HYDROCARBON DEGRADATION IN POULTRY DROPPINGS AND CASSAVA PEELS-AMENDED TYPIC PALEUSTULTS IN SOUTHEASTERN NIGERIA.

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ABSTRACT
This greenhouse study was aimed at determining the potentials of poultry droppings (PD) and cassava peels (CP) for nutrient-enhanced biodegradation of petroleum hydrocarbon (THC) in a well drained Typic Paleustults using the THC levels and degradation duration as remediation indices. The performance of the organic amendments was compared against the performance of NPK fertilizer. The soil was contaminated with crude oil at the rate of 50 t/ha, after which the soil amendments were applied in single and combined forms (25 t PD/ha + 25 t CP/ha, and 50 t PD/ha + 50 t CP/ha), with the control as 0 t/ha (without soil amendment). Soil samples were collected from the contaminated soils at 14, 45, 76, 131 and 201 days after the application of the amendments respectively. There were significant (P = 0.05) effects of the amendments and rates of application on the THC concentrations. A comparison of the effects of PD, CP, NKP fertilizer and PD + CP on the THC concentrations at 201 days showed decrease in the contaminant concentrations in the order: NPK fertilizer < PD < CP + PD < CP < Control. The percent THC degradation at 201 days for 50t CP and 100t CP were 23 % and 24 % respectively, while that of 50t PD and 100t PD were 34 % and 36 % respectively. For soils treated with NPK fertilizer and CP + PD, the percent THC degradation was 40 % and 41 % for 50t NPK and 100t NPK fertilizers respectively, and 26 % and 31 % for 25t CP + 25t PD and 50t CP + 50t PD respectively. The time required to completely degrade the THC in the soil followed the same trend. Although PD showed great potential for THC degradation, it will take less time to achieve 100 % degradation of THC in NPK fertilizer-amended soil when compared with the other nutrient supplements.

Keywords: Biodegradation; Organic amendments; Soil contamination; Total hydrocarbon

INTRODUCTION

Since oil pipeline network is widespread across almost all the agro-ecological zones in Nigeria, oil spillages are no longer restricted to the oil-producing zones. Incidences of crude oil spills at non-oil producing zones, and the consequent contamination of terrestrial ecosystems abound. Accidental spillages due to oil well blow-outs or pipeline leakages; and continuous low level inputs such as natural seepage from oil reservoirs in sea-beds, the discharge of oil containing effluent from oil terminals and quality control centers and discharges from boat traffic are the main types of petroleum leakage into the environment (Isinguzo and Odu, 1983). Under natural environment, crude oil pollution resulted in increased percentage organic carbon and decreased phosphorus (Ogboghodo *et al*., 2004). These manifest in the alteration of the ecological equilibrium such as change in biodiversity and soil biomass, and alteration of soil physico-chemical status, with the consequent abandonment of such lands; leading to reduction of productive land resource base available to rural farmers in such zones.

The undesirable ecological and socio-economic effects associated with oil spills have led to the development of remediation techniques aimed at reducing the adverse effects of oil in the environment. Such technologies include strategies designed to stimulate indigenous subsurface hydrocarbon–degrading bacteria (Thomas *et al*., 1987, Lee *et al*., 1988), air stripping funnel and gate systems and vapor extraction systems which are of a typically high cost or have limited application (Curtis and Lammey, 1988), and require monitoring and control for optimum performance, and do not usually result in a complete destruction of the contaminant (Yerushalmi *et al*., 2003). Due to continual fiscal constraints and adverse effect on the environment, an environmentally friendly approach to effectively mitigate or remediate hydrocarbon releases into the environment, becomes inevitable. Biological degradation of contaminant or pollutant in the environment has been described as a proven method of remediating petroleum–contaminated soils, and soils contaminated by many other organic chemicals (Marc St- Cyr *et al*., 1992; Jørgensen *et al*., 2000). Although this technique is a site-specific process and its efficiency may be limited by microbiological and physico-chemical conditions

in the soil (Yerushalmi *et al*., 2003), bioremediation is widely accepted as environmentally friendly, economically and politically viable (Rhykerd *et al*., 1999).

