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PHYTOREMEDIATION: GERMINATION AND GROWTH OF *Peltophorum pterocarpum* (DC.) Baker ex Heyne IN CRUDE OIL-CONTAMINATED SOIL

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ABSTRACT

This study assessed the ability of *Peltophorum pterocarpum*, a nitrogen-fixing plant, to remove pollutants from crude oil-contaminated soil. The experiment was conducted in a screen house following standard procedures. Data were collected on rate of emergence, plant height and stem girth, number of leaves and number of active root nodules. Physicochemical properties (organic matter, pH, nitrogen, phosphorous, potassium, calcium and magnesium) of the soil were assessed using standard procedures. *Peltophorum pterocarpum* had germination rates of 80%, 74%, 62%, 55%, 48% and mean height of 64.60cm, 52.40cm, 48.00cm, 40.20cm, and 24.10cm in 0 mL, 25 mL, 50 mL, 75 mL, and 100 mL concentrations of crude oil respectively at 16 weeks after planting, The corresponding mean girth were 0.60 mm, 0.40 mm, 0.35 mm, and 0.21 mm; and the mean number of nodules were 18, 8, 10, 8, 4, while mean number of leaves were 15.00, 14.00, 11.00, 10.00, and 8.00, respectively. Crude oil concentration increased in *P. pterocarpum*, while organic matter, pH, nitrogen, phosphorus, potassium, calcium, magnesium, and salt content of the soil decreased. The rate of emergence, height, girth, number of leaves, and number of nodules of *Peltophorum pterocarpum* decreased as crude oil content increased. This indicated that crude oil had detrimental impacts on the plant. The ability of *Peltophorum pterocarpum* to survive in crude oil in various concentrations suggests that it might be utilized to clean up soil that has been contaminated by crude oil.

Keywords: Crude oil, *Peltophorum pterocarpum*, Soil Contamination, Phytoremediation, Root nodules.

INTRODUCTION

All organisms are capable of withstanding certain levels of environmental pollutants and may use them in the course of their growth processes. However, at greater concentrations, these pollutants can become harmful (Bradshaw, 1991). Pollutants do not have any known physiological functions in plants, except some which act as metabolic precursors for plants. The primary sources of pollution include fossil fuel extraction and burning (coal, oil, and natural gas), producing facilities and foundries, mines, urban agricultural runoff, and the use of agricultural chemicals like pesticides, herbicides, and fertilizers in sewage effluent (Acton and Gregorich, 1995; Seward and Richardson, 1990; Alexander, 1995).

Nigeria has a long history of exploring for and exploiting crude oil (Olujimi *et al*., 2011). Despite its economic importance, the exploration and exploitation have caused a number of areas to become contaminated with crude oil and petroleum byproducts (Bauman, 1991). According to a United Nations for Environmental Protection (UNEP) assessment on oil pollution in Ogoni, the Niger Delta region of Nigeria has seen a number of contaminations with petroleum hydrocarbon and other associated pollutants (Nwachukwu, 2006). Deep research into the environmental issues that develop during the exploration of crude oil is required potential pollution and contamination issues connected to handling of crude oil and its bye products. Worldwide use of crude oil production is estimated at roughly 90 million barrels per day (Mabros, 2006), with Nigeria being the top-crude oil manufacturer in Africa and the sixth biggest oil producer in the world, having a utmost daily output crude oil of 2.5 million barrels (Nigerian National Petroleum Company, 2013). The products are utilized in the production of a wide variety of goods. The implications and negative effects of crude oil exploration and exploitation have led to environmental degradation on a global scale, including environmental contamination and youth unrest in polluted areas. According to Bishop (2000), pollution occurs when contaminants are present in the ground and atmosphere to such a degree that they have a negative impact on regular environmental processes.

