

# **Evaluation of Physicochemical Properties, Micro and Macro- Minerals of Soil Contaminated With Crude Oil and Remediated With Cow Horn-Powder**

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**ABSTRACT:** The objective of this paper was to present the evaluation of physicochemical properties, Micro and Macro-Minerals of soil contaminated with Crude Oil and remediated with cow Horn-Powder using standard methods. The results of the physicochemical properties (pH, Moisture Content, Soil Organic Matter and Conductivity) of remediated soils ranged between  $0.293\pm0.003$  and  $92.01\pm1.15$ . The results of the Micro Minerals (Zn, Cu, Na and Fe) ranged between  $6.569 \pm 0.054$  and  $1293 \pm 0.008$ . The results of the Macro minerals (P, N, Ca, K) and Mg) ranged between 0.293±0.003 and 173.8±0.002. Accordingly, significant differences (P≤0.05) were noticed between the values of physicochemical properties, Micro and Macro minerals of the impacted soils and controls. Results suggest Cow Horn-Powder is potent in raising Physicochemical Properties, Micro and Macro Minerals levels of Crude Oil impacted Soils.

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*\*Corresponding Author Email: [nkpakol@gmail.com](mailto:nkpakol@gmail.com) Tel: 07034426338; 07054628500* It's no news that Crude oil pollution is a Global challenge, with great negative effects on water and lands. Associated with these detrimental environmental impacts come hazards on public health. It has been shown that the pollution induced by Crude Oil has a negative impact on the Physicochemical, Micro and Macro minerals qualities of the soil. Soil Moisture content, Lead, Electrical Conductivity, Nickel, Chloride and Copper level in Soils increases with Crude Oil Contamination (Osuji and Onojake, 2004). Moreover, whereas both Total Organic Matter and Total Organic Carbon levels in polluted surface soils tend to decrease, there is an increase in subsurface polluted soils. The pH level of Crude oil contaminated soil decreases (Osuji and Onojake, 2004). Soil Electrical Conductivity and Organic Matter increases, while the availability of Phosphate, Nitrogen and Exchangeable acidity reduces due to Crude Oil Spillage (Chukwu and Udoh, 2014). When Crude Oil spills occur, they disrupt the delicate balance existing between the soil's physical, Biological and Chemical properties. Consequently, plant kingdom, fertility of the soil and the Ecosystems at large receives adverse consequences because of these alterations (Etuk *et al.*, 2013). Without soil minerals, plants won't grow well. These minerals are of great necessity to plants, to thrive. Minerals are divided into two groups or classes: Micro and Macro. Macro minerals are minerals required by plants in large quantities (about 10-100kg or more per hectare). Some examples of Macro minerals are Nitrogen (N), Phosphurus (N), Potassium (K), Calcium (Ca), Magnesium (Mg), and Sulphur (S). Micro minerals are minerals required by plants in low quantities. Zinc (Zn), Manganese (Mn), Copper (Cu), Iron (Fe) and Sodium (Na) are some examples of Micro Minerals. It is of great essence to properly remediate soils impacted with Crude Oil, as to bring about plants growth and supports to other environmental services. Biostimulation method is one of the methods documented to bring about remediation of the environment. For instance, the use of fertilizers to boost the soils nutrients (Ayotamuno *et al*., 2006). This method, according to Margesin and Schinner (2001) and Yakubu (2007) is vast, highly efficient, economical and environmentally friendly. Cow horns contain minerals compositions such as Nitrogen, phosphorus and Potassium that are capable of adding nutrients to microorganisms to enhance their ability to clean up Crude Oil contaminated soils. Therefore, this study was designed to evaluate the physicochemical properties, Micro and Macro-minerals of soil contaminated with Crude Oil and remediated with Cow Horn-Powder.

## **MATERIALS AND METHODS**

*Sources of Materials:* The Cow Horns used for this study were obtained from an Abattoir at Aluu Town, Port Harcourt, Rivers State, Nigeria. The Crude Oil was gotten from Shell Petroleum Development Company (SPDC), Port Harcourt. The soil samples used were obtained from the experimental plot of land at Aluu Town, Port Harcourt, Rivers State, Nigeria.

