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Characterization of Soil Health Using Microbial Community and Maize Germination as Bioindicators in Oil-Contaminated Soil

Udom BE* and Nuga BO

*Corresponding author, Department of Crop and Soil Science, University of Port Harcourt, P.M.B 5323, Port Harcourt, Nigeria. E-mail: ebassidy@yahoo.com, babatundenuga@yahoo.co.uk

Abstract

A field experiment was carried out to evaluate the health status of a sandy soil contaminated with 5% (w/w) spent lubricating oil each for two years using microbial population and germination index of maize crop as bioindicators. Three legume plants: (*Gliricidia sepium*, *Leucaena leucocephala* and *Calapogonium caerulean*) combined or not with poultry manure were tested for their ability to improve the soil health status and maize production. Bio-test of the oil showed that the oil inhibits germination and establishment of maize crop. The contaminated (A_5) soil showed 95.4% reduction in total microbial population compared with the control (C) at 3 months after oil contamination. At 12 months after oil contamination, total counts and hydrocarbon degrading microorganisms (H-dms) showed drastic reduction in the A_5 soil. A few plants that germinated died before 72 days after planting due to lack of adequate oxygen and increase in soil wilting coefficient caused by the oil. The highest H-dms population of 6.2×10^7 cells g^{-1} was recorded for the treatment receiving *Gliricidia* combined with poultry manure ($A_5 + Gl + Pm$). This treatment also give significant ($P < 0.05$) increase in the grain yield of maize plant. Yields of 4.91, 8.25 and 6.4 tons ha^{-1} were obtained in the plots receiving *Gliricidia* combined with poultry manure during the first, second, and third planting season respectively. There was persistent deleterious effects on microbial population, seed germination and maize grain yield after 24 months, as cobs were empty. Spent oil inhibited microbial population and diversity up to 3 months of the application, imposing serious limitations on the soil to sustain biological productivity. The oil significantly lowered the soil pH and increased the total hydrocarbon content of the soil, which adversely affected both the microbial community and maize performance.

Key words: Petroleum products, microbial population, legume plants, maize performance, hydrocarbons.

Introduction

The global emphasis on soil health and sustainable food security is persuading soil scientists to consider rehabilitation of degraded lands, especially where oil contamination limits the use of such soils. With today's increased usage of petroleum products, the probability of major spillage endangering the aquatic or terrestrial environment becomes highly significant. Such accidents are likely to occur with pipeline leaks, train derailment, ship wreckages, and storage tank

raptures and transport accidents along the interstate highways. There is little information available in the literature on the biological effects of petroleum products in the soil environment. Paucity of information in field experiments to characterize soil health using population of microbial community and maize seed germination in soil contaminated with spent lubricating oil necessitated this study. When the soil is contaminated with oil, aromatic compounds such as benzene, toluene, phenol and ethyl benzene (commonly found in petroleum) will act as disinfectants when present in the liquid phase and

often inhibit bacterial growth¹. Such microbiostatic or microbiocidal compounds may also affect bacterial population. Contamination of agricultural ecosystems arising from discharge of petrol oils and grease is becoming a widespread problem that requires urgent attention. Depletion in the nutrient status (nitrogen and phosphorus), inhibition of microbial population and seed germination has been reported in spent-oil-contaminated soils^{2,3}.

Soil health is the continued capacity of the soil to function as a vital living system, within ecosystem and land-use boundaries, to sustain biological productivity, promote the quality of air and water environments, and maintain plant, animal and human health⁴. Several bioindicators of soil health and quality have been developed and reviewed^{5,6}. Among them micro organisms, due to their capacity to respond quickly to environmental changes, are expected to be efficient bioindicators. Microbial bioindicators could be based on functional diversity of the bacterial community. Functional diversity can be defined as the number, type, activity, and rate at which a set of substrate is utilized by a bacterial community⁷. Among the functional diversity indicators, the carbon utilization pattern and the measurement of enzymatic activities, expressed by the whole bacterial community have been suggested as useful tool to evaluate the soil status⁵. Variation in microbial population and activity can be used as a predictor of changes in soil health. Studies showed that, *Pseudomonas* and *Bacillus* micro organisms were prevalent in the oil-contaminated sites, whereas, dramatic reduction occurred in the total microbial community due to the additions of petroleum waste sludge⁸. N-fixing and heterotrophic microbes relevant for maintenance of soil health were gradually eliminated in oil-spill sites⁹. The very low NO₃ nitrogen usually associated with oil-contaminated soils is the limiting factor to N-fixing and heterotrophic microbes. Reduced oxygen content of the soil due to the blockage of pores resulted in increased water stress on the seed and imposed negative effects on germination are a few other effects of oil on soils¹⁰. Higher concentration of spent lubricating oil in the soil inhibited germination of *Capsicum annum* L. and *Lycopersicon esculenta*¹¹. The effect of oil on seed germination has been shown to be inhibitory due to unfavourable soil condition¹¹. Upon drying, soils contaminated with oil become too hard to allow germination. Therefore, the comparison of the density and the composition of soil microbial community and seed germination are useful to evaluate the influence of petroleum products on soil