Some publications have noted that crude oil has a great influence on the development and functionality of plants. According to Gudin and Syratt (1975) and McGill and Rowell (1977), these effects are brought about by polluted soil conditions and crude oil's interference with plants' ability to absorb nutrients. Notwithstanding the importance of crude oil exploration to the economies of many countries, it has had a detrimental effect on the ecosystem's flora and fauna. Everyone urgently has to take notice of the unsustainable devastation of the environment in the Niger Delta region, which is causing food crops, farmlands, fish lakes, and consequent agitation. Oyedeji, *et al.* (2013); Raimi and Isiche (2015); Adewole and Bulu (2012) reported revealed some plants that can remediate crude and waste engine oils. Considering the enourmous challenge that crude and engine oil pollution posed in Nigeria, there is need to determine and evaluate as many plants as possible for phytoremediation

Peltophorum pterocarpum is widely renowned for its ability to thrive in challenging conditions where other crops fail or exhibit poor performance. The leguminous tree *Peltophorum pterocarpum* (DC. Baker), sometimes called the Yellow Flamboyant or Yellow Flame Tree, belongs to the Caesalpiniaceae subfamily of the family Fabaceae. It is a broadleaf fast-growing tree with a diameter at breast height of up to 1 m and a height range of 15–25 m (occasionally amounting to 50 m). The leaves are compound, 30-60 cm in length, and have 16–20 pinnae with 20–40 oval leaflets that are 8–25 mm long and 4–10 mm wide on each pinna. Yellow blooms with a diameter of 2.5 to 4 cm are produced in 20 cm in length complex racemes. Pods make up the fruit with each pod having one to four seeds long and 2.5 cm wide, beginning off red and turning black as it ripens. Trees start to bloom after about four years. The tree is frequently cultivated for decorative purposes in tropical areas, particularly in India, Nigeria and other countries. One of the legumes that grows in Niger Delta's untamed forest is this one

(Osam, 2006). It may be utilized for a wide range of things, such as constructing cabinets; the foliage can be grown for fodder. Its effectiveness in phytoremediating soil contaminated by crude oil was reported by Osam (2006). It is necessary to ascertain the impact of crude oil polluted soil on its germination, growth, and development because there is little to no information on how well it performs during its early growth in soil that has been polluted by oil. The ability of *Peltophorum pterocarpum* to fix nitrogen and its capability to survive in polluted soils led to the choice of this plant as the test plant in the literature that was considered.

MATERIALS AND METHODS

Study sites

The investigations were performed at the Niger Delta University, Wilberforce Island, Nigeria, in the Screen House of the Department of Biological Sciences and Central Research Laboratory of the Faculty of Science. The physicochemical investigation on the soil samples were performed at the Central Research Laboratory, Federal University of Technology Akure, Nigeria.

Source of experimental samples

Clay-loamy top soil was obtained from a 5-year-old fallow plot in the Research and Experimental Farm of Niger Delta University, Wilberforce Island, Nigeria, at a depth of 0 to 10 cm. The crude oil (Bonny light) was obtained at the Oporoma Flow Station of Shell Petroleum Development Company. The experimental plant, *Peltophorum pterocarpum* was sourced from the campus of University of Port-Harcourt, Nigeria.

Viability test of the seeds used

The method employed in this study to test the viability of seeds of *Peltophorum pterocarpum* was the floating technique previously described by Anoliefo and Vwioko (1995). A beaker of distilled water was used to soak about 500 seeds of the plant species for a total of 30 minutes. 300 viable seeds that drowned in the water were chosen at random, while the ones that floated were eliminated.

Laboratory experiment- Germination tests of the tree species

Five medium-sized plant bags were packed with clay-loamy soil from the Research and Experimental Farm of Niger Delta University, Wilberforce Island, Nigeria. The bags, each weighing 3000 g, were set up in the screen house. Various concentrations of crude oil (0, 25, 50, 75 and 100 mL) were artificially added to the soil and well mixed in the different concentrations of oil in the soil (0, 25, 50, 75 and 100 mL) indicate the various treatments (uncontaminated, low, average, high and extremely high contamination correspondingly). Samples (200g) of the contaminated soil from each treatment (0, 25, 50, 75, and 100 mL) were taken using the DTA Series Electronic Scale FED-3000 (Made in China, 2005), liquefied in 1000 mL graduated cylinders, and allowed to soak for 72 hours. Filtered aqueous extracts were then collected, labeled, and stored in 500 mL Pyrex conical flasks. Twenty (20) petri dishes, each double-layered Whatman No. 1 filter paper (Whatman International Ltd, Maidstone, England), were separated into groups of four to simulate the amount of oil treatments for crude in the soil (25, 50, 75 and 100), with five duplicates for each treatment. An identical control experiment was created and replicated five times. An identical control experiment was set up and replicated five times. To test the germination of the seeds of a few different plant species, ten plant seeds were put in a petri dish and dampened each day at 0700 with filtrates of the appropriate concentrations. For ten days after sowing, daily counts of germination were conducted and documented. Utilizing the equation proposed by Kayode and Oyedeji (2012), the percentage of germination was calculated:

