100 to 290 days according to Buckles (1995) and Keatinge *et al* (1996).

*Author to whom all correspondence should be addressed.
E-mail: edacheb@yahoo.com, eb.ochekwu@uniport.edu.ng, Tel: +234 (0)7034501934

The plant also produces clusters of pods which contain seeds known as mucuna beans, the pods are covered with reddish-orange hairs that are readily dislodged and can cause intense irritation to the skin, the species name pruriens (from the Latin word "Itching sensation") refers to the result to be had from contact with the seed pod hairs. The itch caused by the pods is due to the presence of proteins serotonin and mucunain. There are many regions in Africa where the quality of the soil is degraded because of intensified agriculture, *Mucuna pruriens* grows well in these areas to weed out infestation and restore soil fertility. Mucuna is a short-day annual (Duke 1981) so that it flowers in October/November in the northern hemisphere.

The toxicity of unprocessed velvet bean may explain why the plant has few problems with insect's pest, velvet bean is well known for its nematocidic effects when used in rotation with a number of commercial crops. It also seems to possess a notable allelopathic activity, which suppresses competing plants, it can however harbor soil borne pathogens such as *Macrophomina phaseolina,* which is detrimental to velvet bean and other food crop. (Manyam, 2004). The United states produced the largest amount of maize throughout the world, but there are other countries that also produce high qualities of maize such as China, Brazil and South Africa, Maize is susceptible to water logging (Kay 1979).

In 2003 there was an estimated six hundred metric tons of maize produced in areas that do not have extreme cold temperature as it is a cold-intolerant crop, maize is a cereal plant that produces grains that can be cooked, roasted, fried, ground, pounded or crouched to prepare various food items like pap, and various food industries. Soil pollution is a buildup in the soil of toxic chemical compounds salts, pathogens or radio-active materials that can affect plant and animals life (Engelking, 2005). Soil is a mixture of mineral, plant, and animals that form during a long process that may take thousands of years. It is necessary for most plant growth and is essential for all agricultural production when soil is polluted the thin layer of the fertile soil that covers much of the earths land and is vital for the growth of vegetation is damaged (Adam and Duncan, 2003).

Soil pollution weakens the plants they succumb to natural stresses such as insects, disease or climate extremes that they otherwise might have withstood (atlas *et al* 1997). Kerosene, Petrol and Diesel are refined products of petroleum. They are alkane hydrocarbon, crude oil is a complex mixture of hydrocarbon and compounds contain oxygen, sulphur nitrogen and trace amounts of metal, refined crude oil yields such relevant component like kerosene, petrol, diesels, fuel oil, alkane and aromatic pesticides. Kerosene, Petrol and Diesel are important petroleum products obtained through fractional distillation process. In this process petroleum is heated in steel or bubble tower in order to vaporize the different hydrocarbons it contains, the separate fractions which have different boiling point, condense to liquids as they are cooled in the condenser. Kerosene has been recovered from other substances, notably coal, oil shale and wood it is used in lamps canner in insecticide sprays and the most aggressive of all the product.

Diesel is used for power generator and transport motors, kerosene, petrol and diesel are used in industries as primary raw materials and this in turn has resulted in an increased in its production and transportation thereby resulting in pollution of the environment. When these products are released into the environment, they become dangerous pollutants with tremendous adverse effects on the abiotic and biotic compounds of the affected habitats. (Kinako,1981). This work is aimed at knowing the phytoremediation potential of velvet bean and maize to decontaminate soil polluted with different petroleum products such as DPK, AGO and PMS. Also, the objectives aim at determining the effect of these petroleum products on the plant and the ability of these plants to remediate the soil through different growth parameters.

