PRINT ISSN 1119-8362
Electronic ISSN 2659-1502

Full-text Available Online at
https://www.ajol.info/index.php/jasem
http://www.bioline.org.br/ja

J. Appl. Sci. Environ. Manage.
Vol. 26 (11) 1771-1777 November 2022

Effect of Maize Husk Treatment of Crude Oil Contaminated Soil on Morphological and Biochemical Indices of Cowpea Seedlings

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ABSTRACT: The ameliorative potential of maize husk on crude oil impacted soil had been documented. The aim of this study was to evaluate the effect of maize husk treatment of crude oil contaminated soil on morphological and biochemical indices of cowpea seedlings. Treatment of soil with maize husk significantly ($p< 0.05$) increased the morphological and biochemical indices comparable to control seedlings. On the other hand, exposure of cowpea seedlings to crude oil-contaminated soil caused significant ($p< 0.05$) decreases in both morphological and biochemical indices compare to values in control seedlings. However, these values were restored close to control values by treatment of crude oil-contaminated with maize husk. The treatment of soil with maize husk caused improvement of morphological parameters and alteration of biochemical indices of cowpea seedlings grown in uncontaminated and crude oil-contaminated soils. This has affirmed the use of maize husk as possible soil conditioner.

DOI:**https://dx.doi.org/10.4314/jasem.v26i11.6**

Cite this paper as: ARUOREN, O; ONAKURHEFE, P; ONYEUKWU, O. B; OHWOKEVWO, O. A; ACHUBA, F. I (2022). Effect of Maize Husk Treatment of Crude Oil Contaminated Soil on Morphological and Biochemical Indices of Cowpea Seedlings. *J. Appl. Sci. Environ. Manage.* 26 (11) 1771-1777

Keywords: Cowpea seedlings; Crude oil; Maize husk; Metabolism; Morphological indices

Contamination with hydrocarbons has been one of the major environmental challenges associated with crude oil production (Achuba, 2018; Achuba, 2019). Crude oil is rich in various classes of hydrocarbons and their interaction with the soil result in the restriction of oxygen and water flow due to petroleum-mediated altered soil permeability (Otunyo, 2010; Nazir, 2011; Ewetola, 2013; Akinwumi *et al*., 2014; Jesna and Hari, 2015; Ayininuola and Kwashima, 2015) The restriction of oxygen availability to biological system in soil perturbs both aerobic and anaerobic respirations in exposed organism. The altered respiration is heightened by the presence of hydrocarbon because both processes are simultaneously dependent on each other (Osubor and Anoliefo, 2003; Achuba 2015; Achuba and Oshiokpu, 2019). The altered respiration stimulates oxidative damage which predisposes exposed plants to an array of metabolic and pathological maladies (Achuba 2015; Achuba and Ja-anni, 2018; Achuba and Oshiokpu, 2019).Biological approaches towards bioremediation with low economic cost and eco-friendly natural products, have led researchers to employ organic wastes as an alternative to high cost inorganic fertilizers. In recent times, an array of agricultural wastes has been tried either as organic manure or soil reconditioning material against crude oil toxicity in exposed plants (Achuba and Erhijovwo, 2017; Achuba and Ja-anni, 2018; Achuba and Ohwofasa, 2019; Achuba, 2019). Organic wastes (animals and plant) are host to large groups of microbes during the process of decomposition. During decomposition, microorganisms are in constant breed, thereby making them abundantly available, which are effective

degraders of hydrocarbons. The degradation of hydrocarbons by microorganism ensures improved soil structure, soil aeration and supply of basic soil nutrients.The use of organic wastes in crop cultivation is an age long practice. Despite being non-beneficial materials to human nutrition, it is not readily available round the year. This is because there are no well-defined processing, preservation and storage facilities for most of these wastes. Storage of these materials via drying could address the challenge of its availability all-round the year. However, whether the effectiveness of these wastes could be maintained, if dried, and stored for along time still needs to be evaluated. One important agricultural waste produced in most West African countries is maize husk. The efficacy of this waste in remediating crude oil polluted soil has been reported earlier (Abiodun *et al.*, 2016). Therefore, the aim of this study was to evaluatethe effect of maize husk treatment of crude oil contaminated soil on morphological and biochemical indices of cowpea seedlings.