health. Changes in microbial population or activity can precede detectable changes in soil physical and chemical properties, providing an early sign of soil improvement, or an early warning of soil degradation. Different works characterized the influence of artificial chemical contamination on microbial community by using microcosms experimental set up in the laboratory¹². Field experiments to improve population of microbial community and seed germination in soil contaminated with crankcase oils and grease is important to contribute to our understanding on how to manage such soils. The aim of this work was to provide valuable input data in bioremediation measures to improve the productivity of soil contaminated with petroleum products. In this study, we characterized the soil health status on the basis of microbial community, germination, and yield of maize crop and the potential of some legumes plants and organic nutrients to improve the soil health status were evaluated.

Experimental

The study was carried out at the University of Nigeria, Nsukka, Research Farm (Lat 06°52' N and Long. 07°24' E). The soil is sandy loam and described as *Typic Kandistult*¹³. The plots were impacted with 5% (w/w) (equivalent of 50,000 mg kg⁻¹) mono-and multi- grade crankcase oils from petrol and diesel engines, together with gear oils and transmission fluids and applied in a single dose each for two years. By the second year, oil contaminated plots had spent oil application load of 100,000 mg kg⁻¹, representing a total load of 10% (w/w). Three (3) legumes: *Gliricidia sepium*, *Calapogium caerulean*, and *Leucaena leucocephala*, alone or in combination with 0.5% (w/w), (equivalent of 500 mg kg⁻¹) of poultry manure. The experiment was arranged as a Randomized Complete Block Design (RCBD), with nine (9) treatments, viz: Uncontaminated (control) soil (C), 5% spent oil (A₅), *Gliricidia* spp. (A₅ + Gl), 5% spent oil + *Leucaena* spp. (A₅ + Le), 5% spent oil + *Calapogonium* (A₅ + Ca), 5% spent oil + poultry manure (A₅ + Pm), 5% spent oil + *Calapogonium* spp + 0.5% poultry manure (A₅ + Ca + Pm), 5% spent oil + *Gliricidia* spp + 0.5% poultry manure (A₅ + Gl + Pm), and 5% spent oil + *Leucaena* spp + 0.5% poultry manure (A₅ + Le + Pm), with five replications. The legume seeds and poultry manure were introduced during early rains to the plots seven (7) days after the oil contamination and allowed to incubate for fourteen (14) days before planting maize crop. The second application of 5%

(w/w) spent oil was done 360 days after the first application.

Sampling

Soil samples for microbial population counts were collected from 0-30 cm depth at 3, 12, 24, and 36 months after oil contamination. The implications of the oil and treatments on maize were evaluated using germination index measurement, at 2-4 weeks after planting and the dry grain yield measured at 14% moisture content.

Measurement of microbial density

The number of viable counts and hydrocarbon-degrading microorganism (H-dms) were measured by direct microscopic counts after treating the soil samples with MacConkey agar crystal violet and *tryptic* soy agar plate media^{14,15}. Fifty grams (50 g) of fresh soil from each treatment were placed in plates containing titration series of nutrient media solutions in triplicate. After incubation for 5 days in the dark at 25°C, bacterial density was recorded by the most probable number (MPN) method. Values of bacterial growth were calculated using the number of bacterial-colony-forming units (cfus), and expressed as cfug⁻¹ of fresh soil.