Materials and Methods

The Parameters measured is used to determine the stages of development in velvet bean and maize plant growing under contaminated soil, from the second week to twelve week of planting. Growth can be measured as increase in length, leaf area, stem, number of leaves and girth increase; increase in mass is often determined by harvesting the entire plant or the part of interest and weighing it immediately before too much water evaporates from it. Also increase in dry mass of a plant or plant parts is used to measure growth. The dry mass is commonly obtained by drying fresh plant for 24-48hours at 70-80 ^{o} C. Weighing balance, Petroleum products: Premium Motor Spirit (PMS): Dual Purpose Kerosene (DPK) and Automobile Gasoline Oil (AGO). Materials used include, Petri dishes, Loamy Soil (20kg) each for 40 bags, Conical flask, cylinders, pH, meter (Jenway 3015^{PH}), Foil paper, methyl orange indicator (H_2SO_4), Seed (velvet bean and maize).

The experiment was carried out in the University of Port Harcourt, Plant Science and Biotechnology Laboratory and Botanical garden. Collection of Materials (Source of Petroleum product used for this experiment was bought from NNPC mega filling station, on East West Road fueling station at east west road Port Harcourt. Soil sample were collected during each sampling for physicochemical analysis, soil sampling started two weeks after soil has been polluted, the following physicochemical properties were taken after soil have been polluted with different petroleum products; the parameter calculated for are conductivity, soil pH, Alkalinity, Total heterotrophic bacteria count, Total heterotrophic fungi count, Total bacterial count, Total fungi count and heavy metals. The data collected were statically analyzed using SAS (2007, version 9.1) statically Package for treatments tested. The mean were separated using least Significant Difference (LSD) at 5% level of probability.

Results

At two weeks after Planting of maize plant, Treatment 4 was significantly ($p \leq 0.05$) higher than treatment 1, 2 and 3 for Plant Height and Leaf Area. For number of leaves, significant ($p \leq 0.05$) differences did not occur among the treatments (Table 1). At four weeks after Planting of the maize plant, Treatment 4 is significantly ($p \leq 0.05$) higher than treatment 1, 2, and 3, for leaf area and plant height. For number of leaves, significant ($p \leq 0.05$) different does not exist among the treatments (Table 2). At eight weeks after Planting of the maize plant, Treatment 4 is significantly ($P \leq 0.05$) higher than 1, 2 and 3 for plant height and leaf area, for number of leaves significant ($P \leq 0.05$) differences does not exist among the treatment (Table 3). At twelve weeks after Planting of the maize plant, Treatment 4 is significantly ($p \leq 0.05$) higher than treatment 1,2 and 3 for plant height and leaf area, for number of leaves significant ($p \leq 0.05$) differences does not exist among the treatments (Table 4). At two weeks of planting of velvet bean, Treatment 4 is significantly ($P \leq 0.05$) higher than treatment 1,2, and 3 for plant height, number of leaves and leaf area (Table 5). At four weeks of planting of velvet bean, Treatment 4 is significantly ($P \leq 0.05$) higher than treatment 1,2, and 3 for plant height, number of leaves and leaf area (Table 6). At eight weeks of planting of velvet bean, Treatment 4 is significantly ($P \leq 0.05$) higher than treatment 1,2, and 3 for plant height, number of leaves and leaf area (Table 7). At twelve weeks of planting of velvet bean, Treatment 4 is significantly ($P \leq 0.05$) higher than treatment 1,2, and 3 for plant height, number of leaves and leaf area (Table 8).

Table 1: MAIZE Mean of growth parameters measured at 2 weeks after planting

Treatment	Mean PH	Mean NL	Mean LA
1	3.920^{bc}	3^{a}	4.6^{b}
2	11.720^{b}	3.2^{a}	4.7^{b}
3	5.160^{c}	2.4^{a}	3^{b}
4	20.060^{a}	3.4^{a}	16^{a}
L.S.D.	6.0104	1.5252	7.6331

The values represent the means of 5 replicates, means in the same column with the same letters are not significantly different from each other.