MATERIALS AND METHODS

The sources of soil sample and cowpea seeds used were previously described (Achuba and Okoh, 2015; Achuba, 2018b). The physicochemical features of soil sample were published earlier (Achuba, 2019). Analytical grade reagents were used for determination of each parameter.

Collection and treatment of maize husk: Maize husk is a product of the cob which abounds in market places and drainage systems during the on-season of maize harvest. The husks were collected from commercial fresh maize sellers in Obiaruku, Delta State, Nigeria. They were carefully sorted to remove debris and other extraneous materials. Thereafter, the husks were dried under partial shade for four days. It was subsequently ground and sieved through a 2 mm mesh and stored in dry polythene bags under laboratory conditions for three months.

*Experimental design and planting of cowpea seeds:*In the experiment, a total of eighteen plant pots were used. They were randomly divided into six groups containing three plant pots in each group.
Group 1: 600g of unpolluted soil sample (Unpolluted control)
Group 2: 600g of unpolluted soil sample + 50g of ground maize husk
Group 3: 600g of unpolluted soil sample + 100g of ground maize husk
Group 4: 600g of polluted soil sample (Polluted control)
Group 5: 600g of polluted soil sample + 50g of ground maize husk
Group 6: 600g of polluted soil sample + 100g of ground maize husk
Planting of viable seeds followed method described earlier (Achuba, 2006).

Determination of morphological parameters: Morphological indices such as root and shoot lengths were measured with ruler, Leaf area of the seedlings was determined using a ruler and its value converted by multiplying with a correction factor. The values obtained from manual measurement compared with determination with software as reported by De Carvalho et *al.* (2017).

Preparation of homogenate for biochemical assays: The leaf from each group (0.5 g) was harvested, washed with ice-cold water (4 ^{o}C), and homogenized with 0.1g butylatedhydroxy toluene (BTH) in 10 mL of 0.05M Phosphate buffer, pH 7.4 at 4 ^{o}C . The mixture was filtered with cheese cloth. The filtrate was centrifuged at 7000 g for 20 minutes (4 ^{o}C) to produce the supernatant (S_1) used for the estimations of various biochemical parameters.

Determination of Chlorophylls and carotene: The leaf from each group (1.0 g) was measured. They were separately homogenized before extracting with 80% acetone. Each mixture was filtered and subjected to centrifugation at 2500 rpm for ten minutes. The absorbances of the supernants were read at various wavelengths, 480, 510, 645and 663 nm. The formula of Duxbury and Yentsch (1956) was adopted in estimating the concentrations of various types of chlorophylls and carotene.

Determination of macromolecules: The sugar content was estimated according to protocol documented by Tietz (1982). The total carbohydrate was determined using the method employing anthrone reagent (Pearson, 1976). Total amino acid and protein were estimated as described by Achuba (2006) and Lowry et al. (1951), respectively.

Determination of Lipid peroxidation and non-enzymatic antioxidants: Malondialdehyde (MDA) which is a measure of lipid peroxidation with a molar extinction coefficient of 1.56 x10^5 M/cm was estimated according to the method of Gutteridge and Wilkins (1982) using the supernatant (S_1). The supernatant (S_1) was used to estimate reduced glutathione following the protocol of EIlman (1959) which is dependent on maximum light absorption at 412 nm. The leaf from each group (2.0 g) was added to 20mL of 0.05M phosphate buffer, pH 7.4 that was acidified with 5% metaphosphoric acid (5: 1 v/v) and homogenized. The mixture was filtered and the filtrate

used to estimate ascorbic acid via titration using 2,6-dichlorophenol-indophenol (DCIP) as electron acceptor (Achuba, 2008).Commercial kit (Teco Diagnostics, USA) was used to estimate uric acid in the plant extract following a protocol outlined by Caraway, (1963).

Determination of enzymatic antioxidants: The method of Misra and Fridovich (1972) was used to estimate the activity of superoxide dismutase, a method based on the ability of the enzyme to cause 50 % inhibition of epinephrine to adrenochrome at a wavelength of 480 nm. The activity of Manganese dependent SOD was estimated by adding 1 mMNaCN to inhibit Cu-ZnSOD activity, and the activity of cytosolic Cu-ZnSOD was then estimated using the difference between total and cyanide -sensitive enzyme activity (Crapo *et al.*, 1978).Catalase activity was estimated using the procedure of Cohen *et al*., (1970) based on discharge of the purple color of potassium permanganate at 480 nm measured at intervals 30-60 seconds due to oxygen produced by the action of catalase on hydrogen peroxide. The procedure described by Rani *et al.* (2004) was adopted in the estimation of glutathione peroxidase. This procedure is based on reduction of hydrogen peroxide and organic peroxides by glutathione to alcoholic molecule with maximum absorption at wavelength of 412 nm.

Determination of xenobiotic metabolizing enzymes: The reaction between 1-chloro-2, 4, dinitrobenzene (CDNB) and reduced glutathione produced a chromophore that absorbs maximally at 340 nm and was used to estimate the activity of glutathione-s-transferase (GST) (Habig *et al.*, 1974). The reaction betweenxanthine as the substrate and oxygen as electron acceptor was utilized to estimate xanthine oxidase activity according to Stirpe and Della Corte (1969).The conversion of benzaldehyde to benzoate using 2,6–dichlorophenol (DCIP) as the electron by aldehyde oxidase (AO) was used to estimate the activity of the enzyme as described by Omarov *et al*. (1998). The conversion of sulphite to sulphate using 2,6–dichlorophenol (DCIP) as the electron acceptor by sulphite oxidase (SO) was used to estimate the activity of the enzyme as described by Macleod *et al.* (1961).

*Statistical Analysis of data:*Statistical analysis of data was done with one-way Analysis of variance (ANOVA) and significant difference between means was set at p values < 0.05.

RESULTS AND DISCUSSION

Morphological indices were negatively and positively affected by crude oil and maize husk treatment of soil, respectively (Table 1). This implies that while crude oil treatment of soil depressed the growth of cowpea seedlings, the addition of maize husk to the untreated soil improved the growth of the seedlings compared to seedling grown in untreated soil. These observations are in line with earlier observations by several authors (Achuba 2006; Sivaraj *et. al.*, 2014; Achuba and Iserhienrhien, 2018; Achuba, 2019).

Table 1: Effect of maize husk treatment of crude oil polluted soil on morphological parameters of cowpea seedlings.

Groups	Stem Length (cm)	Leave Length (cm)	Leave width (cm)	Plant height (cm)	Root Length (cm)
1	16.73 ± 0.59^{a}	6.13 ± 0.23^{a}	3.70 ± 0.20^{a}	30.10 ± 1.64^{a}	13.03 ± 0.60^{a}
2	18.83 ± 0.76^{a}	6.63 ± 0.49^{a}	3.72 ± 0.21^{a}	31.67 ± 2.68^{a}	12.77 ± 0.64^{a}
3	18.33 ± 1.26^{a}	5.97 ± 0.81^{a}	3.73 ± 0.15^{a}	30.20 ± 0.59^{a}	8.17 ± 1.26^{b}
4	8.63 ± 2.58^{b}	4.71 ± 0.53^{b}	2.93 ± 0.29^{b}	19.92 ± 1.30^{b}	7.37 ± 1.46^{a}
5	10.00 ± 1.00^{b}	2.90 ± 0.20^{c}	2.13 ± 0.32^{b}	14.30 ± 2.08^{c}	4.13 ± 0.81^{c}
6	17.83 ± 0.76^{a}	5.07 ± 0.67^{b}	3.23 ± 0.63^{c}	29.20 ± 4.26^{a}	10.50 ± 2.18^{a}