*Baseline Studies of Experimental Oil:* Soil samples were collected, prepared and analyzed from all the plots for Physico-chemical Properties (pH, Moisture Content, Soil Organic Matter (SOM) and Conductivity), Micro Minerals (Zn, Cu, Na, Mn and Fe) and Macro Minerals (Total Phosphorus, Nitrogen, Ca, K and Mg). This was done to know the true state of the experiment site.

*Crude Oil Contamination of Soil:* Zero point nine (0.9) liters of Crude Oil was used to artificially contaminate the Soil per 0.18m<sup>2</sup> . As described by Ayotamuno *et al*. (2006), a rubber can that has holes was used. This was driven into the soil using fork. The plot designated Normal Control (NC) was not contaminated.

The contaminated Plots were left for fourteen days. The plots were then tilled systematically through homogenization, ploughing and windrows construction to bring about good surface areas for breaking down of microbes, weathering, vaporization, temperature etc. This was done after every two days.

*Preparation of Bio stimulating Agent (Cow Horn-Powder*): Thirty- four (34) pieces of Cow Horns were purchased from an Abattoir in Aluu-Town, Port Harcourt, taken to the Laboratory, washed thoroughly with deionized water to remove dirt and impurities from the surfaces. They were cut into pieces and airdried for 72 hours. They were ground with electric grinder and finally applied to the soil.

*Soil Biostimulation:* After two weeks of impacting the soils with Crude Oil, Soil samples were collected and analyzed. Thereafter, the partitioned plots encoded XgCH, YgCH and ZgCH were biostimulated with 50g, 75g and 100g respectively of ground Cow Horn Samples.

*Experimental Design:* The Experimental design used in this study was carefully done to encompass the collection, measurement and analysis of data. Application of randomization was done. In order to have an excellent finalization of results, quantitative experiment was taken into consideration. The study was carried out on a plot of land by size 220m<sup>2</sup>. The plot was divided into five sub plots, separated apart by two meters. The sub-divided plots were plot 1 (Normal Control), plot 2 (Negative Control), plot 3 (Treated Plot), plot 4 (Treated Plot) and plot 5 (Treated plot). The plots were respectively coded NC, -Ve C, XgCH, YgCH and ZgCH.

While NC received no contamination and biostimulation, -Ve C was contaminated but not biostimulated. The remaining three plots were contaminated and treated (bio stimulated using Cow Horns). The partitioned plots were made into beds of 40cm by 40cm with depths of 30cm. This was done to mitigate against contaminants flowing to nearby plots; especially if/ when it rains.

*Soil Samples Collections:* The Soil Samples were collected from 0-25cm depth, and were thoroughly homogenized. Soil samples were collected as following: before Soil contamination, before Soil biostimulation, on the  $6<sup>th</sup>$  and  $8<sup>th</sup>$  weeks respectively. The Soil Samples were collected and put in plastic bags that have been sterilized, in alignment with the opinion of Kale *et al*. (2018). The samples were immediately transferred to the laboratory for analysis.

*Soil Sample Preparation:* The Soil samples were left undisturbed overnight, haven been soaked with deionized water. They were subsequently pounded in an agate mortar and with the aid of plastic vial, got filtered.

*Soil Sample Clean-up:* In order to remove Polar Hydrocarbon, moisture, interferences, colour and any possible impurity, the sample clean-up was done. *Digestion of Soil:* Soil digestion was done using a mixture of Nitric acid and Hydrochloric acid.

*Instrumentation and Methods of Assay: Determination of Physiochemical Parameters, Micro and Macro minerals of the Soil:* Soil physiochemical parameters (Moisture content, Total Organic Matter, Conductivity and pH ), Micro (Zn, Cu, Na, Mn and Fe) and Macro Minerals (Phosphorus, Nitrogen, Ca, K and Mg) were determined.

*Determination of Phosphorus:* The method of Shyla *et al* (2011) was followed. Spectrophotometric method of measurement was used in this research to determine phosphorus.

*Determination of Moisture Content:* AOAC (2020) method was followed in the determination of the soil moisture content.

*Determination of Total Organic Matter (TOM):* Bisutti *et al*. (2004) method of soil total organic matter determination was followed.

*Determination of Total Nitrogen in the Soil:* The method of Kalambe (2021) was followed.

Nitrogen in soil was determined using the Kjeldahl Digestion method. They involved three steps: the digestion step, distillation and titration.

*Soil pH Determination:* ASTM D4972 (2019) method was used in determining pH of the soil. A ratio of 1:20 soil to water was prepared as follows: Five grams of air- dried soil was put into a one hundred (100) ml beaker and eighty (80) ml of deionized water was added to it and continuous Stirring of the suspension for half an hour was done. Determination of the pH was done by immersing electrodes in the suspension.

#### *Electrical Conductivity Determination*

(i)Fifty grams of air dried soil samples was weighed into a one hundred (100) ml glass beaker.

(ii)Fifty (50) ml of deionized water was added, using a graduated cylinder.

(iii)It was mixed well using a glass rod, and allowed to stand for thirty minutes.

(iv)The suspension was stirred every ten minutes.

(v)The combined electrode was put into the suspension; about 3cm deep. The reading to one decimal place was taken after thirty seconds.

(vi)The combined electrode was removed carefully from the suspension, and thoroughly rinsed with deionized water in a separate beaker.

*Instrumentation:* In this Research work, the determination of the concentrations of the Physicochemical properties, Micro and Macro Minerals were done respectfully with the instrumentality of UV/Vis spectrophotometer, multipara meter, insitu meter and 250D laboratory tech oven.

*Statistical Analysis:* Statistical package for the Social Sciences (SPSS) was used to determine the differences among treated and control groups. Comparison among groups were done using one way ANOVA. Significant differences between control and treated were assessed by the least significant difference (LSD). All data were expressed as mean  $\pm$  Standard error of the mean; pvalues less than or equal to 0.05 were considered to be significant (Igwe *et al*., 2020).

## **RESULTS AND DISCUSSION**

*Baseline Properties of the Clean Experimental Soil:*  The baseline studies of the physicochemical parameters, Micro and Macro Minerals from the research study site are summarized and presented in table 1. Presented in Table 1 are the baseline results of the clean experimental soil. The pH of the soils  $(4.26\pm0.10)$  was acidic. This is normal for an agricultural soil. This finding was in consonance with the work of Belonwu *et al*. (2007) which established pH from baseline studies to be acidic. Gray *et al*. (1998) documented that the pH of soils has a complementary relationship with the nutrients available in soils to plants. The values of the Moisture Content, Soil Organic Matter and Electrical Conductivity were relatively low. This may be due to the soil texture. The values of Mn, Cu, Zn, Na and Fe were found to be higher than the recommended values by the WHO. Oviasogie and Ndiokwere (2008) had opined that, soils that are low in pH would bring about higher concentrations of metals in soils. Conversely, the values of Nitrogen, Phosphurus, Calcium, Magnesium and Potassium were lower as compared with WHO recommended values.

**Table 1:** Baseline Properties of the Clean Experimental Soil

S/N	<b>Parameters</b>	<b>Units</b>	<b>Values</b>			
1	pΗ		$4.26 \pm 0.10$			
2	Moisture Content	$\frac{0}{0}$	$32.3 + 0.44$			
3	<b>Total Nitrogen</b>	Mg/kg	$5.04 \pm 0.03$			
4	Soil Organic Matter	Mg/kg	$13.1 \pm 0.07$			
	(SOM)					
5	Phosphorus	Mg/kg	$0.350 \pm 0.003$			
6	Conductivity	Us/cm	$38.7 \pm 1.16$			
7	K	Mg/kg	$127 \pm 0.04$			
8	Ca	Mg/kg	$5.07 \pm 0.02$			
9	Na	Mg/kg	$36.0 \pm 0.12$			
10	Mg	Mg/kg	$135 \pm 0.02$			
11	Mn	Mg/kg	$88.9 \pm 0.05$			
Values are means of triplicate determinations $\pm$ standard						

*deviation.*

*ND = Not Detected*

*Effects of Enhanced Bioremediation on Soil Physicochemical Properties:* Table 2 below shows the analytical results of the Physico-chemical properties

asessement of the soils after contamination with Crude Oil (day 0) and biostimulated with Cow Horn Powder after six and eight weeks respectively. The parameters were pH, Moisture content, Soil Organic Matter and Electrical Conductivity. Table 2 (Effects of Enhanced Bioremediation on Soil Physicochemical properties) results reveals higher values of pH in the impacted soils than normal control, after two weeks of soil pollution. This is in consonance with the findings of Osuji and Adesiyan (2005); Obsasi *et al.* (2013) and Egobueze *et al.* (2019) that, the pH of crude oil contaminated soil gained significant increase after the second week of contamination. The Moisture content of the contaminated soils gained increase significantly when compared with the controls. The reason for this high moisture content may be adduced to infiltration which is lower in the soil, sequel to the density, which is large in size brought about by the contamination (Agim *et al*., 2021). Increase in the moisture content of soils by crude oil pollution had been reported by Osuji and Onojake (2004). No statistical difference was observed between the values of soil organic matter (SOM) from the contaminated beds and controls after two weeks of soil pollution. This may be attributed to the fact that, the crude oil caused an impairment in the metabolic processes that would have brought about increased soil activities (Osuji and Onojake, 2004). Lower values of electrical conductivity from all the contaminated beds than the control bed were recorded; with YgCH bed having the lowest concentration and XgCH bed the highest concentration of electrical conductivity (E.C). This agrees with the findings of Osuji and Nwoye (2007) who reported lower E. C values in oil -affected soils. The table (2), shows a decrease in the pH of the soils after six weeks of remediation and an increase in the pH of the soils after eight weeks of remediation from the three beds when compared with the controls. The initial decrease observed in the soil's pH corroborates the work of Egobueze *et al.* (2019) who recorded a decrease in the pH of soils contaminated with crude oil and amended with palm oil wastes. It may be that the nutrients added to the soils were not sufficient to cause an increase in the soil's pH. The increases recorded in the pH of all the bio-stimulated soils are in agreement with the documentations of Ijah and Antai (2003). The increase after eight weeks recorded in the pH of soils polluted with crude oil but bio-stimulated with cow horn-powder also agrees with the assertions of Egobueze *et al.* (2019) who recorded an increase in soil pH of Fadama crude oil polluted soils amended with poultry wastes. Table 2 also reveals a significant decrease in soil's Moisture Content from the various bio-stimulated beds after the sixth and eight weeks of remediation respectively. The decreases observed may be attributed to uncompensation of water lost in the soils by the Organic Matter degradation by-product water produced (Ghaly *et al.,* 2013). It may also be that adequate water was not supplied to the soils, resulting in the soil being dried and hindering microbe's activities (Nwankwo, 2012).



*Values are means of triplicate determinations ± standard deviation; NC = Normal Control; -Ve = Negative Control; XgCH = Treated Plot (50g of Cow Horn powder); YgCH = Treated Plot (75g of Cow Horn powder); ZgCH = Treated Plot (100g of Cow Horn powder)*

Also, the table (2) reveals a decrease in Soil Organic Matter from the remediated plots, after six and eight weeks of remediation respectively. The decreases observed in the Soil Organic Matter Content may be due to fast decomposition of soil matter by microorganisms having sufficient oxygen. General increases were notice in the Electrical Conductivity of soils from the remediated beds after six and eight weeks of remediation respectively. This finding agrees with the work of Osuji and Onojake (2004) who recorded increases in the values of electrical conductivity on a remediated crude oil contaminated soils