Key-

Treatment 1	-	AGO	Treatment 2	-	PMS
Treatment 3	-	DPK	Treatment 4	-	control

Table 2: Mean of growth parameters at 4 weeks after planting

Treatment	Mean PH	Mean NL	Mean LA
1	1.260^{b}	9.00^{a}	10.20^{b}
2	21.800^{b}	9.60^{a}	13.60^{b}
3	11.400^{b}	7.20^{a}	8.60^{b}
4	37.200^{a}	9.60^{a}	29.80^{a}
L.S.D.	11.173	3.0295	14.077

The values represent the means of 5 replicates, means in the same column with the same letters are not significantly different from each other.

Table 3: Mean of growth parameters at 8 weeks after planting

Treatment	**Mean PH**	**Mean NL**	**Mean LA**
1	22.800^{b}	10.2000^{a}	18.60^{b}
2	30.600^{b}	11.0000^{a}	20.00^{b}
3	22.800^{b}	9.6000^{a}	16.60^{b}
4	55.160^{a}	10.8000^{a}	48.60^{a}
LSD	16.891	2.1606	22.646

The values represent the means of 5 replicates, means in the same column with the same letters are not significantly different from each other.

Table 4: Mean of growth parameter at 12 weeks after planting

Treatment	**Mean PH**	**Mean NL**	**Mean LA**	**Fresh Weight**	**Dry weight**
1	25.00^{b}	10.2000^{a}	18.60^{b}	5.274	1.758
2	30.60^{b}	11.000^{a}	20.00^{b}	5.928	1.976
3	22.20^{b}	9.6000^{a}	16.60^{b}	5.31	1.770
4	55.360^{a}	10.800^{a}	48.60^{a}	21.456	7.152
LSD	16.918	2.1606	22.646	17.2455	5.7485

The values represent the means of 5 replicates, means in the same column with the same letters are not significantly different from each other.

Table 5: Velvet bean - Mean of growth parameter at 2 weeks after planting

Treatment	Mean PH	Mean NL	Mean LA
1	6.52^{b}	5.20^{b}	6.60^{b}
2	20.80^{b}	6.60a^{b}	11.20^{b}
3	9.56^{b}	3.20^{b}	6.80^{b}
4	49.44^{a}	19.60^{a}	71.20^{a}
LSD	15.479	13.816	7.6028

The values represent the means of 5 replicates, means in the same column with the same letters are not significantly different from each other.

Table 6: Mean of growth parameters at 4 weeks after planting

Treatment	Mean PH	Mean NL	Mean LA
1	13.06^{b}	10.20^{b}	12.60^{b}
2	45.38^{b}	9.60^{b}	21.80^{b}
3	21.62^{bc}	6.00^{b}	13.80^{b}
4	93.16^{a}	19.00^{a}	41.60^{a}
LSD	30.479	5.2216	12.24

The values represent the means of 5 replicates, means in the same column with the same letters are not significantly different from each other.

Table 7: Mean of growth parameters at 8 weeks after planting

Treatment	Mean PH	Mean NL	Mean LA
1	43.56^{c}	35.60^{b}	33.40^{c}
2	121.0^{b}	82.40^{b}	71.80^{b}
3	71.40^{bc}	38.00^{b}	50.60^{bc}
4	186.00^{a}	132.60^{a}	100.40^{a}
LSD	58.876	51.017	22.408

The values represent the means of 5 replicates, means in the same column with the same letters are not significantly different from each other.

Table 8: Mean of growth parameters at 12 weeks after planting

Treatment	Mean PH	Mean NL	Mean LA	fresh weight	Dry weight
1	44.16^{c}	36.00^{b}	34.20^{b}	8.055	2.685
2	118.96^{b}	78.80^{b}	72.40^{b}	25.8	8.60^{b}
3	71.60^{cb}	53.40^{b}	51.20^{cb}	14.316	4.772
4	187.00^{a}	132.60^{a}	100.80^{a}	38.37	12.824
LSD	65.2	60.256	27.544	31.6569	10.5523

The values represent the means of 5 replicates, means in the same column with the same letters are not significantly different from each other.