Isolation of hydrocarbon-degrading bacteria

In order to select strains able to biodegrade hydrocarbon, 10 ml of each dilution were spread on MacConkey agar added with Cycloheximide. The density of hydrocarbon-degrading bacteria (cells g⁻¹ of fresh soil) in each soil samples were determined and colonies were then randomly selected, isolated and maintained on MacConkey agar plates added with the spent oil. The hydrocarbon-degrading bacteria isolated consisted of the *Bacillus*, *Pseudomonas*, *Beijerinckia* and *Methanobacterium*.

Measurement of total hydrocarbon content, organic carbon, nitrogen and pH

Total hydrocarbon (TH) at each sampling data was determined gravimetrically by toluene extraction (cold extraction) method¹⁶, to provide an estimate of organic and bioavailable form of total hydrocarbon content (THC). The liquid phase of the cold extract was measured with a Spectrophotometer and fitted into standard curve from fresh spent oil treated with toluene. Total organic carbon (TOC) was determined by the Walkley and Black wet dichromate oxidation method¹⁷. Total nitrogen was measured by the macro-kjeldahl digestion procedure¹⁸. Soil pH was

measured with a glass electrode in a 1:2.5 soil/water aqueous solution¹⁹.

Results and Discussion

pH, Total hydrocarbon content, total organic carbon, and C:N ratio

The soil pH ranged from strongly acid to extremely acid at the top 0-30 cm soil. There were significance ($P < 0.05$) increases in the soil pH with treatments relative to the contaminated plots (A_5) (Table 1). Apart from the inherent acidic nature of the highly weathered soil of the South-Eastern Nigeria earlier reported²⁰, the oil contributed largely to the extreme acidity of the soil, with concomitant deleterious effects on microbial population. The C/N ratio was very high in contaminated plot, a 16:1 ratio in 3 months, 13:1 and 14:1 in 12 and 36 months after oil contamination, respectively (Table 1). The legume plants lowered and maintained the C/N ratio of the treated plots at 6:1 in the end of 36 months, thus confirming the previous observations²¹, that organic matter generated by these legume plants are in good amount and quality. The distribution of total hydrocarbon content (THC) of the soil as modified by the treatments is shown in Table 1. In 12 months after oil contamination, the THC for $A_5 + G1 + Pm$, $A_5 + Le + Pm$ and $A_5 + La + Pm$ were 15471, 15549 and 15816 mg kg⁻¹ respectively compared to 30648 mg kg⁻¹ recorded for A_5 plots. The significantly low residual THC observed for the plots treated with *Gliricidia* spp, *Calopogonium* spp, *Leucaena* spp and poultry manure was not surprising. The degradation process may have been enhanced by the positive changes in the soil pH by the legume plants. Secondly, the plants may have participated in hydrocarbon degradation via their support of symbiotic root-associated micro-organisms that actually accomplished hydrocarbon degradation^{21,22}. Since different species of plant have varying effects on rhizosphere micro-organisms, the *Gliricidia*, *Leucaena* and *Calopogonium* spp, supplemented with poultry manure showed promise in improving the soil health.

Bacterial growth

A comparison of the relative values of bacterial cfus in contaminated and non-contaminated plots and those treated with legumes and poultry manure is shown in Table 2. At 3 months after oil contamination, the viable microbial population for the 0-30cm soil ranged from 1.2×10^6 to 2.6×10^9 Cfug⁻¹, whereas, hydrocarbon degrading microorganisms (H-dms) ranged from 1.9

Table 1. Changes in Total hydrocarbon contents, total organic carbon and pH of the soil by treatments after 36 months.