*Effects of Enhanced Bioremediation on Soil Micro Minerals:* The results of Zinc (Zn), Copper (Cu), Sodium (Na), Manganese (Mn) and Iron (Fe) from all the impacted plots and control are clearly presented in Table 3. The concentrations of the Micro Minerals of the Soil are presented in table 3. It was observed that Cu, Mn, Zn, Na & Fe were present in the impacted soils. While statistical differences were observed in the values of all the metals (Mn, Na, Zn and Fe), no statistical difference was observed in the values of Cu. Generally, these findings are consistent with the work of Rasheed *et al.* (2013). They documented that, the presence of micro and macro minerals in polluted soils sequel to crude oil contamination may be due to chemical reactions that are near the soil surface. After six weeks of remediation, reductions were observed in the values of manganese (Mn) from all the remediated plots, with significant differences to the controls. However, after one month of remediation, increases were observed from the 3 different remediated plots. Statistical differences were recorded when compared to the controls. The Table (3), further reveals a decrease of 75%, 30.4% and 6.0% respectively in the copper (Cu) contents from the remediated plots after six weeks of remediation, with statistical differences to the controls. On the contrary, increases of 28%, 5.3% and 40.0% were observed after one month of remediation. The decreases recorded after six weeks of remediation may be attributed to significant increase in the numbers of microorganisms. This may have placed high demand on the Copper (Cu) generated from the soils, thus, leading to a decrease in the Cu content of the soils (Agbogidi *et al.,* 2007). The general increases in the concentrations of Cu after one month of remediation may be adduced to the fact that, the cow horn powder were potent in improving the soil's nutrient. This is in absolute agreement with the findings of Mbah *et al*. (2009). General increases were noticed in the values of Zinc (Zn) and Sodium (Na) from the remediated plots after six weeks and one month respectively. Significant differences were observed when compared to the controls. These results shows the effectiveness of cow horns in adding nutrient (Zinc and Sodium) to soils impacted with crude oil. The obtained results conforms to the work of Nigam *et al.* (2014) who opined that manures are needed to increase the concentration of zinc and Sodium in soil for healthy growth of plants. Increases of 53.0%, 62.3% and 64.1% were observed in the Iron (Fe) content of soils after six weeks of remediation from the XgCH, YgCH and ZgCH respectively, with significant differences to the controls. Similarly, 52.4%, 65.0% and 67.0% increases were respectively recorded from the remediated plots after one month of remediation. These current study results, agrees with the findings of Akenga, *et al.* (2014) who investigated the concentration of Fe in soils at Kakamega North District, Kenya. The regulation of plant's growth requires an enzyme system of which Fe is part of. It is a micronutrient largely needed by plants and animals.



*Values are means of triplicate determinations ± standard deviation; NC = Normal Control; -Ve = Negative Control; XgCH = Treated Plot (50g of Cow Horn powder); YgCH = Treated Plot (75g of Cow Horn powder); ZgCH = Treated Plot (100g of Cow Horn powder)*

*Effects of Enhanced Bioremediation on Soil Macro Minerals:* The results of Phosphorus (P), Nitrogen (N), Calcium (Ca), Potassium (K) and Magnesium (Mg) from all the impacted plots and control are clearly presented in Table 4. Table 4 is the result of Enhanced Bioremediation on Soil's Macro Minerals. Significant decreases were recorded in the values of total nitrogen from all the contaminated soils when compared with the controls after two weeks of soil contamination. This was corroborated by Chukwu and Udoh (2014) who reported a decrease in the availability of total nitrogen upon crude oil contamination. Increase in the number of microbes which may have caused demand on the nitrogen earlier generated from the soil may be the reason for the decrease in the total nitrogen content of the soils (Agbogidi *et al.,* 2007). There were 0.5%, 2.0% and 5.0% increases in the Total Nitrogen content of soils after six weeks of remediation respectively for XgCH, YgCH and ZgCH. In the same vein, increases of 11.0%, 11.0% & 20.0% respectively were observed for XgCH, YgCH & ZgCH after eight weeks of remediation when compared with controls. The total nitrogen content of the soils ranged from 4.45 to 4.68 after six weeks of remediation and 1.50 to 5.23 after eight weeks of remediation. Statistical differences were noticed among the various remediated plots and controls. Different researchers have recorded various variation in the total nitrogen content of soils biostimulated or augmented with substances (Nwankwo, 2012). 78%, 79% and 79.3% decreases respectively were recorded from the three contaminated beds for phosphorus when placed side by side with the controls after the second week of pollution. The observed decrease in available phosphorus may be attributed to the quick usage of small quantities of phosphorus to break down hydrocarbons in the soil by microorganisms. In continuation, Table 4 shows 44.0%, 52.1% and 34.0% increases for Phosphorus when compared with the controls after six weeks of remediation from XgCH, YgCH and ZgCH plots. It further shows 29.4%, 52.1% and 34.1% increases from the XgCH, YgCH and ZgCH plots respectively after eight weeks of remediation. Statistical differences were observed between the values from the remediated plots and controls. These results were corroborated by Nwankwo (2012) who recorded a decrease in the level of phosphorus on a crude oil impacted soil remediated with feedstock. Osuji and Nwoye (2007), Adoki and Orugbani (2007a) have differently recorded to low quantity of N in the remediating agent (Cow horns). In the same vein, increases were recorded in the values of Calcium (Ca), Magnesium (Mg) and Potassium (K) after the sixth and eight of Bioremediation respectively. The general increases in the concentrations of Ca, Mg and K after one month of remediation may be adduced to the fact that, the cow horn powder were potent in improving the soil's nutrient. This is in absolute agreement with the findings of Mbah *et al*. (2009), who documented that, adding organic wastes to oil contaminated soil led to improved agronomic properties of the soils.