Table 9: Values of Conductivity, Alkalinity and pH

	CONDUCTIVITY	ALKALINITY	pH
AGO	3.6	4.8	3.2
PMS	3.2	4.5	3.7
DPK	3.4	4.7	3.8
CONTROL	3.0	4.0	5.0

Table 10: Soil Test

	THB (ml/g)	HUB (CFU)	THF(CFU)	HUF (CFU)
PMS	67+27 94/2 = $4.7x10^{5}$	$9.9x10^{4}$	$2.51x10^{5}$	$17x10^{4}$
DPK	86+38 124/2 = $6.2X10^{5}$	$5.6x10^{3}$	$1.73x10^{5}$	$1.5x10^{4}$
AGO	3.6+26 62/2 = $3.1x10^{5}$	$1.23x10^{5}$	$1.05x10^{5}$	$1.5x10^{4}$
Control	47+29 76/2 = $3.8x10^{5}$	1.0610^{5}	$1.7x10^{4}$	$1.2x10^{4}$

Where ml/g =mill per gram; CFU = Coliform Forming Unit

- HUB =Hydrocarbon utilizing bacteria
- HUF =Hydrocarbon utilizing fungi
- THBC =Total heterotrophic bacteria count
- THFC =Total heterotrophic fungal count

Table 11: Heavy metal accumulation in soil

Metal content (g/ml)	**Before planting**	**12 weeks after planting in soil of maize**	**12 weeks after planting in soil of velvet bean**
AGO	355	350	170.5
Iron (Fe)			
Zinc (Zn)	240	220	100.5
Copper (Cu)	100	105	60.4
PMS	75.0	70.0	60.5
Iron (Fe)			
Zinc (Zn)	3.0	2.3	2.0
Copper (Cu)	155.5	110	101
DPK	150	105.5	103
Iron (Fe)			
Zinc (Zn)	30.0	27.2	24.8
Copper (Cu)	150	75.5	55.0

Table 12 Heavy metal accummulation in plant

Metal content (g/ml)	Before planting in soil	12 weeks after planting of Maize	12 weeks after planting Velvet bean
AGO	355	20.0	69.9
Copper (Cu)			
Zinc (Zn)	240	16.7	100
Iron (Fe)	100	30.5	51.2
PMS	75.0	20.6	34.3
Copper (Cu)			
Zinc (Zn)	3.0	1.5	2.1
iron (Fe)	155.5	3.6	10.7
DKP	150	16.0	39.3
Copper (Cu)			
Zinc (Zn)	30.0	20.6	24.1
Iron (Fe)	150	35.4	55.7

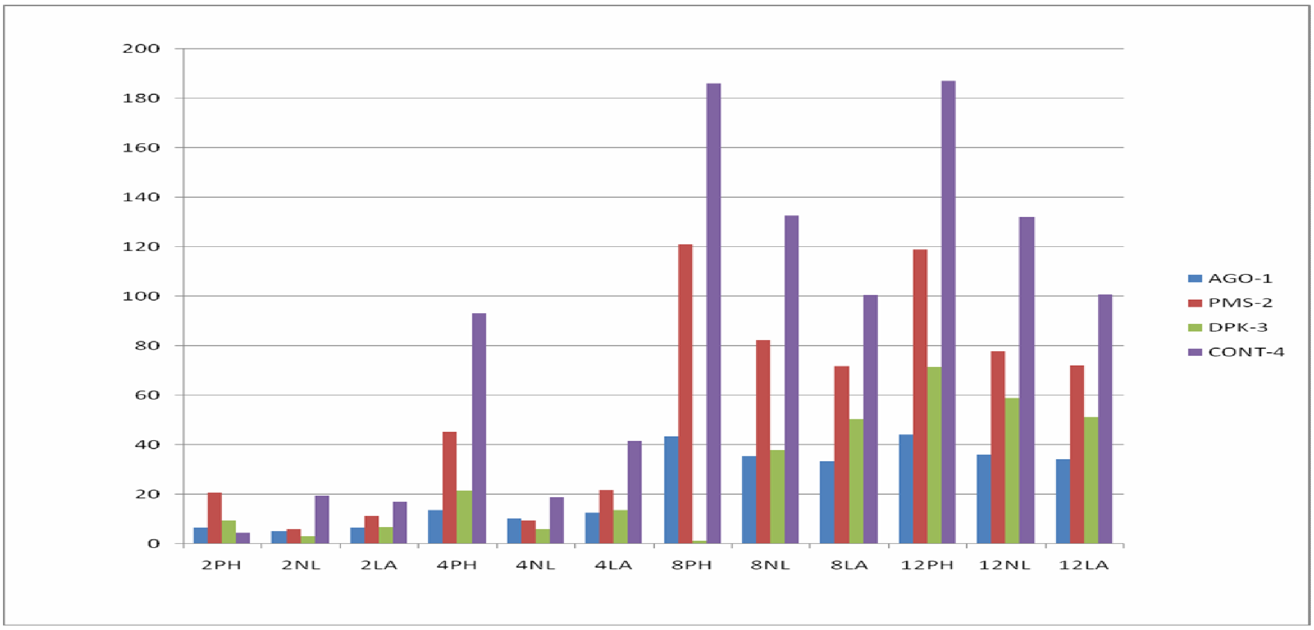

Fig 1: General treatment (Maize)

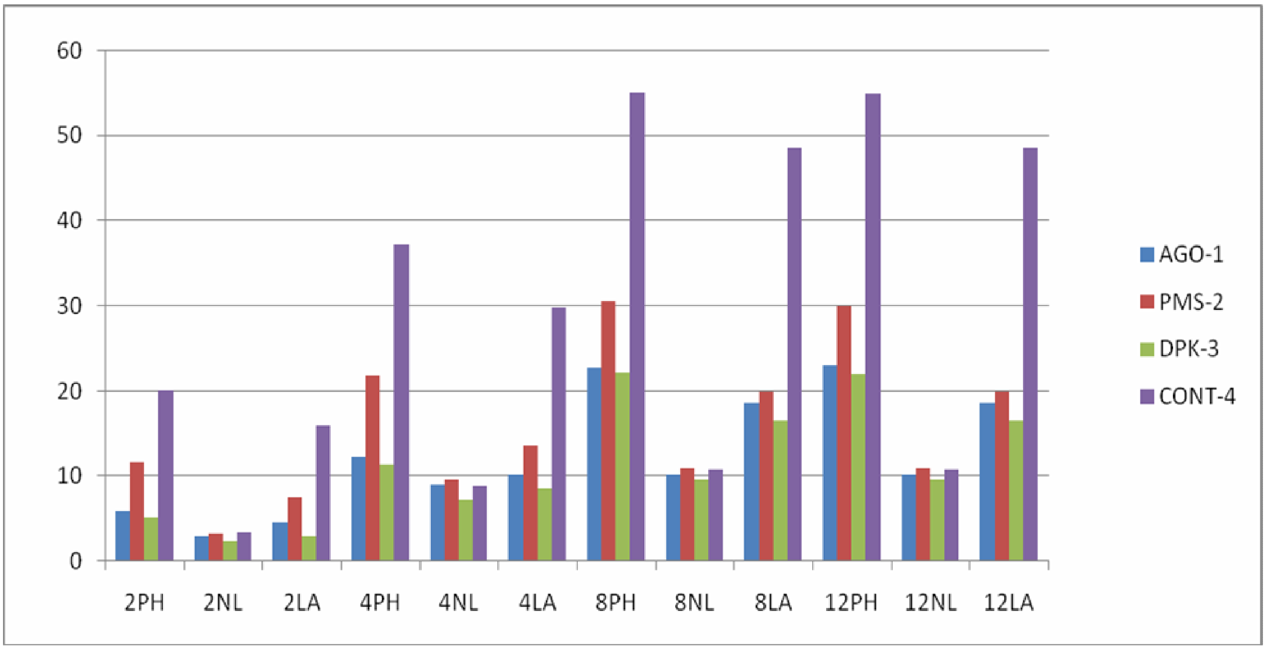

Fig 2: General treatment (Velvet bean)