Treatment	pH	THC (mg/kg)	TOC (g/kg)	C:N
3rd Month				
A ₅	3.7	35492	14.17	16:1
A ₅ ^{1/2} + Gl	3.8	34784	13.38	13:1
A ₅ + Le	4.0	34652	13.36	11:1
A ₅ + Ca	3.9	33964	12.92	12:1
A ₅ + Pm	4.0	34413	23.55	5:1
A ₅ + Gl + Pm	4.2	28413	23.42	6:1
A ₅ + Le + Pm	4.4	28519	23.07	7:1
A ₅ + Ca + Pm	4.1	28944	23.35	10:1
C	4.0	2390	8.65	10:1
LSD _{0.05}	0.67	9646	0.49	1:4
6th Month				
A ₅	3.1	32841	13.6	15:1
A ₅ ^{1/2} + Gl	3.6	29328	13.41	8:1
A ₅ + Le	3.8	28553	13.38	10:1
A ₅ + Ca	3.8	29422	12.96	8:1
A ₅ + Pm	4.1	29666	23.69	6:1
A ₅ + Gl + Pm	4.4	19783	23.98	6:1
A ₅ + Le + Pm	4.3	19946	23.69	8:1
A ₅ + Ca + Pm	4.4	79134	23.93	8:1
C	4.3	2117	6.35	3:1
LSD _{0.05}	0.36	8986	0.68	2:1
12th Month				
A ₅	3.2	30648	13.36	8:1
A ₅ ^{1/2} + Gl	4.1	17742	13.47	6:1
A ₅ + Le	3.8	17886	13.41	6:1
A ₅ + Ca	4.5	17421	13.35	4:1
A ₅ + Pm	3.8	16638	22.97	6:1
A ₅ + Gl + Pm	4.8	15471	24.07	7:1
A ₅ + Le + Pm	4.2	15549	23.90	6:1
A ₅ + Ca + Pm	4.1	15816	24.62	7:1
C	4.3	2075	6.24	3:1
LSD _{0.05}	0.85	8437	0.53	1:03
18th Month				
A ₅	3.0	41033	13.11	13:1
A ₅ ^{1/2} + Gl	4.6	36617	13.74	5:1
A ₅ + Le	4.1	36214	13.61	5:1
A ₅ + Ca	4.3	36347	13.59	4:1
A ₅ + Pm	3.7	39118	21.44	6:1
A ₅ + Gl + Pm	4.8	35473	23.30	5:1
A ₅ + Le + Pm	4.6	35718	25.00	7:1
A ₅ + Ca + Pm	4.8	35736	24.69	6:1
C	4.2	1964	6.35	9:1
LSD _{0.05}	0.81	7449	0.49	1:09
24th Month				
A ₅	3.1	36416	12.17	13:1
A ₅ ^{1/2} + Gl	4.8	29011	14.32	11:1
A ₅ + Le	4.3	29930	14.73	8:1
A ₅ + Ca	4.4	30662	13.94	4:1
A ₅ + Pm	3.8	31457	22.66	6:1
A ₅ + Gl + Pm	4.8	21974	25.92	6:1
A ₅ + Le + Pm	4.8	22603	24.16	6:1
A ₅ + Ca + Pm	4.6	23146	24.71	6:1
C	4.2	29103	6.10	7:1
LSD _{0.05}	0.26	14558	1.31	0.18
30th Month				
A ₅	3.4	33164	12.13	13:1
A ₅ ^{1/2} + Gl	4.8	23943	14.98	11:1
A ₅ + Le	4.6	22866	14.81	7:1
A ₅ + Ca	4.8	28744	14.11	4:1
A ₅ + Pm	3.7	29411	21.62	5:1
A ₅ + Gl + Pm	4.8	20997	25.96	6:1
A ₅ + Le + Pm	4.6	21009	24.65	6:1
A ₅ + Ca + Pm	4.8	20843	24.85	6:1
C	4.2	19089	6.08	8:1
LSD _{0.05}	0.14	2137	0.52	1:0
36th Month				
A ₅	3.4	31731	12.11	14:1
A ₅ ^{1/2} + Gl	4.8	20619	15.00	7:1
A ₅ + Le	4.8	21174	14.50	5:1
A ₅ + Ca	4.7	24366	14.16	4:1
A ₅ + Pm	3.8	28694	21.48	5:1
A ₅ + Gl + Pm	5.0	20416	25.98	6:1
A ₅ + Le + Pm	4.8	20544	24.71	6:1
A ₅ + Ca + Pm	5.1	20712	24.89	6:1
C	4.1	19004	6.08	10:1
LSD _{0.05}	0.37	1618	1.25	0.84

Table 2. Viable and hydrocarbon-degrading micro-organism populations in the contaminated soil as influenced by the treatments

Treatment	Variable counts (cfug ⁻¹)	H-dms (cellsg ⁻¹)
3 months		
A ₅	1.2 x 10 ⁶	5.3 x 10 ⁴
A ₅ + Gl	1.7 x 10 ⁶	8.1 x 10 ⁴
A ₅ + Le	1.4 x 10 ⁶	7.0 x 10 ⁴
A ₅ + Ca	1.8 x 10 ⁶	5.6 x 10 ⁴
A ₅ + Pm	2.1 x 10 ⁶	1.9 x 10 ⁴
A ₅ + Gl + Pm	2.1 x 10 ⁷	4.6 x 10 ⁵
A ₅ + Le + Pm	1.5 x 10 ⁷	4.2 x 10 ⁵
A ₅ + Ca + Pm	1.3 x 10 ⁷	3.1 x 10 ⁵
C	2.6 x 10 ⁹	2.4 x 10 ²
LSD _{0.05}	568496	14931
12 months		
A ₅	6.1 x 10 ⁴	2.8 x 10 ⁴
A ₅ + Gl	8.2 x 10 ⁸	5.8 x 10 ⁵
A ₅ + Le	7.5 x 10 ⁸	5.0 x 10 ⁵
A ₅ + Ca	5.2 x 10 ⁸	4.9 x 10 ⁵
A ₅ + Pm	4.6 x 10 ⁸	1.2 x 10 ⁵
A ₅ + Gl + Pm	3.6 x 10 ¹⁰	6.2 x 10 ⁷
A ₅ + Le + Pm	3.0 x 10 ¹⁰	5.8 x 10 ⁷
A ₅ + Ca + Pm	2.5 x 10 ¹⁰	5.3 x 10 ⁷
C	3.0 x 10 ⁸	1.3 x 10 ²
LSD _{0.05}	951172	40746
24 months		
A ₅	2.8 x 10 ⁶	1.8 x 10 ⁵
A ₅ + Gl	7.2 x 10 ⁹	1.2 x 10 ⁵
A ₅ + Le	6.1 x 10 ⁵	3.6 x 10 ⁵
A ₅ + Ca	5.1 x 10 ⁹	3.0 x 10 ⁶
A ₅ + Pm	3.8 x 10 ⁷	1.6 x 10 ⁵
A ₅ + Gl + Pm	8.3 x 10 ¹⁰	8.5 x 10 ⁷
A ₅ + Le + Pm	7.0 x 10 ¹⁰	5.5 x 10 ⁷
A ₅ + Ca + Pm	6.5 x 10 ¹⁰	2.1 x 10 ⁷
C	3.8 x 10 ⁷	<10 ³
LSD _{0.05}	490677	58355
36 months		
A ₅	2.1 x 10 ⁶	1.5 x 10 ⁴
A ₅ + Gl	7.5 x 10 ⁹	75.1 x 10 ⁵
A ₅ + Le	7.3 x 10 ¹⁰	1.6 x 10 ⁵
A ₅ + Ca	26.0 x 10 ¹⁰	2.0 x 10 ⁵
A ₅ + Pm	2.3 x 10 ⁸	1.3 x 10 ⁴
A ₅ + Gl + Pm	8.6 x 10 ¹¹	7.2 x 10 ⁵
A ₅ + Le + Pm	7.3 x 10 ¹¹	7.4 x 10 ⁵
A ₅ + Ca + Pm	6.0 x 10 ¹⁰	6.1 x 10 ⁵
C	4.8 x 10 ⁷	1.1 x 10 ²
LSD _{0.05}	937981	14691

H-dms =Hydrocarbon degrading micro-organisms

x 10⁴ to 8.1 x 10⁴ cell g⁻¹ during the same period. The contaminated (A₅) soil showed 100% reduction in total microbial population compared with the control (C) at 3 months after oil contamination. At 12 months after oil contamination, total counts and H-dms showed drastic reductions in the A₅ soil (6.1 x 10⁴ cfu g⁻¹ and 2.8 x 10⁴ cells g⁻¹ soil) respectively. Highest H-dms population of 6.2 x 10⁷ cells g⁻¹ was recorded for the A₅ + Gl + Pm soil. This implies that spent oil inhibits microbial population and diversity in the first 3 to 12 months of oil application in the soil, imposing serious limitation on the soil to sustain biological productivity. Plots treated with poultry manure only showed high values of total viable counts of micro organism at 3, 12 and 18 months after oil contamination, but the H-dms were relatively low (1.9 x 10⁴, 1.2 x 10⁵ and 1.6 x 10⁵ cell g⁻¹ soil) respectively. This development implies that poultry manure application do not encourage the proliferation of hydrocarbon-degrading micro organisms, rather, the availability of petroleum hydrocarbon and a suitable plant species. The relatively high H-dms recorded for plots treated with legumes plants and contaminated soil (A₅), is in conformity with^{15,16}, that the number of hydrocarbon-utilizing organisms were most abundant in oil polluted sites than in unpolluted sites. Inference drawn from this result is that substantial adapted population of micro organisms were abundant in hydrocarbon contaminated zone with the bacterial biomass increasing as organic contaminant are metabolized. Previous studies²³, had made similar observation on contaminated sites with organic pollutants. Large population of H-dms observed in soils treated with legumes plants may have been stimulated by the legume root exudates or that their widely branched root systems which provided large root surface for the growth of large population of H-dms²⁴.

