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Influence of Mycoremediation and Selected Surfactants on Growth Performance of Fluted Pumpkin In Crude Oil Impacted Soil and the Nephrotoxic Effects of Leaves Aqueous Extract on Wistar Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author KTN designed the study, performed the statistical analysis, wrote the protocol and first draft of the manuscript. Author KTN also managed the literature searches. Authors FCN and EPB managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

This study investigated the mycoremediation effects of *Pleurotus ostreatus* and selected surfactants (Triton x-100 and meshed *Costus afer* stem) on the growth performance of fluted pumpkin (*Telfairia occidentalis*) in crude oil impacted soil and their effects on the electrolytes, urea and creatinine levels of Wistar rats fed with aqueous leaf extract of fluted pumpkin (*Telfairia occidentalis*) cultivated on the amended soil. Crude oil highly impacted soil excavated from an oil spill site at Obeche community in Ogba/Egbema/Ndoni Local Government Area of Rivers State, Nigeria was used.

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Remediation was induced using white rot fungus (*Pleurotus ostreatus*), phyto-emulsified surfactant (*Costus afer* stem) and chemical surfactant (Triton x-100). Seven experimental cells (20cm diameter x 20cm high polypropylene bags) labelled A-G, each containing 2kg of polluted soil were used. The application of amendments to the crude oil impacted soil affected the growth of fluted pumpkin. Cell D (PSS + Triton x-100) was observed to have the highest number of leaves (15.00 ± 0.70), highest vine length (45.00 ± 0.00), highest fresh weight of leaves (16.50 ± 0.70) when compared with other cells. The control cell, cell A (CISS) and cell F (CISS + *Pleurotus ostreatus* + Triton x-100) also were observed to have a positive effect on the growth performance of fluted pumpkin. When the aqueous leaf extract of fluted pumpkin was administered to the Wistar rats, potassium level was observed to be decreased in groups C to G with cell D having the lowest value (3.99 ± 0.00) when compared with group A (29.39±34.44). The results also indicated that groups C, D, F, and G were decreased when compared with group A for sodium, only group E was decreased when compared with group A for Chlorine, all the groups for Calcium where significantly ($p \leq 0.05$) decreased when compared with group A. There was a significant ($p \leq 0.05$) difference when group A is compared with group G for Bicarbonate. Histopathological evaluation of the kidney of Wistar rats revealed the presence of congested renal vessels and haemorhage.

Keywords: Pleurotus ostreatus; Telfairia occidentalis; mycoremediation; pollution; remediation.

1. INTRODUCTION

The soil is a primary recipient by design or accident of a myriad of waste products and chemicals used in modern society. Pollution caused by petroleum and its derivatives is the most prevalent problem in the environment. Since commercial exploration of petroleum started in Nigeria in 1958 [1]. Petroleum has continuously grown to be the mainstay of the Nigerian economy. However, the exploration of petroleum has led to the pollution of land and waterways [2].

The presence of oil and refined petroleum products in the soil can lead to toxic effects on plants and soil microorganisms and acts as a source of groundwater contamination [3]. Most of the crude oil reservoirs and oil refineries in Nigeria are located in areas with agricultural activities and urban areas in the Niger Delta. It is believed according to UN reports, that an average riverine dweller of the Niger Delta is exposed to polluted air, polluted water, and polluted food, hence facing health hazard resulting to reduced life expectancy [4]. Consequently, the remediation of soil impacted by oil production and transport is not only of importance considering environmental problems but also for the preservation of agricultural productivity and human health. Chemical and physical methods applied for remediation of petroleum-contaminated soils such as thermal treatment, soil washing, solidification, and stabilization are expensive, disruptive to the environment and involved high-energy consumption [5]. Therefore, natural remediation techniques have been developed to provide more environmentally friendly and cost-effective cleanup of sites impacted by petroleum spills [6].

The exploration of petroleum products has rendered agricultural lands less productive [7,2], and the creeks and the aquatic lives have become more or less dead [8,9,2].