<b>Table 4:</b> Effects of Emilianced Bloremediation on Soft Macro Millierals							
<b>Parameters</b>	NC	$-VeC$	<b>XgCH</b>	YgCH	ZgCH		
Nitrogen (Mg/Kg)							
Day 0	$4.67 \pm 0.33$	$1.24 \pm 0.09$	$4.09 \pm 0.91$	$4.18 \pm 0.83$	$4.31 \pm 0.51$		
Week 6	$4.43 \pm 0.58$	$1.33 \pm 0.07$	$4.45 \pm 0.55$	$4.50 \pm 0.50$	$4.68 \pm 0.33$		
Week 8	$4.20 \pm 0.80$	$1.50 \pm 0.24$	$4.71 \pm 0.30$	$4.70 \pm 0.30$	$5.23 \pm 0.21$		
Phosphorus (Mg/Kg)							
Day 0	$0.357 \pm 0.003$	$0.167 \pm 0.003$	$1.619 \pm 0.014$	$1.666 \pm 0.003$	$1.722 \pm 0.004$		
Week 6	$0.422 \pm 0.003$	$0.171 \pm 0.003$	$0.237 \pm 0.001$	$0.202 \pm 0.003$	$0.280 \pm 0.009$		
Week 8	$0.354 \pm 0.006$	$0.192 \pm 0.003$	$0.250 \pm 0.010$	$0.256 \pm 0.003$	$0.293 \pm 0.003$		
Calcium $(Mg/Kg)$							
Day 0	$1.006 \pm 0.001$	$3.501 \pm 0.001$	$289.7 \pm 0.001$	$388.5 \pm 0.001$	$412.6 \pm 0.001$		
Week 6	$4.934 \pm 0.001$	$2.007 \pm 0.001$	$34.84 \pm 0.001$	70.47±0.001	$37.53 \pm 0.001$		
Week 8	$2.952 \pm 0.001$	$10.70 \pm 0.001$	$4.30 \pm 0.001$	$11.00 \pm 0.001$	$48.10\pm0.001$		
Magnesium (Mg/Kg)							
Day 0	$11.06 \pm 0.09$	88.85±0.017	235.9±0.046	$263.6 \pm 0.001$	$137.0 \pm 0.069$		
Week 6	$55.91 \pm 0.008$	$56.43 + 0.032$	$14.91 + 0.008$	52.59±0.009	$49.01 \pm 0.009$		
Week 8	54.32±0.009	141.9±0.009	$62.21 \pm 0.001$	$80.95 \pm 0.001$	$170.0 \pm 0.001$		
Potassium (Mg/Kg)							
Day 0	85.26±0.001	$100.4 \pm 0.001$	$229.5 \pm 0.001$	$216.3 \pm 0.001$	$89.4 \pm 0.001$		
Week 6	$88.30 \pm 0.001$	$73.92 \pm 0.001$	92.82±0.001	$116.8 \pm 0.001$	$122.2 \pm 0.001$		
Week 8	$123.6 \pm 0.001$	$136.3 \pm 0.001$	$84.32 \pm 0.001$	$102.8 \pm 0.001$	$173.8 \pm 0.001$		
0.11 $\mathbf{r}$	$\cdots$ $\cdots$ $\cdots$						

**Table 4: Effects of Enhanced Bioremediation on Soil Mac** 

*Values are means of triplicate determinations ± standard deviation; NC = Normal Control; -Ve = Negative Control; XgCH = Treated Plot (50g of Cow Horn powder); YgCH = Treated Plot (75g of Cow Horn powder); ZgCH = Treated Plot (100g of Cow Horn powder)*

*Conclusion:* Results from this research work suggest Cow Horn-Powder is potent in raising Physico-

Chemical Properties, Micro and Macro Minerals levels of Crude Oil impacted Soils. This research also

demonstrated that Cow Horns are good sources of manure.

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