PHYTOSTIMULATION OF AGRICULTURAL SOIL DIFFERENTIALLY CONTAMINATED WITH CRUDE OIL, USING *VIGNA UNGUICULATA.*

T. E. OGBULIE And J. N. OKEREKE
Department of Biotechnology,
School of Science,
Federal University of Technology,
Owerri, Imo State, Nigeria

And

E. C. CHINNAKWE
Department of Industrial Microbiology,
School of Science,
Federal University of Technology,
Owerri, Imo State, Nigeria

ABSTRACT

Phytostimulation of crude oil contaminated agricultural soil was carried out using *Vigna unguiculata*. Different volumes of Bonny light crude oil vis-a-vis 100ml, 200ml, 400ml and 800ml were used to pollute the soil after planting. Gas Chromatographic analysis revealed the total petroleum hydrocarbon (TPH) of the samples in the green house to be 56mg/kg, 129mg/kg, 146mg/kg and 552mg/kg for the four concentrations respectively. The TPH of the samples from field experiment also recorded 58mg/kg, 122mg/kg, 267mg/kg and 613 mg/kg for the different concentrations respectively. Results obtained from the gas chromatographic (GC) analysis during this treatment process also depicted the different alkane groups that were removed from the interaction to be within the range of C_7 to C_{12}; and C_{33} to C_{40} alkanes for samples in the field. Samples in the greenhouse also gave similar results except for the pot polluted with 800ml of crude oil where the only alkane groups degraded were C_7 toC_9. Although, the values of the growth rate of the plants indicated that plants in the greenhouse performed much better than the plants in the field both before and after pollution, there was a general decrease in the rate of growth per plant after some days of pollution. Nevertheless, a steady growth was observed from 192h till a slight increase ensued at 384h. Statistical analysis using T-test of SPSS Software oil for Windows Evaluation Version however showed that there was no significant difference in the rate of crude absorption by plants in the green house and in the field. Generally, the results showed that *V. unguiculata* can be a promising process in stimulating polluted soil for effective remediation.

INTRODUCTION

Crude oil is made up of a complex mixture of several polycyclic aromatic compounds and other hydrocarbons (Atlas, 1981; Leahy and Colwell, 1990). Diverse components of crude oil and petroleum such as polycyclic aromatic hydrocarbons (PAH) have been found in both aquatic and terrestrial environments as a result of pollution from industrial effluents and petrochemical products (Ogbulie and

Iwuala, 2006). Sources of petroleum and its products in these environments include accidental spills and intentional rupture of oil pipelines (Anderson, 1990; Leahy and Colwell, 1990; Mentzer and Ebere, 1996; NNPC 2004a, b; Okpokwasili and Amanchukwu, 1988 and SVMS, 2001).

The terrestrial and aquatic environments of the oil-rich Niger Delta region of Nigeria and its adjoining areas are the main recipients of crude oil spills (Ogbulie and Iwuala, 2006). Most times this leads to enormous pollution of their ecosystem (Ogbulie *et al.*, 2010) resulting in loss of microbial communities, habitats of economically important fish species and other aquatic animals, damage to wetlands along the coast as well as areas of vegetation meant for agricultural purposes etc. This also poses a serious threat to public health [Abed *et al*, 2002; Mishra *et al*, 2001; Nwachukwu and Ugorji, 1995 and Page *et al*, 2002).

Indeed, the environmental impact of oil exploration and exploitation is one of the inheritable consequences of economic development and civilization in a technical age; but most of the terrestrial ecosystems and shorelines in these oil producing communities are important agricultural lands and are under continuous cultivation (Ogbulie and Iwuala, 2006).

Consequently, there is need for innovation methods to restore these polluted sites, especially in an inexpensive, environmentally friendly manner. And among the many techniques employed to clean up heavy metals and oil polluted sites, bioremediation (*in situ*) – a process that involves action of autochthonous microorganisms or other biological systems, is the most widely used (Caplan, 1993; Dua *et al*, 2002; Horsfall and Spiff, 1998; Koren *et al*, 2003; Macnaughton *et al*, 1998; Sayler *et al*, 1997 and Young and Cerniglia, 1995). This study was carried out to evaluate the ability of *V. unguiculata* (vegetable cowpea) to possibly stimulate crude oil contaminated agricultural soil for effective remediation.