Germination test Percentage: $Gt\,\% = \frac{Number\ of\ seeds\ germinated}{2aNumber\ of\ seeds\ planted}\,X100$

Screen house experiment – Initial growth response of the tree species in crude oil-polluted soil

The topsoil was distributed into fifty (50) medium-sized plant bags (each measuring 200 cm^3) that were set up in the screen house. They were split up into five separate groups. Each separate group was made up of 10 planting bags, lined up in a row. Different amount of oil in the soil: 0, 25, 50, 75 and 100 mL, were used to contaminate the groups, which represented the control (unpolluted), low, medium, high and extremely high pollution rates, correspondingly. Each pot in the treatments and its control were planted with three viable seeds two weeks after pollution (2WAPo) after all the treatments and control bags had been set up in the Green House and had been irrigated for two weeks at intervals of 72hours at 0700. Two weeks after planting (2WAP), it was thinned to one per bag. Measurements of plant height, girth and number of leaves each tree species were made fortnightly for 16 weeks, in an effort to understand how they responded to the soil's initial exposure to crude oil in terms of growth. A Vanier Caliper was used to measure the seedling girth (mm) at the first node above soil level, and a meter was used to measure the seedling height (cm) between the soil level and the last node toward the upper part of the shoot. Mean height and girth of each treatment were measured and documented. At 16 WAP, RGR measurements were obtained for each species across all treatments including growth suppression (GS). The RGR were computed according to the method of Kayode and Tedela (2000).

Analysis of the physical properties of the soil samples

The various physicochemical parameters of the soil were determined following standard procedures as reported in literatures including bulk density, soil organic carbon, organic matter, soil pH (Ibitoye, 2006), moisture content (Osuji and Onojake, 2004), volume of air in soil, soil water capillarity and porosity (Akinsanmi, 1975), soil nitrogen, calcium and magnesium (Anderson and Ingram, 1996), available phosphorous (Bray and Kurtz, 1945).

RESULTS AND DISCUSSION

The percentage germination of *P. pterocarpum* polluted with crude oil is presented in Figure 1. At varying concentration of 0 ml, 25 ml, 50 ml, 75 ml and 100 ml of crude oil on germination of *P. pterocarpum* was 80% (62 COV), 74% (59 COV), 62% (53 COV), 55% (46 COV) and 48% (40 COV) respectively. *P. pterocarpum* in crude oil-polluted soil showed a decreased order in germination potentials. The amount of oil present in the soil that water extracts determines how much decrease there is. According to Osuji *et al.* (2005); Kayode *et al*. (2009); Kayode and Oyedeji (2012), crude oil has a negative impact on soil conditions, microorganisms and plants. This investigation validates such reports.

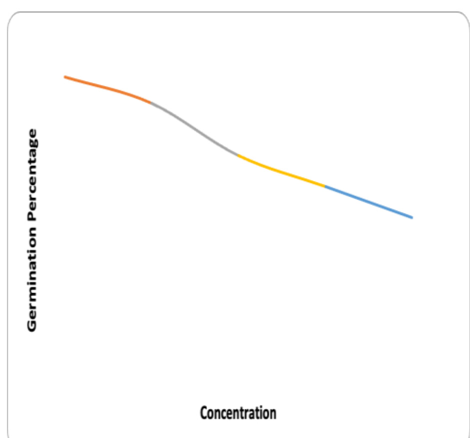

Figure 1: Percentage germination of *P. pterocarpum* in crude oil-polluted soil water extracts