Effects of oil on maize germination and yield

The plots treated with a combination of legume plants and poultry manure showed high percent germination (93, 90 and 88%) for A₅ + Gl + Pm, A₅ + Le + Pm and A₅ + Ca + Pm respectively, (Table 3). After 24 months, the residual effects of the oil in untreated plot reduced germination counts of the maize seed by 66% during the third planting season. Grain yield of 4.91 tha⁻¹ was obtained during the first planting and 8.25 t ha⁻¹ and 6.46 t ha⁻¹, respectively, during the second and third planting seasons for A₅ + Gl + Pm. In the three planting seasons, no yield was recorded for A₅ and control plots, because of the oil and the inherent low

Table 3. Effects of treatment on germination and grain yield of maize

Treatment	Maize grain yields (tons ha ⁻¹)			Germination count (%)		
	Planting Season			Planting season		
	Fist	Second	Third*	Fist	Second	Third*
A ₅	0.0 ^a	0.0 ^a	0.0 ^b	41 ^a	36 ^b	34 ^a
A ₅ + Gl	3.06 ^c	5.16 ^b	4.18 ^a	57 ^b	53 ^a	67 ^a
A ₅ + Le	2.32 ^a	5.05 ^b	4.12 ^a	55 ^b	50 ^d	65 ^a
A ₅ + Ca	1.87 ^a	4.79 ^b	3.47 ^a	60 ^b	63 ^c	89 ^b
A ₅ + Pm	4.01 ^b	6.23 ^b	4.50 ^b	58 ^b	63 ^c	63 ^a
A ₅ + Gl + Pm	4.91 ^c	8.25 ^b	6.46 ^a	62 ^b	80 ^d	93 ^b
A ₅ + Le + Pm	3.91 ^b	6.90 ^c	5.03 ^b	63 ^b	81 ^d	90 ^b
A ₅ + Ca + Pm	4.16 ^b	7.02 ^a	6.21 ^a	66 ^b	79 ^d	88 ^b
C	0.0 ^a	0.0 ^a	0.0 ^b	78 ^c	70 ^d	78 ^c
LSD _{0.05}	0.85	0.81	0.80	3.0	5.0	7.0

Mean followed by different alphabets within rows and columns are significantly different at P<0.05.

*Residual Effect.

fertility of the soil. Seventy-two days after planting (72 DAP), the few maize plants that germinated in contaminated soil without treatment died prematurely. The reasons for the no yield and/or death of plants after a few weeks after germination was due to insufficient aeration of the soil, caused by the displacement of air from the pore spaces by the oil, and an increase in the demand for oxygen brought about by the activities of oil-decomposing micro organisms³. It could also be due to the fact that the oil penetrated and accumulated in the seeds, causing damage to cell membranes and leakage of cell content in agreement with previous reports²⁵.

Conclusion

The spent lubricating oil clearly had detrimental effects on germination, growth and yield of maize crop. Microbial population and diversity in the soil were adversely affected by the oil. Therefore, the use of different biological indicators, such as bacterial density, and diversity and germination of maize is efficient and informative in characterization of soil health status. The legume plants used in this study combined with poultry manure are effective in restoring the soil health when disposal of petroleum products is the dominant problem. Particularly the, *Gliricidia*, *Leucaena* and *Calopogonium* spp, supplemented with poultry manure significantly reduced soil acidity, THC and increased the soil micro-organisms. Therefore, these legume plants are promising species in improving the productivity of oil-contaminated soils.

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