Bioremediation uses naturally occurring organisms to clean up polluted sites by transforming toxic and other undesirable materials into more benign or volatile substances. In order to enhance the natural degradation process, sufficient nitrogen and phosphorus are required to balance the available hydrocarbons in order for microbial growth and hydrocarbon reduction to occur (Mark and Jeffery, 1991). Viñas *et al*., (2005) attributed a remarkable shift in the composition of bacterial community to both the biodegradation processes and the addition of nutrient.

Cassava peels (CP) derived from the tropical root crop cassava (*Manihot* sp.) and poultry droppings (PD) have been used as amendments on degraded soils in southeastern Nigeria (Agbim, 1985; Mbagwu, 1992), and for nutrient-enhanced biodegradation of hydrocarbon in crude oil-contaminated wetland soil (Akamigbo and Jidere, 2002). So far, there is limited information on the potentials of these waste materials for nutrient-enhanced biodegradation of hydrocarbon in Typic Paleustults, of southeastern Nigeria. This information will be useful in rehabilitating abandoned land resource base available for crop production. The objective of this greenhouse study was therefore to compare the potentials of PD, CP and inorganic fertilizer for nutrient enhanced-biodegradation of hydrocarbons in crude oil-contaminated Typic Paleustults using hydrocarbon level and projected remediation duration as remediation index.

MATERIALS AND METHODS
Location
The study was carried out at the Faculty of Agriculture Farm, University of Nigeria, Nsukka, using topsoil (0 – 15cm) from the Nkpologu soil series, classified as Typic Paleustults (Soil Taxonomy, 1999), and Ferric Acrisols (FAO-UNESCO, 1988). The soils are derived from false-bedded sandstones. The area is located by Latitudes 06° $30'$ N and Longitudes 07° $10'$ E, has an elevation of about 400 m above mean sea level and slopes of between 2-4%. It is characterized by bimodal rainfall patterns with wet and dry seasons lasting from April to October and November to March respectively (Obi, 1982). The average total annual rainfall of the area is 1600mm and the vegetation is a derived savannah. The mean monthly temperature varies between 25^oC and 32^oC.

Materials
The physical characteristics of the soils prior to the start of the study are presented in Table 1. The particle size distribution shows that the soil is predominated by sand fraction (80 %) while the clay and silt fractions are 15 % and 5 % respectively, thereby placing the soil in a textural class of Sandy Loam. With the bulk density, gravimetric water retention at saturation and total porosity of 1.69 g/cm^3,

21 % and 36 % respectively, the soil is well drained and aerated all year round.

The crude oil used for the study was Nigerian Bonny Light obtained from Shell Petroleum Development Company (SPDC) Ltd Port Harcourt, Nigeria. Table 2 shows the chemical characteristics of the soil before and after contamination with crude oil.

The poultry droppings used for the study was collected from the battery cage poultry farm of University of Nigeria, Nsukka, while the cassava peels were obtained from local cassava processing centers. The NPK fertilizer 15:15:15 was sourced from the local market. The chemical characteristics of these soil amendments are shown in Table 3.

Greenhouse study
The topsoil (0-15 cm), poultry droppings (PD) and cassava peels (CP) were air-dried, ground and sieved to pass through 2 mm sieve. The soil was contaminated with crude oil at the rate of 50 t/ha, after which the soil amendments were applied in single form (50 t PD/ha, 100 t PD/ha, 50 t CP/ha, 100 t CP/ha, 50 t NPK/ha and 100 t NPK/ha) and combined forms (25 t PD/ha + 25 t CP/ha, and 50 t PD/ha + 50 t CP/ha), with the control as 0 t/ha (without soil amendment). The amendments were mixed thoroughly with 3 kg of the polluted soils. Each amendment was applied after one week of contamination and replicated three times, giving rise to twenty seven pots. The soils were placed in ceramic pots with drainage holes at the base, plugged with cotton wool to retain the soil. The pots were arranged in a completely randomized design in the greenhouse and allowed to stand for 201 days. The pots were watered to field capacity once in a week, while soil samples were taken from the surface of the pots at 14, 45, 76, 131 and 201 days after the application of the amendments respectively.

Jidere, C. M. and Akamigbo, F.O.R.