KEY: 2PH: Plant height at 2weeks after planting; 2NL: number of leaves; LA: Leaf Area at 2Weeks after planting; 4PH: Plant height at 4weeks after planting; 4NL: number of leaves at 4weeks after planting; 4LA:Leaf area at 4weeks after planting; 8PH: Plant height at 8weeks after planting, 8NL: number of leaves at 8weeks after planting; 8LA: Leaf area at 8 weeks after planting, 12PH: Plant height at 12 weeks after planting; 12NL: Number of leaves at 12 weeks after planting; 12LA: Leaf area at 12 weeks after planting.

Discussion

The concentration of heavy metals varied among velvet bean and maize plant. The data indicates that heavy metal accumulation was higher in Velvet bean plant. As seen from the heavy metal analyzed for Zn, Fe and Cu. Velvet bean and maize in AGO contaminated soil (Fe: 355g/ml., Zn: 240g/ml and Cu: 100g/ml., PMS soil (Fe: 75.0g/ml, Zn: 3.0g/ml, and Cu: 155.5g/ml), DPK soil (Fe: 150g/ml, Zn: 30.0g/ml, Cu 150g/ml). After 12 weeks of planting velvet bean, heavy metal in AGO contaminated soil (Fe 170.5g/ml, Zn 100.5g/ml and Cu 60.4g/ml), PMS soil (Fe 60.5g/ml, Zn 2.0g/ml and Cu 101g/ml), DPK soil, (Fe 103g/ml, Zn 24.8g/ml and Cu 55.0g/ml). For Maize soil, the heavy metals in AGO soil (Fe 350g/ml, Zn 220g/ml and Cu 105g/ml), PMS soil (Fe 70.0g/ml, Zn 2.3g/ml and Cu 110g/ml)., DPK soil, (Fe 105.5g/ml, Zn 27.2g/ml and Cu 75.5g/ml.

Velvet bean showed more phytoremediation potential compared to maize which had inhibitory effect of these pollutants on the crop. This may be due to the interference with the metabolic and enzyme activities of the crop as seen from the values above. There was higher increase in growth parameters measured for velvet bean than that of maize plant. The yellowing of leaves observed might be due to deficiency of nitrogen fixing bacteria in the plant, these pollutants in soil caused decrease in decomposable micro organism's composition in soil (Atlas *et al*., 1976) and is also known to inhibit non-symbiotic nitrogen fixation by rhizobuim.

For maize plant the pollutants reduced the crops productivity by affecting the photosynthetic activities and mineral absorption by roots thereby interfering with manufacturing and distribution of food. This may be due to the ability of oil to penetrate the cells of tissues, which resulted to their death and also its interference with the enzymic and phytochromonal systems of the plants (Minshall and Helson 1991). However velvet bean plant showed a higher phytoremediation potential than maize plant.

The study has demonstrated that soil polluted with petroleum product of AGO, DPK and PMS can lead to gradual degradation and heavy metal build up in velvet bean and maize plant. The heavy metals present in the contaminated soils when absorbed by plants are capable of making the plant leaves potentially toxic and harmful to man and livestock. Petroleum hydrocarbon contamination of top soil renders the soil in the surrounding area unsuitable for plant growth reducing the availability of nutrient which may result to reduction in crop yield and the plant and also intoxicate the soil which may adversely affect the microorganisms in the soil, oil exerts adverse affects on soil conditions, on micro organism and on plants. Oil pollution has damaging effect on plants species (Kinako, 1981) plant usually go extinct mostly after oil spill. The plants studied showed that Velvet bean remediate pollutants more in the soil compared to maize plants.

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