The primary mechanism for eliminating spilled oil from the environment is the microbial degradation [10]. This remediation option which involves the use of microorganisms to detoxify or remove organic and inorganic compounds from the environment also offers green technology solution to the problem of environmental degradation. The technological process relied upon microbial enzymatic activities to transform or degrade the contaminants from the environments [11]. It is a form of bioremediation that harness fungal mycelium to transform complex or simple chemical compounds into non-hazardous forms thereby resulting in materials of higher nutritive value or simply reducing the final bulk of the product [12]. Fungi use is expected to be relatively cheap as they can be cultivated on a number of inexpensive agricultural or forest wastes such as corncobs and sawdust [13]. The natural degradative ability of white rot fungi can be used in the decontamination of polluted soil by ramifying the substratum and digesting it through the secretion of extracellular enzymes which are non-specific. The extracellular lignin-degrading enzymes such as manganese peroxidase, laccase and lignin peroxidase can be secreted by white rot fungi to decontaminated polluted soil [14]. [15] stated that spent white-rot fungi (*Pleurotus ostreatus*)

substrate can be used to biotreat Nigerian oil-based drill cuttings containing Polyaromatic Hydrocarbons (PAH's) under laboratory conditions. [16] reported an improvement in the nutrient contents of the soil, bioaccumulation of heavy metals, degradation of total petroleum hydrocarbon (TPH), lignin, and increased activity of polyphenol oxidase and peroxidase due to biodegradation of spent cutting fluids by *Pleurotus tuberregium.*

Costus afer is found in the forest belt from Senegal east to Ethiopia and south to Tanzania, Malawi, and Angola. It is often planted in home gardens for medicinal purposes. *Costus afer* belongs to the family Costaceae a monocot and a relatively tall, herbaceous, unbranched tropical plant with creeping rhizome. It is commonly found in moist or shady and river banks forest of West and Tropical African countries including Senegal, South Africa, Guinea, Nigeria, Ghana and Cameroon [17,18]. *Costus afer* is pantropical and comprises of about 70 species, which about 40 species are found in tropical America, about 25 in tropical Africa and about 5 in South-East Asia [19]. *Costus afer* is commonly called bush cane [20,21]. It is kenned as "Okpete" or "Okpoo" in Igboland, "Kakizuwa" in Hausa, "tete-egun" in Yoruba and "Mbritem" in Efik, all in Nigeria. Anglophone Cameroon calls it "Monkey sugar cane" [21,22]. The aim of the study is to investigated the mycoremediation effects of *Pleurotus ostreatus* and selected surfactants (Triton x-100 and meshed *Costus afer* stem) on the growth performance of fluted pumkin (*Telfairia occidentalis*) in crude oil impacted soil and their effects on the electrolytes, urea and creatinine levels of wistar rats fed with aqueous leaf extract of fluted pumkin (*Telfairia occidentalis*) cultivated on the amended soil.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Collection of samples

a) Soil sample

Soil sample were collected from the contaminated site from the depth of about 0-25cm and 2kg weighed out into cellophane bag measuring 20cm in height and 20cm in width.

b) Mushroom (*Pluerotus ostreatus*) spawn

The fungus *P. ostreatus* used for this research was obtained from the mycology unit of the department of plant science and biotechnology, University of Port Harcourt, Choba campus. The culture was subcultured in malt extract agar to get pure growing culture.

c) Plant used as phyto-surfactant (*Costus afer*)

Fresh stems of *Costus afer* were obtained from Omuike village in Aluu Community of Ikwerre L.G.A. of Rivers State. They were authenticated by a plant taxonomist of the department of plant science and biotechnology, University of Port Harcourt. The stems were washed, cut into pieces and macerated using electric blender and stored in an air tight container.

d) Fluted pumpkin (*T. occidentalis*) seeds

Healthy looking seeds of *T.occidentalis* were purchased from Aluu community market. They were first soaked in alcohol for 30 seconds to kill seed pathogens and then soaked in water for 24 hours before sown deeply into the amended soil samples.

e) Triton x-100

One hundred milliliters (100ml) of Triton x-100 was purchased from Sigma-Aldrich company, