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Use of Macro-Minerals of Fluted Pumpkin in Evaluation of Remediation Potentials of Crude Oil Impacted Soil by Cow Horn Powder at Ikwerre Local Government Area, Rivers State, Nigeria

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ABSTRACT: The objective of this work is to assess the remediation potentials of cow horn powder using fluted pumpkin grown on crude oil contaminated soil at Ikwerre Local Government Area, Rivers State, Nigeria using appropriate standard methods. Data shows that the ZgCH group showed a better treatment than XgCH and YgCH in enhancing the level of the minerals (Na =67.1±0.00, N = 111±0.41, Ca = 493±0.00) when compared with the level of the minerals in the -Ve C (Na = 39.0±0.06, N = 65.4±0.03, Ca = 367±0.00). The results after the 8th week of remediation showed that cow horn powder significantly improved the macro minerals concentration of fluted pumpkin leaves grown on crude oil-impacted soil across all the cow horn powder treated groups compared to the -Ve C and NC groups. In conclusion, cow horn powder can enhance the mineral concentration of plants grown on crude oil-impacted soil.

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The increased rate and use of Crude Oil as necessary energy source has resulted to higher use of poor sub-standard, non-conventional, and unprofessional methods for producing and processing Crude Oil (Whitworth *et al.,* 2018). The unprofessional method of Crude Oil processing involves illegal extraction and most times standard protocols, measures and conditions are not kept. Hence, the unprecedented adverse effect of the toxic components of Crude Oil has become evident and of great concern to individuals' health in the occupational, environmental and agricultural sectors (O'Callaghan-Gordo *et al*., 2016; Johnston *et al*., 2019). These toxic Chemicals associated with Crude Oil pose a great menace leading to Environmental degradation involving air, water, and soil pollution. Hence, the aquatic life becomes negatively affected, as well as soil products required to sustain domestic, social, and human lives. When Crude Oil and its products get into the Environment, they undergo numerous weathering processes that interfere with their physicochemical properties. Heavy metals such as Lead, Cadmium, Nickel, Zinc, Chromium, Arsenic and Vanadium are equally found in Crude Oil (Onojake and Frank, 2013; Fu *et al.,* 2014). Fluted pumpkin, botanically known as *Telfairia occidentalis,* belonging to the Family Cucurbitaceae, is a common vegetable grown in West Africa, predominantly in Nigeria for its edible seeds and leaves (Gbile 1986). The economic importance of the plant has been utilized in its use for the treatment of anaemia, malaria, and attacks of convulsion (Gbile 1986; Okokon *et al.,* 2009). This agrees with the scientific reports on the anti-anemic property of fluted pumpkins (Alada 2000). Oboh (2004) also reported

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fluted pumpkin leaves to prevent garlic-induced hemolytic anemia in rats. As a medicinal plant fluted pumpkin is also confirmed to possess some antioxidant, antidiabetic and antimicrobial features and characteristics (Aderibigbe *et al.,* 1999; Oboh *et al.,* 2006; Eseyin *et al.,* 2010). Also, its action against high cholesterol has been observed and reported (Eseyin *et al.,* 2005; Adaramoye *et al.,* 2007). The seed of fluted pumpkin has also been reported to protect the testes against oxidative damage due to aging or toxic chemicals (Abarikwu *et al* 2022; Ezim *et al.,* 2023). These Scientific reports justify the common use of the plant in Nigeria by pregnant women constantly The nutritional weapon and driving force of fluted pumpkin against diseases and illnesses including inflammation, cancer, and arthritis are due to the presence of some essential substances like Zinc, Potassium, Phosphorous, Selenium and Magnesium which are present or identified in pumpkin seeds (Batool *et al.,* 2022) However, environmental contamination of soil with Crude Oil will results to grave depression in the growth of plants and the loss of the Mineral components responsible for the Medicinal and economic importance of plants such as fluted pumpkin. Cow horn powder has been reported by researchers to improve the growth of plants and enhance the biological activities of the soil even at its minute quantity (Turinek *et al.,* 2009). The importance and effect of Cow horn powder as an agricultural stimulator have also been studied. However, there are limited studies on the effect of Cow horn powder as a bioremediation substance to improve the fertility of crude oil-impacted Soil, as well as maintain and improve the mineral content of fluted pumpkin grown on crude oil-impacted soil. Hence, this study was therefore designed to assess the remediation potentials of cow horn powder on fluted pumpkin seed grown on crude oil contaminated soil at Ikwerre Local Government Area, Rivers State Nigeria.

MATERIALS AN METHODS

Chemical/ Reagents: Reagents and chemicals were purchased from BDH chemicals limited and Hopkins and Williams Essex (England). All reagents and chemicals were of the analytical grade. All glassware and containers utilized were washed using soap, rinsed, soaked in 10% nitric acid for twenty-four hours, cleaned carefully with distilled water, and dried. The chemicals/reagents used include: Acetone (C3H6O), Acetic acid (C2H4O2), Ammonia (NH3), Ammonium, Hydroxide (NH4oh), Argon (Ar), Baric acid (H2BO4), Calcium Chloride (CaCl2), Hydrochloric acid (Hcl), Dichloromethane, Ethanolic extract, Ethanol, Helium (He), Hexane (C6H14), Hydrofluoric acid (HF), Nitric acid (HNO3), Potassium Chloride (KCL), Potassium Dichromate (K2aCr2O2), Potassium, Phosphate (K2PPO4), Sodium Carbonate (Na2Co3), Sodium Chloride (NaCl), Sodium Fluoride (NaF), Sodium Hydroxide (NaOH), Sodium Silicate, Sodium Sulphate (NaSO4), Toluene.

Equipment and Apparatus: Gas Chromatography, Atomic Absorption Spectroscopy, Ultraviolet/Visible Spectrophotometer, Multipara meter, 250D Lab tech Oven, Digestive tube, Manual Hammering Hallow meta pipe,, Measuring cylinder, Microwave digester, Retort stand, round bottom flask, Thermometer, Crucible, Petrich dish, Pipette, Plastic Vial, Teflon Vassel, Kjehdal Flask, Conical flask, Distillation, Heating mantle.

Demographic Description of the Sample Area: The contaminated soil and research area is located at Ikwerre Local Government Area, River State. The L.G.A. covers an area of 667.5km, with 265,400 inhabitants. Which has coordinated Latitude: $6^0$58N to $7^0$6'N and Longitude: $4^0$40'E to $4^0$55'N.3470E. The soil is sandy loam. The field experiment was conducted in randomized block design with five replica. After ploughing, the seeds were sown on 21 February 2022. Germination of Fluted Pumpkin was seen 14 days after Plantation. Collection of 1st Plant samples was at 4 weeks after sowing (25/3/22), then at 6 weeks for the collection of 2nd Plant samples (8/4/22), and at 8 weeks for the collection of 3rd Plant samples (22/4/22).

The average maximum temperature of this research area on daily bases and throughout the researched period is 28.64 °C. Dry seasons with the greatest average daily temperatures are observed in the months of February, March, and April. March had temperature of 36 °C, whereas February temperature was 35.1 °C for ten years spanning from 1985 to 1994. This same space-based pattern could be seen for average daily temperature. It is usually possible to predict monthly rainfall that increases in months of July up till August before it declines in dry season which start from November down to February. From July up till September, experiences the most rain period. Although exact occurrence period differs.

Source of Materials: The fluted pumpkin seeds were sourced from a farm located in Choba, Port Harcourt. These seeds were confirmed by a Taxonomist in UNIPORT Herbarium. The Crude Oil was gotten from Shell Petroleum Development Company, Port Harcourt, Rivers State. The Cow Horns were gotten from an Abattoir in Aluu Town.

Cow Horn powder Preparation: The thirty-four pieces of Cow Horns procured were washed with deionized water, air-dried for 72 hours, and ground with electric grinder into powder.

Experimental Design: The research investigations were carried out over a period of 10 weeks. Crude Oil of 0.9 L was used to Contaminate 0.18 m^2 of land. This was a modified method of Ayotamuno et al. (2006). Ayotamuno et al. (2006) contaminated 0.16 m^2 of Land using 0.8 L of Crude Oil. The soil was partitioned into five plots of 40 cm by 40 cm. Plot 3, 4, and 5 were amended with the application of cow horn manure of various weights of 50g, 75g, and 100g respectively, three days before planting pumpkin seeds. Fifteen viable seeds of fluted pumpkin were sown on each of the plots.

Plot 1 = (NC = Normal Control: Not remediated nor contaminated)
Plot 2 = (-Ve C = Negative Control: Contaminated but not remediated)
Plot 3 = (XgCH = Contaminated and remediated with 50g of cow horn powder)
Plot 4 = (YgCH = Contaminated and remediated with 75g of cow horn powder)
Plot 5 = (ZgCH = Contaminated and remediated with 100g of cow horn powder).
4 weeks, 6 weeks, and 8 weeks after application of cow horn powder.

Sample Processing: The leaves of the fluted pumpkin were harvested, washed thoroughly using distilled water to remove impurities; then dried in air by being spread in the laboratory at normal room temperature 25ºC, crushed with mortar and pestle to powdered form. The soil samples collected were also dried using forced draft drying chamber at temperature of below 50ºC for nearly twenty-four hours, grinded to powdered form and analysed using specific methods. Available nitrogen, in the soil sample, was determined using regular micro Kjeldal technique and tabulated in percentage units.

Determination of Mineral Composition: A 2 g of samples were correctly weighed into porcelain crucible then turned to ash at 450ºC to 500ºC and then cooled to normal temperature in desiccator. The ash that was digested with 5 mL mixture of HNO3, HClO4, and HCl in ratio 3:1:1; digested solution was painstakingly moved into 100 mL flask and filled up to mark using distilled water. The macro minerals content (Na, Ca) was ascertained using atomic absorption spectrophotometry ("AA-6800) analysis. Triplicate digestion for these samples and blank were conducted to ensure precision.

Statistical Analysis: All the figures are shown as mean ± SD in the respective groups. Substantial changes among the different groups were evaluated using GraphPad for Windows with a one-way analysis of variance. The ANOVA was followed by Tukey's test as a post hock test used for the assessment of data difference between the test groups and the control group. A significant change was considered substantial at the value $P < 0.05$.

RESULTS AND DISCUSSION

Physicochemical parameters of the normal soil: Table 1 shows the summary statistics of the physicochemical properties of soil before contamination.

Table 1: Physicochemical parameters of the Soils

Parameters	Values
Na	36.0±0.12
P	0.35±0.00
N	5.04±0.0
Ca	5.07±0.00
K	127±0.00
Mg	135±0.00
pH	4.26±0.10

Values are means of triplicate determinations ± standard deviation.

***S**elected Macro Minerals of the Soils after Contamination with Crude Oil:* Contamination with crude oil decreased P and Nitrogen, but increased Ca, K and Mg levels compared with normal control. Remediation with cow horn powder at 50, 75 and 100g increased the level of all macro minerals exception of K at 100g remediation. All selected macro minerals increased in bioremediation soil

Table 2: Selected Macro Minerals of the Soils after Contamination with Crude Oil

Samples	P	N	Ca	K	Mg
-N C	0.357 ± 0.003^{cde}	4.67 ± 0.33^{yqzw}	1.006 ± 0.001^{bcde}	85.26 ± 0.000^{zqyw}	11.06 ± 0.009^{bcde}
-Ve C	0.167 ± 0.003^{cde}	1.24 ± 0.09^{cdea}	3.501 ± 0.001^{acde}	100.4 ± 0.000^{xqyw}	88.85 ± 0.017^{acde}
XgCH	1.619 ± 0.014^{abde}	4.09 ± 0.91^{xbyw}	289.7 ± 0.001^{abde}	229.5 ± 0.000^{xzyw}	235.9 ± 0.046^{abde}
YgCH	1.666 ± 0.003^{abce}	4.18 ± 0.83^{xbqw}	388.5 ± 0.001^{abce}	216.3 ± 0.000^{xzqw}	263.6 ± 0.000^{abcd}
ZgCH	1.722 ± 0.004^{abcd}	4.31 ± 0.51^{xbqy}	412.6 ± 0.001^{abcd}	89.40 ± 0.000^{xzqy}	137.0 ± 0.069^{abcd}

Values are means of triplicate determinations ± standard deviation; NC = Normal control (Uncontaminated and untreated); -Ve C = Negative control (Contaminated and untreated). XgCH = Treated group 1: (contaminated and treated with 50g of cow horn powder). YgCH = Treated group 2: (contaminated and treated with 75g of cow horn powder). ZgCH = Treated group 3: (Contaminated and treated with 100g of cow horn powder).

Elemental Compositions of cow horn samples: The cow horn samples as seen in Table 3 has a very high content of Na, Mg, K, Ca, P, , and N in a descending manner.

Selected Macro minerals of the fluted pumpkin leaves after the 4th week of remediation: The results on the macro minerals of fluted pumpkin leaves after 4th week of remediation show that the N, Ca, and Mg content of the fluted pumpkin leaves increased significantly in groups treated cow horn powder (XgCH, YgCH, and ZgCH) when compared to the -Ve C group. The K content of fluted pumpkin leaves was seen to decrease in groups YgCH and ZgCH with no statistical difference observed in group XgCH when compared to the -Ve C. An increase in P mineral of fluted pumpkin leaves was observed in groups (XgCH and YgCH, with no statistical difference observed in group ZgCH when compared to the -Ve C

Table 3: Selected Macro minerals of the fluted pumpkin leaves after the 4th week of remediation