MATERIALS AND METHODS

Samples Collection

The plant seed sample used for this study is seed of leguminous crop, vegetable cowpea (*Vigna unguiculata var unguiculata*). This plant seed were collected from South Eastern part of Nigeria. The crude oil used was Bonny light crude oil and was collected with sterile containers from Akiri in Oguta, Imo State Nigeria. Whereas the soil sample for planting was collected from an agricultural soil using surface sterilized soil auger and containers, at the depth of 1-30cm.

Seed Preparation prior to Cultivation

The seeds were surface sterilized by washing and shaking in 75% ethanol for 30seconds, rinsed three times with sterile water for 10 minutes each, after which they were washed with 5.25% sodium hypochlorite solution for 15mins and then rinsed three times again with sterile water for 10 minutes per wash (Wu *et al.,* 2006). The seeds were allowed to germinate before planting. The germination was by incubating the seeds on wet sponges pre-sterilized by soaking them in a 2.5% sodium hypochlorite solution for 30 minutes, rinsed and autoclaved in a foil – covered beaker containing water making sure that the sponge was not submerged (Yee, *et al*, 1998). Following this, the surface sterilized seeds were placed on the sterile wet sponges and kept in a growth chamber with a light cycle consisting of 11h of darkness and 13h of light and with

65% humidity at 25°C (Yee, *et al*, 1998).

Seed Cultivation

A total of fifteen germinated (15) seeds were planted in plastic pots (with drainage holes) containing 450g of the agricultural soil and 300ml of sterile tap water added as described by (Yee, *et al*, 1998). A total of nine plastic pots were used, four (4) pots each for green house and field experiment, whereas the last pot was used as control. Four pots per location contained samples polluted with the four different concentrations of the crude oil sample used for the study while one pot contained only soil without any crop plants used as control. The potted plants were exposed to light for 13h each day. The plants were however, watered with 150ml of sterile tap water every 48h as described by Yee *et al* (1998).

Measurement of Growth Rate

The growth rate of the seeds was determined soon after germination ensued. This was done by measuring the length of the plant in centimeters using a rule and also placing of a graph paper prepared for measuring in days and centimeters as described by DeRoo (2010). This measurement continued till the end of the analysis to ensure comparison in the plant growth before and after pollution.

Exposure of the Plants to varying concentrations/ volumes of crude oil

After 28 days of plant growth, different volumes of the oil as 100ml, 200ml, 400ml and 800ml of crude oil together with 50ml of sterile water were added to the potted plants (Ogbulie, *et al.*, 2011). Thereafter, no additional water was added during the remaining period of the experiment as described by Yee *et al.* (1998).

Determination of the Total Petroleum Hydrocarbon (TPH) level of crude oil.

After 30 days of pollution, the total petroleum hydrocarbon content (TPH) was determined using the Gas Chromatograph (GC) with GC recorder interfaced with a computer. This was carried out by Anal Concept Nigeria Limited in PortHarcourt, River State.

RESULTS

The results of the growth rate of the study plants are as shown in Tables 1 and 2. Comparatively, the plants in the greenhouse performed much better than the plants in the field both before and after pollution, although there was a general decrease in the rate of growth per plant after some days of pollution. Furthermore, *Vigna unguiculata* var *unguiculata* showed a steady growth from 192h till a slight increase ensued at 384h.

The resultant TPH values obtained from Phytostimulation undergone in this study is as depicted in Table 3. Generally, the TPH values of the samples in comparison to that of the control sample showed that there was reduction in the concentration of crude oil. The result also indicated degradation of alkanes from the crude oil sample and the various C chains removed are as shown in Figures I – IV for plants kept in the green house and Figures V-VIII for plants kept in the field.

Table 1: Growth rates of the study plants before pollution.

Plants and locations	Time(h) of measurement 48	96	144	192	240	288	336	384	432	480	528	576	624
	Growth rates (cm/h)												
Greenhouse													
V. unguiculata var unguiculata	9.5	14.6	18.1	19.5	20.5	22.0	23.8	24.0	25.0	27.6	29.8	31.7	33.7
Field													
V. unguiculata var unguiculata	8.8	12.1	14.5	17.9	20.3	22.5	28.6	35.0	37.2	40.0	42.0	44.0	45.0

Table 2: Growth rates of the study plants after pollution.