Values are mean ± standard deviations of five determinations. Values not sharing a common superscript on the same column differ significantly (p<0.05). Key: Group 1 = Control (unpolluted); Group 2 = Unpolluted with 75g of maize husk; Group 3 = Unpolluted with 150g of maize husk; Group 4 = Polluted control; Group 5 = Polluted with 75g of maize husk; Group 6 = Polluted with 150g of maize husk

Table 2: Effect of maize husk treatment of crude oil contaminated soil on total chlorophyll, chlorophyll a, chlorophyll b and beta –carotene in cowpea seedlings

Parameters	1	2	3	4	5	6
Total chlorophyll (mg g^{-1})	210 ± 5.83^{a}	219 ± 2.91^{a}	222 ± 2.41^{a}	166 ± 2.39^{b}	184 ± 4.28^{a}	201 ± 1.92^{a}
Chlorophyll a (mg g^{-1})	130 ± 2.00^{a}	131 ± 4.60^{a}	133 ± 3.21^{a}	98.6 ± 6.02^{b}	125 ± 2.79^{a}	124 ± 3.36^{a}
Chlorophyll b (mg g^{-1})	82.6 ±4.39^{a}	87.20 ± 1.48^{a}	91.0 ± 4.47^{a}	74.0 ± 5.00^{b}	78.2 ± 6.46^{b}	2.30 ±2.24^{a}
Beta-carotene (mg g^{-1})	24.8 ±2.59^{a}	26.2 ± 1.92^{a}	27.4 ± 1.67^{a}	19.6 ± 2.19^{b}	23.0 ± 2.24^{a}	23.0 ±2.24^{a}

Values are means ± standard deviations of five determinations. Values not sharing common superscript on the same row differ significantly (p<0.05)

The increase in morphological indices of cowpea seedlings growing in maize husk treated crude oil polluted soil showed a potential ability of maize husk to remediate toxic effects of crude oil as reported by Abiodun *et al*. (2016). In fact, the ability of cellulosic-rich materials to enhance soil enzyme activities and improve the performance of plants in soils polluted by hydrocarbon has been documented earlier (Achuba 2018; Achuba, 2019). This observation also gives credence to the postulation that organic materials reduce the impact of crude oil on plants (Ekpo and Nya, 2012; Sivaraj *et al.* 2014; Achuba and Ja-anni, 2018; Achuba and Ohwofasa, 2019). The treatment of soil with maize husk positively modulated the effect of exposure of cowpea seedlings to crude oil polluted soil. This is indicated by the restoration of pollution-mediated decrease in macromolecules and photosynthetic pigments in the cowpea leaves close to values in control seedlings (Tables 2 and 3). These observations are similar to the reports in previous studies which also showed that petroleum induced metabolic imbalances were also modulated by organic materials (Achuba, 2018; Achuba and Ja-anni, 2018; Achuba and Iserhienrhien, 2018). Also similar to these results were the findings of Sivaraj *et al.* (2014) and Achuba (2019) that poultry manure and oil palm leaves applications affected positively the concentrations of metabolic macromolecules in cowpea seedlings exposed to hydrocarbon impacted soil.

Table 3: Effect of maize husk treatment of crude oil contaminated soil on total protein, total amino acid, total carbohydrate and total sugar in cowpea seedlings

Parameters	1	2	3	4	5	6
Total protein (mg g^{-1})	20.2 ± 1.41^{a}	21.8 ± 1.12^{a}	22.3 ± 0.86^{ab}	24.5 ± 1.63^{b}	19.0 ± 0.73^{a}	20.7 ± 0.58^{a}
Total amino acid (mg g^{-1})	5.92 ± 1.25^{a}	7.03 ± 0.68^{a}	7.53 ± 0.53^{a}	9.96 ± 0.26^{b}	8.25 ± 0.53^{a}	7.93 ± 1.01^{ab}
Total carbohydrate (mg g^{-1})	69.0 ± 3.74^{a}	72.2 ± 4.81^{ad}	81.4 ± 3.58^{b}	58.2 ± 3.12^{c}	71.0 ± 3.54^{a}	75.2 ± 2.28^{ad}
Total sugar (mg g^{-1})	32.0 ± 3.53^{a}	37.0 ± 1.58^{a}	38.8 ± 1.92^{b}	42.4 ± 2.97^{b}	34.6 ± 1.82^{a}	34.6 ± 1.52^{a}

Values are means ± standard deviations of five determinations. Values not sharing common superscript on the same row differ significantly ($p<0.05$)

Table 4: Effect of maize husk treatment of crude oil contaminated soil on lipid peroxidation, glutathione levels, Vitamin C and uric acid in cowpea seedlings.

Parameters	1	2	3	4	5	6
Lipid peroxidation (nMol cm^{-3})	1.04 ±0.03^{a}	0.99± 0.08^{a}	0.99± 0.07^{a}	1.29 ± 0.03^{b}	1.23 ± 0.02^{b}	1.19 ± 0.05^{b}
Glutathione(ug g^{-1})	0.49 ± 0.04^{a}	0.53 ±0.02^{a}	0.56 ±0.02^{a}	0.35 ± 0.03^{b}	0.40 ± 0.04^{b}	0.40 ± 0.03^{b}
Vitamin C (ug g^{-1})	3.84 ± 0.17^{a}	3.93 ±0.33^{a}	4.38 ± 0.24^{a}	3.06 ± 0.19^{a}	3.27 ± 0.21^{b}	3.39 ± 0.09^{b}
Uric Acid (ug g^{-1})	3.73 ± 0.16^{a}	3.06 ±0.16^{a}	3.09 ± 0.12^{a}	2.10 ± 0.37^{b}	2.66 ± 0.11^{a}	2.91 ± 0.05^{a}

Values are means ± standard deviations of five determinations. Values not sharing common superscript on the same row differ significantly ($p<0.05$)

Table 5: Effect of maize husk treatment of crude oil contaminated soil on total SOD, Cu/ZnSOD, MnSOD, catalase and glutathione peroxidase activities in cowpea seedlings.

Parameters	1	2	3	4	5	6
Total SOD (Unitsg^{-1}tissue)	2.67± 0.25^{a}	2.47± 0.12^{a}	2.64± 0.33^{a}	1.90± 0.19^{b}	2.44 ± 0.08^{a}	2.37± 0.07^{a}
Cu/ZnSOD (Unitsg^{-1}tissue)	2.03 ±0.21^{a}	2.01 ±0.12^{a}	2.15 ±0.31^{a}	1.63 ±0.09^{b}	1.83 ± 0.08^{b}	1.94 ±0.03^{b}
MnSOD (Unitsg^{-1}tissue)	0.96 ± 0.07^{a}	1.01 ±0.05^{a}	1.02 ± 0.06^{a}	0.56 ±0.07^{b}	0.80 ± 0.05^{a}	0.88 ±0.05^{a}
Catalase (nMol min^{-1}gfw)	1.32 ± 0.07^{a}	1.34 ±0.04^{a}	1.40 ±0.07^{a}	1.07 ±0.05^{b}	1.20 ± 0.02^{a}	1.23 ±0.03^{a}
Glutathione peroxidase (nMol min^{-1}gfw)	8.02 ± 0.08^{a}	8.02 ±0.66^{a}	8.85 ± 0.15^{a}	6.53 ±0.43^{b}	7.57 ± 0.35^{a}	7.51± 0.39^{a}

Values are means ± standard deviations of five determinations. Values not sharing common superscript on the same row differ significantly ($p<0.05$)

Metabolic derangements in plants have been attributed to crude oil-induced oxidative stress (Achuba, 2014; Achuba and Ja-anni, 2018; Achuba, 2019). This leads to increased lipid peroxidation with corresponding reductions in antioxidant indices (Table 4). However, treatment of soil with maize husk indicated improvement in these indices as compared to control thus giving further credence on the ability of organic materials to act as a sequestering agent against the toxic potentials of crude oil and the ability of maize husk to enhance soil productive capacities (Achuba and Erhijivwo, 2017; Achuba and Ja-anni, 2018; Achuba and Ohwofasa, 2019; Abiodun *et al.*, 2016).

Table 6: Effect of maize husk treatment of crude oil contaminated soil on glutathione-s-transferase, xanthine oxidase, aldehyde oxidase and sulphite oxidase activities in cowpea seedlings

Parameters	1	2	3	4	5	6
Glutathione-s- transferase (Unit g^{-1}fw)	1.86 ±0.11[a]	2.12 ± 0.08[a]	2.22 ±0.03[a]	1.50±0.05[b]	1.63 ±0.05[b]	1.72 ± 0.03[a]
Xanthine oxidase (Unit g^{-1}fw)	3.32 ±0.23[a]	3.68 ±0.14[a]	4.03 ±0.06[a]	2.53 ±0.51[b]	3.36 ±0.17[a]	3.59 ± 0.13[a]
Aldehyde oxidase (Unit g^{1}fw)	1.70 ±0.08[a]	1.74 ± 0.04[a]	1.78 ±0.02[a]	1.18 ±0.02[b]	1.46 ±0.04[a]	1.50 ± 0.03[a]
sulphite oxidase (Unit g^{-1}fw)	3.29 ±0.32[a]	3.68 ± 0.10[a]	3.70 ±0.09[a]	1.96 ±0.41[b]	2.70 ±0.14[a]	2.76 ± 0.10[a]

Values are means ± standard deviations of five determinations. Values not sharing common superscript on the same row differ significantly ($p<0.05$)

In fact, the deleterious impact of hydrocarbon on the living systems is predicated on its ability to stimulate production of reactive oxygen species in the course of metabolism (Achuba and Osakwe, 2003; Achuba, 2014; Achuba, 2019). However, plants and animals are naturally fortified with antioxidant defense system to protect against the damaging impact of reactive oxygen species. This is responsible for the crude-enhanced lipid peroxidation and downward trend in the status of antioxidant systems in cowpea seedlings exposed to crude oil-treated soil (Tables 4 and 5).

Moreover, treatment of contaminated soil with maize husk exhibited positive changes in the cowpea seedlings by reducing lipid peroxidation in addition to enhancement of the antioxidant indices of the cowpea seedlings. This insinuation is consistent with a variety of previous studies on the efficacy of organic materials in reducing petroleum toxicity (Achuba and Ja-anni, 2018; Achuba and Ohwofasa, 2019, Achuba, 2019). Also, the efficacy of the maize husk in decimating the noxiousness of crude oil on the cowpea seedlings is expatiated by the increase in xenobiotic-metabolism enzymes (Table 6).

These enzymes are responsible for the metabolism and incapacitations of foreign substances in plants and animals. Previous reports also hinted on the enhancement of these enzymes by plant and other organic material (Achuba and Okunbor, 2016; Achuba and Oshiokpu, 2019; Achuba, 2019). This has given credence to the usefulness of maize husk, a cellulose rich organic substance, in mitigating the negative consequences of crude oil on crop plants.

Conclusion: It is pertinent to conclude that maize husk should be harnessed, processed and preserved during the on-season. This will ensure its availability throughout the year to be used for the treatment of crude oil impacted soil in oil producing communities of the world. The efficacy of maize husk is indicated by the reversal of the impact of crude oil on the growth and metabolism of cowpea seedlings.

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