Samples	P	N	Ca	K	Mg
-N C	5.05±0.02^{bcde}	63.0±0.06^{bcde}	443±1.48^{zcyw}	17.1±0.01^{bcde}	301±0.27^{bcde}
-Ve C	10.4±0.50^{acdz}	80.4±0.07^{acde}	468±62.4^{xqyw}	19.5±0.01^{acde}	667±0.60^{acde}
XgCH	13.2±0.01^{abde}	79.5±0.07^{abde}	536±47.1^{azyw}	20.4±0.01^{abde}	921±0.83^{abde}
YgCH	14.3±0.30^{abce}	96.0±0.09^{abce}	457±64.1^{xzqw}	12.8±0.01^{abce}	1854±1.67^{abce}
ZgCH	10.4±0.40^{abcd}	95.0±0.09^{abcd}	512±21.0^{xzqy}	13.2±0.01^{abcd}	1208±1.09^{abcd}

Values are means of triplicate determinations ± standard deviation.

Macro minerals of the fluted pumpkin leaves after the 6th week of remediation: The results on the macro minerals of fluted pumpkin leaves after 6th week of remediation show that the P, N, Ca and Mg content of the fluted pumpkin leaves increased significantly in groups treated with cow horn powder (XgCH, YgCH and ZgCH) when compared to the -Ve C group. The K content of fluted pumpkin leaves was seen to decrease in groups YgCH and ZgCH with a significant increase observed in group XgCH when compared to the -Ve C. Additionally, the macro minerals P, N, K, and Mg of the fluted pumpkin leaves were all observed to have a reduced concentration in the -N C compared to the -Ve C.

Table 4: Selected Macro minerals of the fluted pumpkin leaves after the 6th week of remediation

Samples	P	N	Ca	K	Mg
-N C	5.00±0.00^{bcde}	52.2±0.02^{bcde}	445±0.01^{bcde}	15.0±0.00^{zqyw}	261±0.15^{bcde}
-Ve C	10.1±0.01^{acde}	65.4±0.03^{acde}	396±0.03^{acde}	18.0±0.00^{xqyw}	642±0.44^{acde}
XgCH	11.4±0.01^{abde}	79.1±0.04^{abde}	481±0.00^{abde}	24.0±0.00^{xzyw}	415±0.29^{abde}
YgCH	12.2±0.01^{abce}	75.1±0.04^{abce}	383±0.00^{abce}	13.0±0.00^{xzqw}	1486±1.02^{abce}
ZgCH	12.1±0.01^{abcd}	110±0.05^{abcd}	488±0.00^{abcd}	13.0±0.00^{xzqy}	1087±0.75^{abcd}

Values are means of triplicate determinations ± standard deviation.

Selected Macro minerals of the fluted pumpkin leaves after the 8th week of remediation: The results on the macro minerals of fluted pumpkin leaves after the 8th week of remediation show that there was no statistical difference in P concentration when comparing the cow horn powder treated groups and the -Ve C group. however, the N and Ca mineral content of the fluted pumpkin leaves increased significantly in groups treated cow horn powder (XgCH, YgCH and ZgCH) when compared to the -Ve C group. The K content of fluted pumpkin leaves was seen to decrease in groups YgCH and ZgCH with an increase observed in group XgCH when compared to the -Ve C. The Mn mineral content showed an increased level in the -Ve C compared to all other groups.

Table 5: Selected Macro minerals of the fluted pumpkin leaves after the 8th week of remediation

Samples	P	N	Ca	K	Mn
-N C	1.45±0.00^{bcde}	52.2±0.02^{bcde}	397±0.00^{bcde}	14.2±0.00^{bcde}	506±0.35^{bcde}
-Ve C	12.0±0.01^{acde}	65.4±0.03^{acde}	367±0.00^{acde}	17.0±0.00^{acde}	932±0.64^{acde}
XgCH	12.0±0.01^{abde}	79.1±0.04^{abde}	489±0.00^{abde}	21.0±0.00^{abde}	364±0.25^{abde}
YgCH	12.0±0.01^{abce}	75.1±0.04^{abce}	399±0.00^{abce}	15.0±0.00^{abce}	362±0.25^{abce}
ZgCH	12.0±0.01^{abcd}	111±0.41^{abcd}	493±0.00^{abcd}	15.1±0.00^{abcd}	829±0.57^{abcd}

Values are means of triplicate determinations ± standard deviation

Conclusion: The potency of cow horn powder to enhance the mineral content of fluted pumpkin leaves grown on crude oil-impacted soil was investigated 4 weeks, 6 weeks, and 8 weeks after the soil has been contaminated with crude oil. Based on the results of the experiment it was observed that after this length of time, the negative impacts of crude oil on soil fertility can be attenuated with the use of cow horn powder. This is evidenced by improved growth profile and

significant accumulation in fruited Pumpkin leaves of Ca, N (macro nutrients) at the 8th day of remediation.

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