Plants and locations	Time (h) of measurement 48	96	144	192	240	288	336	384	432	480	528
Greenhouse	Growth rates (cm/h)										
V. unguiculata var *unguiculata*	36.0	38.0	42.2	43.0	43.0	43.0	43.0	43.4	44.0	44.6	46.0
Field											
V. unguiculata var unguiculata	48.3	49.4	50.4	51.6	51.6	51.6	51.6	51.6	51.6	53.7	55.0

Figures I -- IV indicated that n-alkane ranging from C_7- C_{12} as well as C_{33}-C_{40} was observed to be absent for treatments with 100ml, 200ml and 400ml whereas only C_7 –C_9 was degraded in treatment with 800ml. This shows the need for increase in number of plant per increased volume of crude oil.

Furthermore, treatments made in Figures V-VIII showed that $C_{7\text{-}15}$ and $C_{33\text{-}40}$ were degraded indicating that the field trial is more promising. Analyzed differences in degradation of crude oil which were carried out using T-test of SPSS Software for Windows Evaluation Version indicated that there was no significant difference in the rate of crude oil absorption by plants in the green house and in the field (Figures IX and X).

Table 3: **TPH concentration after rhizoremediation using indigenous isolates on different crude oil volumes at different locations.**

Plant samples.conc.in mL	**TPH values (mg/kg) at different location**	
	Greenhouse	**Field**
V. unguiculata var Unguiculata/**100mLconc.**	55.66	57.53
V. unguiculata var unguiculata/ **200ml conc.**	128.71	122.21
V. unguiculata var Unguiculata/ **400ml conc.**	146.02	266.97
V. unguiculata var Unguiculata/ **800ml conc.**	552.00	613.37
control (soil + crude oil)	9487.23	10380.01

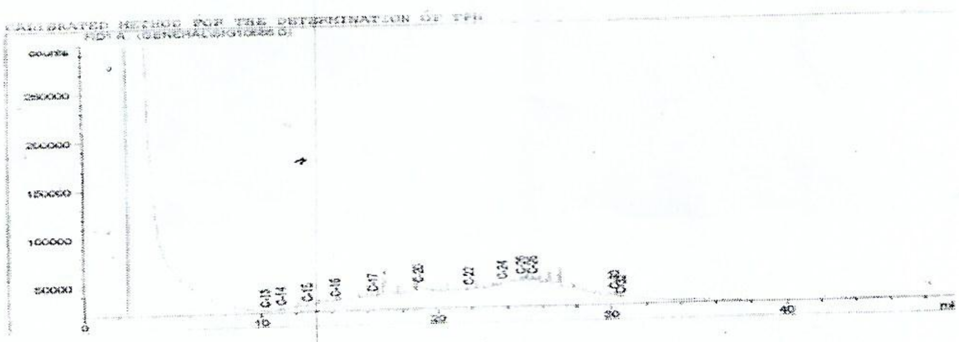

Fig I : Specific alkane group degraded during phytostimulation of agricultural soil polluted with 100ml of crude oil using *V. unguiculata (* green house).

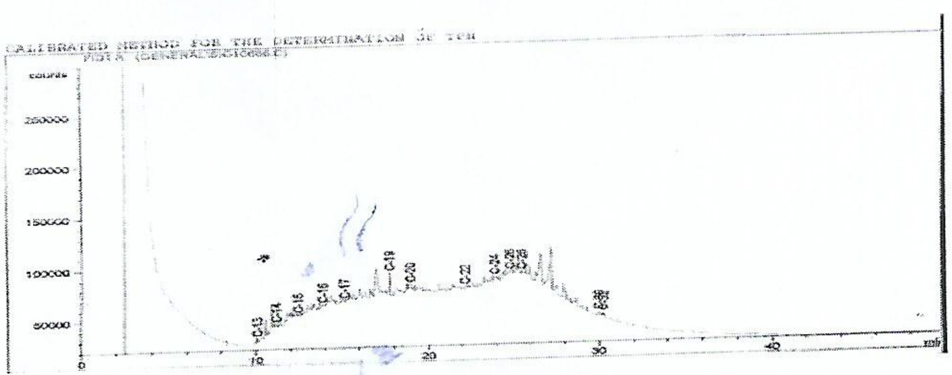

Fig II : Specific alkane group degraded during phytostimulation of agricultural soil polluted with 200ml of crude oil using *V. unguiculata (* green house).

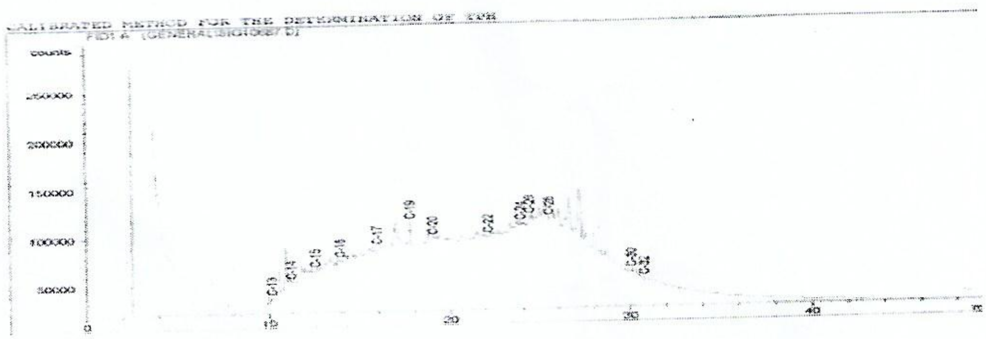

Fig III : Specific alkane group degraded during phytostimulation of agricultural soil polluted with 400ml of crude oil using *V. unguiculata* (green house).

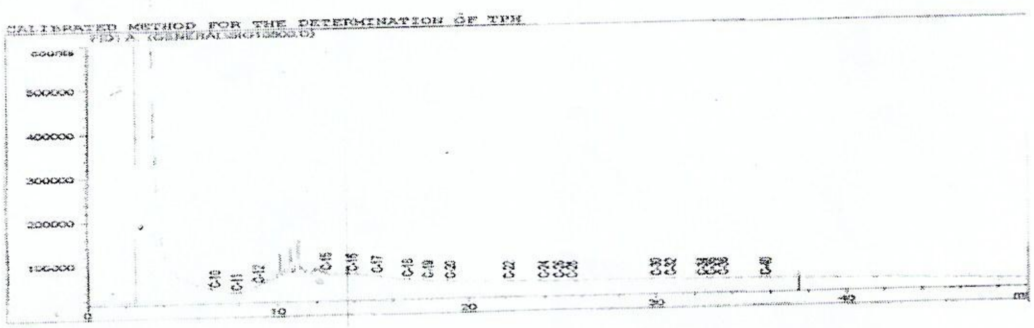

Fig IV : Specific alkane group degraded during phytostimulation of agricultural soil polluted with 800ml of crude oil using *V. unguiculata* (green house).

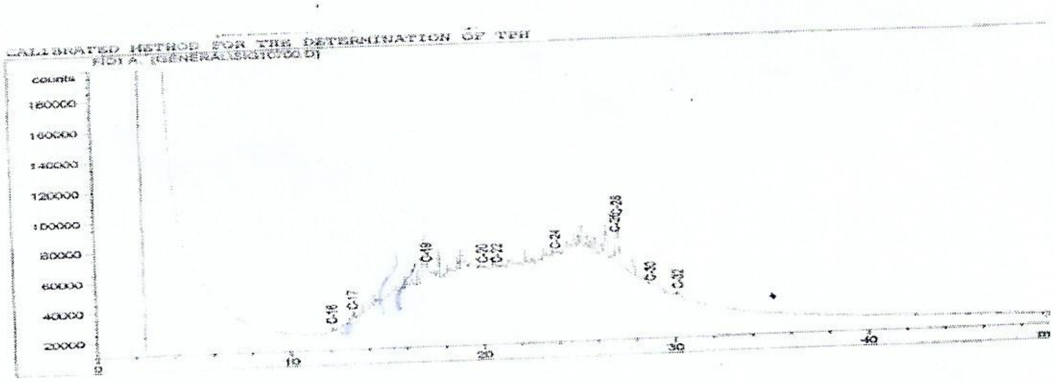

Fig V: Specific alkane group degraded during phytostimulation of agricultural soil polluted with 100ml of crude oil using *V. unguiculata* (field).

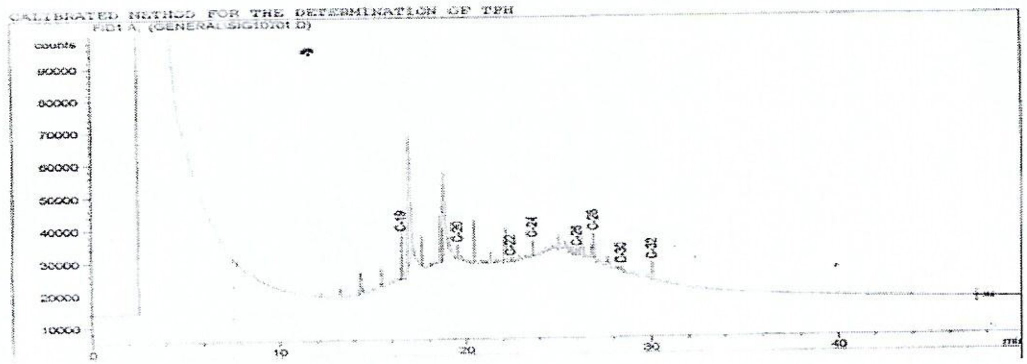

Fig VI: Specific alkane group degraded during phytostimulation of agricultural soil polluted with 200ml of crude oil using *V. unguiculata* (field).

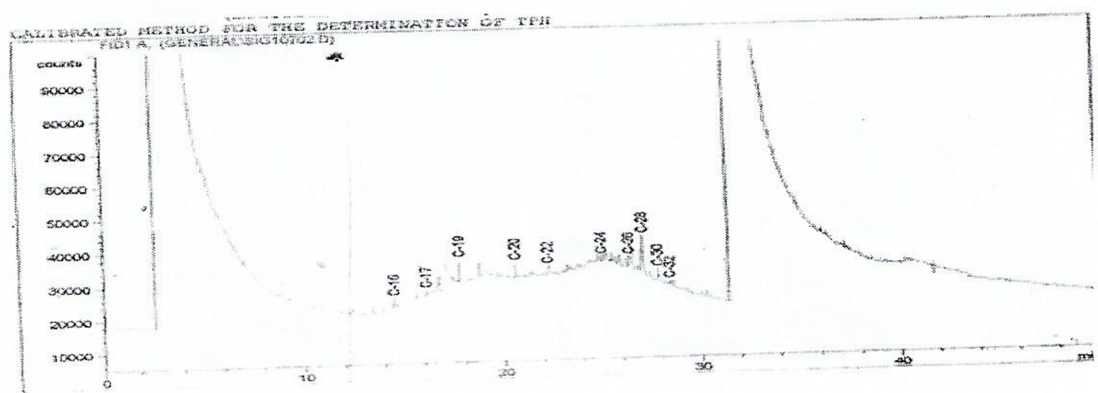

Fig VII: Specific alkane group degraded during phytostimulation of agricultural soil polluted with 400ml of crude oil using *V. unguiculata* (field).

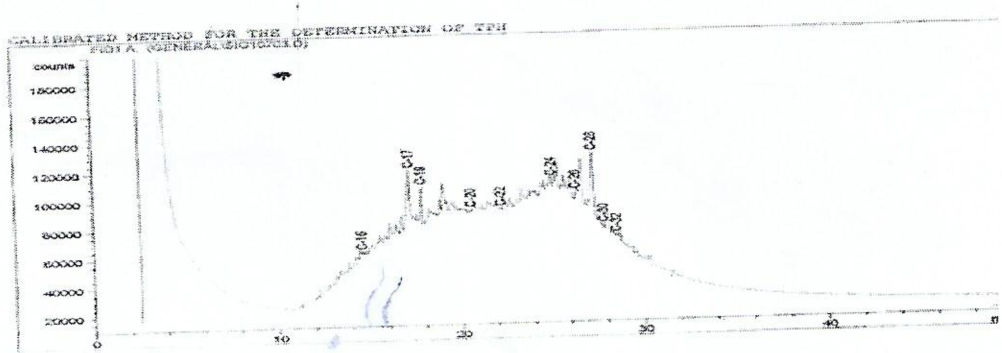

Fig VIII : Specific alkane group degraded during phytostimulation of agricultural soil polluted with 800ml of crude oil using *V. unguiculata* (field).

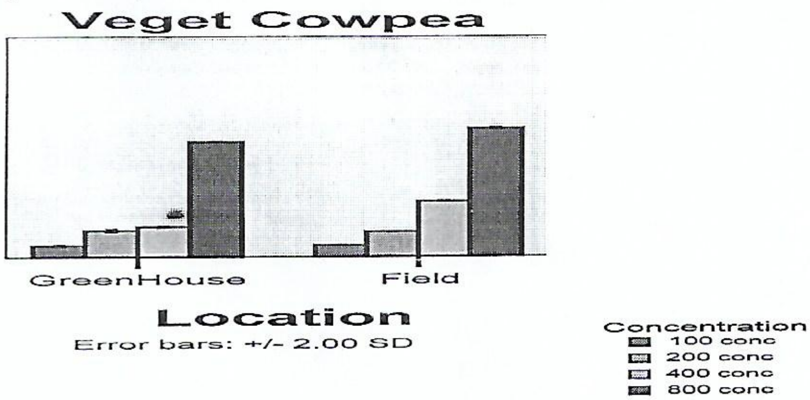

Fig IX: T-test comparing different interactions between each plant per TPH values per location

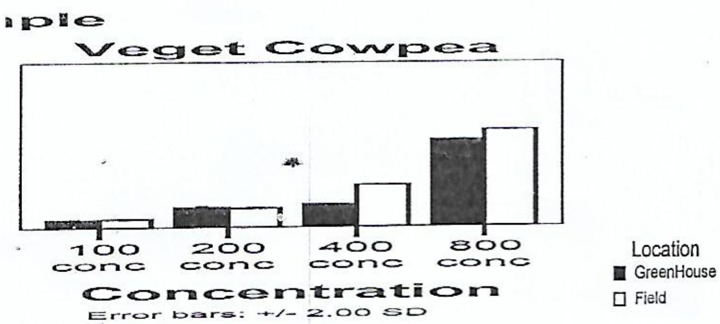

Fig X: T-test comparing different interactions between each plant per TPH values per concentration.

DISCUSSION

Phytostimulation of crude oil contaminated soil was undertaken in this study. The result from the Gas Chromatographic analysis obtained from the research work in comparison with control sample indicates that the use of *Vigna ungulculata* in phytostimulation of crude oil contaminated agricultural soil was effective. This supported the findings of Kuiper *et al.*, (2004) who reported on the use of leguminous plants in remediating PAH contaminated sites. It also lend more weight to the findings of Aprill and Sims, (1990) and Jussila, (2006) who used leguminous plant Glycine max (soya bean) and goat rye in phytoremediation of contaminated soil.

Growth rate of the study plant before and after pollution as shown in the result is in line with the findings of Baek, *et al.*, (2004), who studied the effect of crude oil and oil components on the growth of *Phaseolus nipponensis* (Red bean- a legume). It also supported the findings of Zuofa, *et al.*, (1988).

Biodegradation of both short and long chain n-alkanes as observed in this study supports the findings of Atlas (1981). Indeed degradation of n-alkanes with molecular chain lengths of up to n-C_{44} have been demonstrated which according to Atlas (1981) normally proceeds by monoterminal attack resulting in the formation of a primary alcohol, an aldehyde and a monocarboxylic acid. This therefore is in agreement with this study.

Phytostimulation method undertaken in this study actually indicated that plants can independently clean up crude oil contaminated soil which is in line with the reports of Frick *et al.*, (1999) ; Kuiper *et al.*, (2004) and Jussila, (2006) ; although the TPH values obtained indicates that single application of plant can delay remediation process which is in contrast to the target of effective time saving process hence the need to increase the number of plant per increased concentration of pollutants which is a more promising tool. A plant can therefore be considered as a solar-driven biological pump and treatment system, attracting water with its root system, accumulating pollutants in the rhizosphere and concluding with the degradation or translocation of the pollutants (Ogbulie and Iwuala, 2006).

Statistically, it was observed that there was no significant difference in the rate of degradation of the crude oil by samples in both the green house and in the field. This therefore shows that irrespective of the condition during treatment, *V. unguiculata* could be a better promise in the remediation of crude oil contaminated area. This finding therefore depict that increase in volume of crude oil that polluted the soil will definitely require a larger number of plants to be grown in such area to enhance stimulation for effective time saving remediation.

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