Laboratory analyses
The chemical properties of the contaminated soil and non-contaminated soil, CP, and PD were determined. The soil samples were air-dried, ground, sieved to pass 2 mm sieve and analyzed for the following properties: organic carbon by Walkley and Black analytical method as described by Nelson and Sommes (1982); total nitrogen by macro Kjeldahl method as described by Bremner and Mulvaney (1982); and available phosphorus by Bray II method as described Olsen and Sommers (1982); total hydrocarbon, using Infrared Spectrophotometer. The cation exchange capacity and exchangeable base was determined using ammonium acetate as extracting solution (Thomas, 1982), while the exchangeable acidity was determined according to the method of Mclean (1982). Soil physical properties determined were particle size distribution, using hydrometer method as described by Gee and Bauder (1986), and bulk density, using Blake and Hartge (1986). Total porosity, was calculated from the bulk density using an assumed particle density of 2.65 g/cm3, from f = 100(1 – Dry bulk density/Particle density), where f = Total porosity. This method was described by

Vomocil (1965). The computation of percent hydrocarbon saturation at 201 days, percent degradation and degradation rate respectively were carried out as follows;

Percent hydrocarbon saturation at 201 days = $(C_{201} \div C_{initial}) \times 100$

Percent hydrocarbon degradation = $100 - [(C_{201} \div C_{initial}) \times 100]$

Time required for 100% HC degradation (Year) = $[(201 days \div \%C_{201}) \times 100] \div 365 days$

Degradation rate = $C_{initial} - C_{201} / T$

Where: C_{201} = Hydrocarbon concentration at 201 days

$C_{initial}$ = Hydrocarbon concentration at the beginning of the study.
$\%C_{201}$ = Percentage hydrocarbon degradation at 201 days
T = Duration of study

Data analysis

Analysis of variance using GenStat Discovery Edition 2 computer package was performed on the soil data to assess the effect of the nutrient supplements on hydrocarbon degradation. The mean differences were compared for statistical significance using Fisher's least significant differences of means (5% level) as described by Obi (1986).

RESULTS AND DISCUSSION

The soil pH determined in both water and KCl was higher after the contamination with crude oil than before contamination (Table 2). Organic carbon was very high after contamination (4.62 %) relative to the value before contamination (0.74 %). Similar trend was observed in the total nitrogen, while the C/N ratio of the soil before and after contamination was 14:1 and 33:1 respectively. The contamination of the soil with crude oil increased the total hydrocarbon of the soil from initial value of 10.6 mgkg^{-1} to 3660.48 mgkg^{-1}. The reverse was the case for phosphorus as the value dropped from 14 mgkg^{-1} to 1.6 mgkg^{-1} after contamination. Table 3 indicates that the poultry droppings contain organic matter of 13.60% while the values are 51.5% and 36.10% in cassava peels and PD + CP respectively. This trend was reversed in the nitrogen content of the amendments, thus resulting to a very wide C/N ratio observed in the CP compared with the PD and PD + CP respectively.

The organic carbon (OC) and total petroleum hydrocarbon (THC) concentrations are presented in Table 4. The table showed very high concentrations of OC and THC in all the soils, especially at the first sampling date (14th day of the study). High concentrations of OC and THC are indicators of soil contamination. This was deduced from the fact that

hydrocarbon degradation, time required for 100 % HC

the values of OC and THC increased after contamination with crude oil (Table 2). The contaminant (THC) was significantly ($P < 0.05$) higher in the non-amended soil (control, 0 t/ha) relative to the amended soils throughout the duration of the study (Table 4). In addition, significantly higher concentration of OC was recorded in the control when compared with the amended soils. The higher concentrations of OC and THC in the control suggested relatively slow degradation of THC, probably due to poisoning of microbes, high chain hydrocarbons and/or lack of essential nutrient elements, occasioned by the absence of soil amendments. This was in line with the report of Zobell (1973) that bioremediation of crude oil in a natural ecosystem is relatively slow

Table 1: Physical characteristics of the soil used for the study

Parameter	Value
Clay (%)	15
Silt (%)	5
Sand (%)	80
Textural Class	Sandy Loam
Bulk Density (g/cm^3)	1.69
Total Porosity (%)	36
Water Retention (%)	21
Hydraulic Conductivity (cm/hr)	16.50

Table 2: Chemical characteristics of the soil before and after crude oil contamination

Parameter	Before Contamination	After Contamination
pH (water)	4.2	4.7
pH (KCl)	4.0	4.4
Organic Carbon (%)	0.74	4.62
Total N (%)	0.05	0.138
C/N Ratio	14:1	33:1
Ca^{2+} (Cmol/kg Soil)	1.2	1.6
Mg^{2+} (Cmol/kg Soil)	0.2	0.2
Na$^+$ (Cmol/kg Soil)	0.015	0.190
K$^+$ (Cmol/kg Soil)	0.015	0.024
CEC (Cmol/kg Soil)	6.4	9.6
H$^+$ (Cmol/kg Soil)	0.4	0.8
Al^{3+} (Cmol/kg Soil)	1.8	3.6
Av. P (mgkg^{-1})	14	1.6
THC (mgkg^{-1})	10.6	3660.48

Table 3: Chemical Characteristics of the Amendments

Parameter	Poultry Droppings (PD)	Cassava Peels (CP)	PD + CP (1:1)
Organic Carbon (%)	13.60	51.5	36.10
Total N (%)	3.80	0.36	0.60
C/N Ratio	4:1	143:1	60:1
Ca^{2+} (%)	8.00	1.20	1.36
Mg^{2+} (%)	1.92	0.72	0.96
Na$^+$ (%)	0.76	0.28	0.45
K$^+$ (%)	0.46	0.70	1.20
P (mgkg^{-1})	7.16	0.88	0.166

Table 4: Organic carbon and total petroleum hydrocarbon concentrations of crude oil contaminated soil after incorporation of soil amendments.

Amendment	14 Days	45 Days	76 Days	131 Days	201 Days
			Organic Carbon (%)		
0 t Control	6.43	5.81	5.53	5.18	4.21
50 t CP	5.18	5.14	5.46	4.46	4.42
100 t CP	5.19	5.16	5.33	4.32	3.87
50 t PD	5.61	5.44	5.02	4.22	3.61
100 t PD	5.58	5.26	5.02	4.07	3.41
50 t NPK	5.84	5.62	4.40	3.60	3.46
100 t NPK	5.40	4.82	4.06	3.65	3.42
25 t CP + 25 PD	6.26	5.63	5.32	4.03	3.54
50 t CP + 50 PD	6.05	5.51	5.28	4.29	3.46
Mean	5.72	5.38	5.05	4.20	3.71
LSD$_{(0.05)}$	0.103	0.194	0.056	0.166	0.151
			Total petroleum hydrocarbon (mgkg^{-1})		
0 t Control	3381.79	3265.35	3195.1	3046.4	3012.7
50 t CP	3304.68	3226.48	3045.8	2947.1	2809.1
100 t CP	3266.46	3185.45	2828.1	2861.8	2790.1
50 t PD	3286.31	3110.33	2935.4	2688.8	2417.8
100 t PD	3209.70	3146.58	2804.2	2654.3	2358.9
50 t NPK	3291.62	3161.14	2552.4	2403.0	2185.2
100 t NPK	2901.33	2876.58	2454.2	2305.5	2160.3
25 t CP + 25 PD	3296.22	3134.50	2924.4	2824.3	2691.4
50 t CP + 50 PD	3174.72	3098.23	2884.4	2736.4	2553.7
Mean	3234.76	3133.85	2847.1	2718.6	2553.2
LSD$_{(0.05)}$	7.325	5.076	15.07	12.68	9.97

Table 5: THC degradation (%), degradation rate and time required for complete THC degradation.

Amendment	THC Saturation (%)	Time required for 100% THC degradation (Year.)	THC Degradation Rate (mgkg^{-1}/day)
0 t Control	82	3.1	3.2
50 t CP	77	2.4	4.2
100 t CP	76	2.3	4.3
50 t PD	66	1.6	6.2
100 t PD	64	1.5	6.5
50 t NPK	60	1.4	7.3
100 t NPK	59	1.3	7.5
25 t CP + 25 PD	74	2.1	4.8
50 t CP + 50 PD	69	1.8	5.5
Mean	69.67	1.94	5.5
LSD$_{(0.05)}$	1.72	1.72	1.72

The addition of nutrient supplements is aimed at providing the nutrient elements required for microbial degradation of THC. The least percent THC degradation (18 %) was observed in the control (without nutrient supplement) (Fig. 1). The percent THC degradation at 201 days for 50 t CP and 100 t CP were 23 % and 24 % respectively, while that of 50 t PD and 100t PD were 34 % and 36 % respectively. For soils treated with NPK fertilizer and mixture of CP + PD, the percent THC degradation was 40 % and 41 % for 50 t NPK and 100 t NPK fertilizers respectively, and 26 % and 31 % for 25 t CP + 25 t PD and 50 t CP + 50 t PD respectively. The percent THC degradation was significantly (P = 0.05) influenced by the application of nutrient supplements. Roger *et al*., (1993) asserted that the logic of applying fertilizers as the bioremediation strategy was that under pre-spill conditions, crude oil degrading bacteria was limited by the availability of oil as a carbon source.
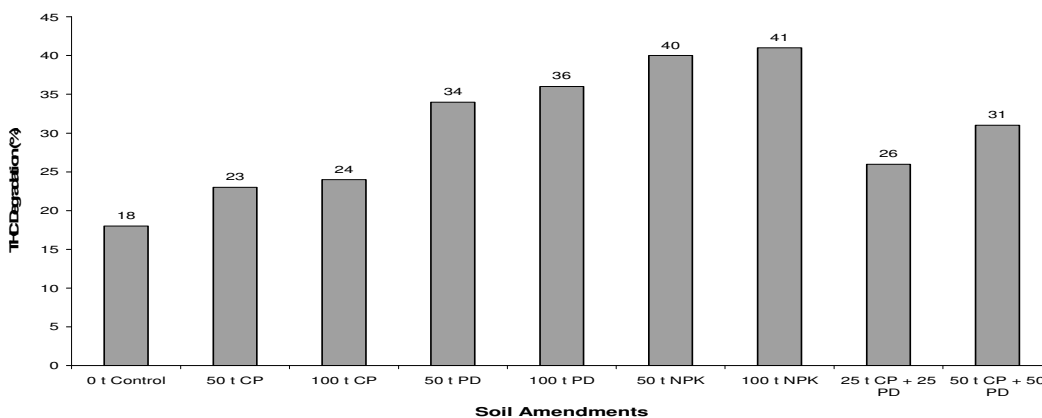
Fig. 1: Percent THC Degradation at 201 days after application of the amendments
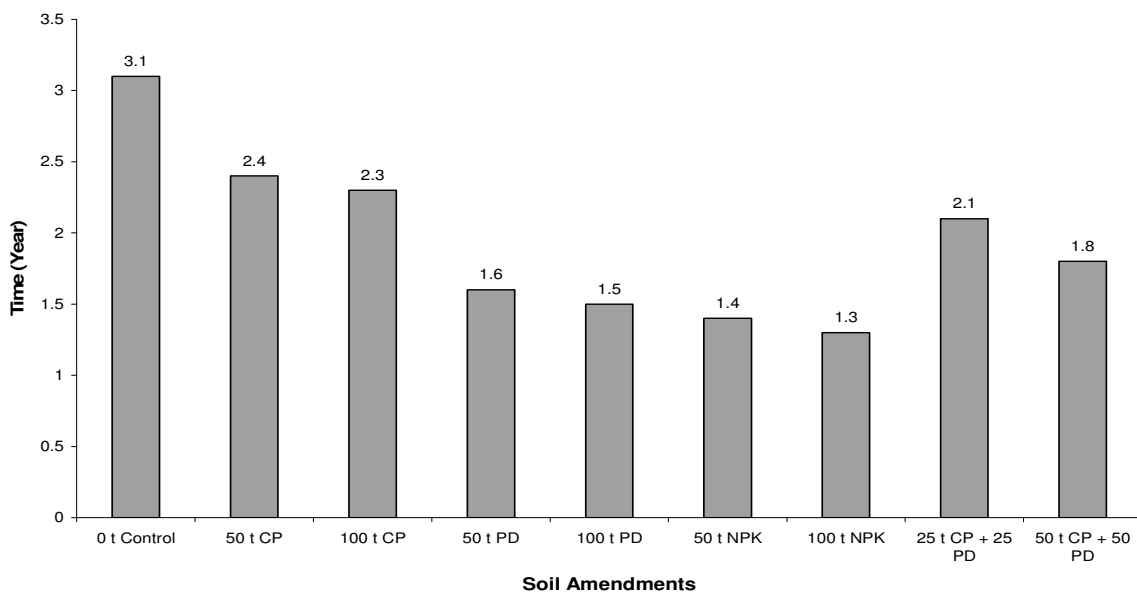

Fig. 2: Effect of the amendments on the time required for complete degradation of THC

The difference in the effect of the amendments on the THC degradation could be attributed to the variation in the potentials of the amendments to supply the essential nutrient elements for THC degradation. Indeed, the ability to supply the nitrogen, phosphorus and potassium varied with soil amendment (Table 3). Sufficient nitrogen and phosphorus are required to balance the available hydrocarbons in order for microbial growth and hydrocarbon reduction to occur (Mark and Jeffery, 1991). A comparison of the effect of PD, CP, NPK fertilizer and mixture of PD and CP on the THC concentrations showed that the concentration of the contaminant decreased in the order: NPK fertilizer < PD < CP + PD < CP < Control (Table 4). The significantly (P = 0.05) low THC level at 201 days observed in the NPK fertilizer-amended soil relative to the other soil amendments could be explained by the fact that NPK fertilizer was able to supply, in readily utilizable form, the essential nutrients required for THC degradation. Odu (1981) reported that lack of

sources of readily utilizable nitrogen and phosphorus might limit growth of micro organisms and hence crude oil degradation in soils.

The THC level was significantly lower in the PD amended soil relative to the other organic amendments (Table 4). Similar result was reported by Adesodun and Mbagwu (2007) in the study of effect of some organic wastes on oil polluted soil. This could be attributed to quick net nitrogen mineralization of PD due to its narrow C/N ratio and the variations in the nutrient element composition of the amendments (Table 3). Organic matter with C/N ratios wider than 30/1 decomposes slowly because they lack sufficient nitrogen for microbes to increase in population (Miller and Donahue 1992). With a wide C/N ratio of 143:1, and low NPK levels, the sole application of CP showed less potential to support microbes for degradation of THC (Table 3). However, the addition of PD to CP narrowed down the C/N ratio of the mixture (PD + CP) to 60:1, and improved its potential as nutrient supplement for microbial degradation of

THC. Consequently, the potentials of organic material for nutrient-enhanced THC degradation are enhanced by narrow C/N ratio.

Although PD showed great potential for THC degradation, however, Table 5 showed that it will take less time to achieve 100 % degradation of THC in NPK fertilizer-amended soil when compared with the other amendments. The time required to completely degrade the THC in the soil is in the order: NPK fertilizer < PD < CP + PD < CP < Control (Fig 2). While the highest THC degradation rate (7.5 ppm/day) was recorded in the 100 t NKP fertilizer-amended soils, the least degradation rate (3.2 ppm/day) was obtained in the control (Table 5), implying that it would take twice more time to degrade equivalent amount of THC in non-amended soil. Similarly, a comparison of the THC degradation rate in the organic waste-amended soil showed that the THC degradation rate was faster in the PD amended soil.

Total hydrocarbon concentration was significantly higher in lower doses of the nutrient supplement when compared with the higher rates of amendment application (Table 4). Similarly, the THC degradation was significantly higher in soils treated with higher doses of the nutrient supplements, thus implying that the percent THC degradation increases with increase in the rate of application of the nutrient supplements until an equilibrium is achieved.

CONCLUSION

This study was aimed at assessing the potentials of locally available organic wastes (poultry droppings and cassava peels), as an alternative to expensive inorganic nutrient supplements for remediation of crude oil contaminated soil. Using the THC level and degradation duration as remediation index, the study indicated that the application of the organic wastes to crude oil contaminated soil reduced the level of THC in the soil. The result of the experiment indicated that the chemical composition of the soil amendments and the application rates of the amendment are vital to the microbial degradation of petroleum hydrocarbon in soil. Although NPK fertilizer performed better in terms of THC degradation, PD showed high potentials for remediation of crude oil contaminated soil as the THC degradation rate in NPK fertilizer-amended soil was highly comparable to that of PD-amended soil. Addition of PD to CP improved the potentials of CP for the degradation of the contaminant and reduced the degradation duration. Therefore, the time required for complete degradation of the contaminant was less in NPK fertilizer-amended soil than the organic wastes-amended soil. The potentials of the nutrient supplements for THC degradation was in the following order: NPK > PD > PD + CP > CP > Control. However, based on environmental friendliness, the PD should be preferred to the NPK amendment.

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