Table 1 shows the mean height of *P. pterocarpum* grown in crude oil-contaminated soil. Mean height of the species were 5.40±1.14 cm, 4.80±2.30 cm, 4.50±2.60 cm, 3.60±2.70 cm and 0.00±0.00 cm in the 0, 25, 50, 75 and 100 ml crude oil-contaminated soil correspondingly at 2 weeks after planting (2 WAP). At 16 WAP, the heights obtained were 64.60±4.60 cm, 52.40±2.10 cm, 48.00±1.50 cm, 40.20±1.36 cm and 24.10±1.80 cm in 0, 25, 50, 75 and 100 mL crude oil-contaminated soils correspondingly. The result showed that the heights deduced decreased with increased concentration of crude oil in the soil. Thus, the percentage growth inhibition increased with increased concentration of the crude oil. This study had similar trend with the works of Anoliefo and Okoloko, (2000) and Kayode *et al*. (2009a, b) that crude oil affects mean height of plants. The results of this investigation also indicated that the heights gotten decreased with increased concentration of crude oil in the soil. This study also exposed that the heights achieved declined with increased concentration of crude oil in the soil. This indicates that the oil contamination inhibited the growth of the seedlings in the polluted soil.

Table 1: Mean number of plant height of *P. pterocarpum* in crude oil-polluted soil water extracts

Time	Plant height (cm)/Crude oil concentration (ml)				
(WAP)	0	25	50	75	100
2	5.40±1.14	4.80±2.30	4.50±2.60	3.60±2.70	0.00±0.00
4	12.60±2.10	10.50±1.10	9.60±1.90	6.30±1.40	0.00±0.00
6	25.40±1.40	18.10±1.70	16.40±1.10	10.50±2.40	4.40±2.60
8	35.60±0.90	28.50±1.80	23.50±1.82	16.60±1.80	7.40±2.80
10	41.70±1.90	34.40±2.40	30.20±1.64	22.80±2.06	10.60±3.50
12	50.60±2.30	41.20±1.90	37.10±1.00	30.40±3.15	15.10±1.75
14	55.20±3.60	47.30±3.20	41.60±1.80	35.50±1.30	18.00±2.60
16	64.60±4.60	52.40±2.10	48.00±1.50	40.20±1.36	24.10±1.80
∑X±SD	291.10±17.94	237.20±16.50	210.90±13.36	165.90±16.17	79.60±15.05
$\Delta H=H_F-H_I$	52.90±3.46	47.60±0.20	43.50±1.10	36.60±1.34	24.10±1.80
RGR	0.18	0.17	0.17	0.17	0
GS	0.00	0.189	0.257	0.377	0.627
%GS	0.00	18.90	25.70	37.70	62.70

RGR = Relative growth rate; GS = growth suppression; ΔH = Change in height
H_S = Starting Height; H_C = Concluding Height; $\bar{X}$ = Mean; (±) = Standard deviation

Mean girth of *P. pterocarpum* raised in crude oil-contaminated soil is presented Table 2. Seedlings of *P. pterocarpum* raised in the treated soil had girth values of 0.60±0.03mm, 0.40±0.03mm, 0.35±0.03mm, 0.25±0.30mm and 0.21±0.04mm for 0ml, 25 ml, 50 ml, 75ml and100 ml respectively at16 WAP. The findings of this study showed a decline in girth with an increase in the concentrations of crude oil in the soil. The biological performance of *P. pterocarpum* in crude oil-polluted soil observed for leaf numbers and number of nodules are presented in Table 3. At 0, 25, 50, 75 and 100 ml crude oil in the polluted soil, the number of nodules for *P. pterocarpum* were 18, 8, 10, 8 and 4 nodules respectively, while the number of leaves were 15.00, 14.00, 11.00, 10.00 and 8.00

respectively. The decrease in number of leaves, nodulation and girth demonstrated that crude oil has some negative impact on plant growth especially at higher concentration of the oil in soil. Therefore, metabolic processes have been hampered by the presence of crude oil in the polluted soil.

Table 2: Mean girth of *P. pterocarpum* grown in crude oil-polluted soil

Experimental	Plant girth (mm)/Crude oil concentration (mL)				
Time (WAP)	0	25	50	75	100
2	0.12±0.02	0.11±0.01	0.12±0.03	0.10±0.01	0.00±0.00
4	0.13±0.01	0.11±0.01	0.12±0.02	0.12±0.02	0.00±0.00
6	0.18±0.01	0.14±0.02	0.14±0.02	0.12±0.02	0.11±0.11
8	0.21±0.02	0.14±0.03	0.16±0.03	0.12±0.01	0.13±0.02
10	0.30±0.04	0.20±0.03	0.16±0.03	0.15±0.02	0.13±0.02
12	0.35±0.03	0.24±0.02	0.20±0.03	0.20±0.03	0.16±0.04
14	0.50±0.04	0.30±0.03	0.21±0.02	0.20±0.03	0.20±0.03
16	0.60±0.03	0.40±0.03	0.35±0.03	0.25±0.30	0.21±0.04
$\Delta G=G_F-G_I$	0.48±0.01	0.29±0.02	0.23±0.00	0.15±0.02	0.20±0.03
$\sum X \pm SD$	2.39±0.20	1.64±0.18	1.46±0.21	1.26±0.44	0.94±0.26

Table 3: Mean number of leaf and nodules of *P. pterocarpum* grown in crude oil-polluted soil

Mean number	0	25	50	75	100	Mean	Variance	SD
Leaf	15.00	14.00	11.00	10.00	8.00	11.60	8.30	2.88
Nodules	18.00	8.00	10.00	8.00	4.00	9.60	26.80	5.18

The physiochemical characteristics of crude oil contaminated soil that *P. pterocarpum* was used for remediation are displayed in Table 5. The contaminated soil pH was in the range of 4.93 (at 0 mL) and 4.38 (100 mL). But the control soil were in the range of 5.37 – 5.14. The volume and kind of soil creatures that transform plant wastes into advantageous soil organisms are regulated by the pH of soils, which in turn determines aggregate stability, which then affects the movement of air and water in soils. This observation shows that nitrogen fixing plant such as *P. pterocarpum* were slightly more efficient in the elevation of the pH. *P. pterocarpum*'s soil pH measurements revealed that the presence of different oil concentrations considerably changed the pH of the soil. The pH of the soil is often acidulous in samples with high oil content. However, growing leguminous plants such as *P. pterocarpum* on such soil offered a significant impact on it at 16 WAP. The organic matter concentration declined as the concentration of the crude oil rose i.e 1.42% (0 ml) - 1.28% (100ml), whereas the control had organic matter in the range of 1.84 – 1.55%. Typically, organic matter content of soils is an index of soil fertility. Another indicator of pollution is organic matter. The carbon mineralizing capacity is strongly connected with the organic carbon content of the soil, and this typically results in a decrease in oxygen level, which in turn affects microbial metabolism. As a result, the organic matter values have broad implications for mineralization. The reduction of the level of organic matter in the remediated soils among the crude oil treated soils found in this work shows that the *P. pterocarpum* have metabolic and absorption capabilities as well as transport systems that specifically sucked up the pollutants from the growth matrix.

The concentration of fertilizer monitoring parameters (nitrogen, phosphrous and potassium) also decreased as the concentration of the crude oil increased. The concentrations were in the range of 0.43 % (0 ml) – 0.34% (100ml) (nitrogen), 7.56 mg/kg (0 ml) – 6.85 mg/kg (100ml) (phosphorous) and 4.26 mg/kg (0 ml) – 3.85 mg/kg (100ml) (potassium) among the contaminated soil. Basically, macro elements measured showed that total nitrogen, phosphorus and potassium were disrupted in the remediated soil samples at 16 WAP in contrast with their controls. The study showed that *P. pterocarpum* is capable of preserving the balance between the available nitrogen and phosphorus in the soil to a point where bioaccumulation or over-reduction resulting to shortage was not possible. According to Cunningham *et al.*, (1996), the root systems of nitrogen-fixing plants like *P. pterocarpum* often feature processes that supply root exudates (energy, carbon, nutrients, enzymes, etc.) to microbial communities in the rhizosphere. As a result of these exudates' induction or enhancement of microbial populations, rhizosphere's ability to degrade organic pollutants is improved. The bacteria carrying out the degrading process might have utilized up the additional nitrogen provided to the polluted soil. In addition, the immobility (lower availability) of phosphorus, which may not have been enough liquefied in the soil to make it accessible while the little that was liquefied may have been swiftly absorbed by soil microbes, was the cause of the low level of phosphorus in the remediated soils.

The concentration of sodium were within an interval of 2.50 mg/kg (0 ml) – 1.80 mg/kg (100ml), calcium were within an interval of 15.25 mg/kg (0 ml) – 12.85 mg/kg (100ml) and magnesium was within an interval of 1.70 mg/kg (0ml) – 1.16 mg/kg (100ml). The exchangeable cation with the highest concentration found in the crude oil-polluted soils was calcium ion, followed by sodium ion, while magnesium ion had the lowest concentration found.

Additionally, the results demonstrated that *P. pterocarpum* was successful in reducing the cation concentrations, with calcium being the most negatively impacted. Soil particles transport plant nutrients which reside within the rhizosphere as ions. With increasing crude oil pollution, the concentrations of the exchangeable cations (calcium, magnesium and sodium) increased. Similar increases in exchangeable cations of soils from crude oil-polluted soil in Ogoni area were observed by Onyeike et al. in 2000. Both the contaminated and control soil samples contained potassium ions and magnesium ions in quantities that were typical of Nigerian soils with low fertility. The high calcium ion levels found in both the polluted and control soils may have human origins.

Table 4: Physiochemical characteristics of crude oil contaminated soil that *P. pterocarpum* was used for remediation

Parameters	Groups	Crude oil concentration (ml)				
		0	25	50	75	100
pH	Contaminated	4.93	4.90	4.70	4.45	4.38
	Control	5.37	5.26	5.19	5.16	5.14
Organic matter, %	Contaminated	1.42	1.38	1.31	1.28	1.28
	Control	1.84	1.75	1.65	1.58	1.55
% N	Contaminated	0.43	0.41	0.37	0.30	0.34
	Control	0.67	0.65	0.48	0.42	0.51

P, mg/kg	Contaminated	7.56	7.35	7.08	7.04	6.85
	Control	11.20	10.30	10.25	10.25	10.72
K, mg/kg	Contaminated	4.26	4.17	4.05	4.05	3.85
	Control	6.87	6.28	5.98	5.75	5.55
Na, mg/kg	Contaminated	2.50	2.10	1.85	1.80	1.80
	Control	3.41	3.70	3.38	5.75	4.87
Ca, mg/kg	Contaminated	15.25	14.45	13.45	12.85	12.85
	Control	19.20	18.50	17.50	16.98	16.98
Mg, mg/kg	Contaminated	1.70	1.68	1.58	1.47	1.16
	Control	2.55	2.50	2.35	2.15	2.15

Table 5 shows the effect of physical properties of unpolluted and crude oil-contaminated soil used in the experiment. Bulk density of 5.80, 6.40, 6.40, 6.8 and 7.70 g/cm^3 were observed in the treatments 0, 25, 50, 75 and 100 ml crude oil- contaminated soil correspondingly. Soil moisture content reduced in the crude oil- contaminated soil samples particularly in the 100 ml crude oil-polluted soil. Similarly, the presence of crude oil in the soil samples affects the soil air, 72.50, 38.60, 30.50, 40.40 and 43.60 % were observed in 0, 25, 50 75 and 100 ml correspondingly. Water holding capacity was also reduced in the crude oil- contaminated soil.

Table 5: Physical properties of unpolluted and crude oil-polluted soil

Treatment (ml)	Bulk Density(g/cm^3)	Moisture content (%)	Soil air (%)	Water Holding capacity (mL)	Soil porosity(ml)
0	5.8	72	72.5	58.4	86.4
25	6.4	44.5	38.6	50	81.5
50	6.4	40	30.5	34.5	60.4
75	6.8	28.5	40.4	24.1	48.3
100	7.7	18.2	43.6	13.7	32.8

The soil's physical characteristics are impacted by the presence of soil. As the crude oil's concentration increased, the soil bulk density, moisture content, soil air, water holding capacity and porosity decreased in the crude oil-contaminated soil. This was in line with the earlier research by Ewetola (2013) and Kayode *et al.* (2009b) which found that the existential of crude oil in soil could obstruct pore spaces within the soil and subsequently impairs soil aeration, porosity and water infiltration capacity which may have detrimental impact on plant growth and productivity.

CONCLUSION

Crude oil has decreasing effects on soil physicochemical characteristics (especially nitrogen, phosphorous, calcium, sodium, magnesium, potassium organic matter) among other. *P. pterocarpum* tolerated crude oil with regard to germination, height, leaf number and nodulation, but decreases with increased concentration of the crude. Therefore, *P. pterocarpum* has phytoremediation potentials of crude oil from the contaminated soil.

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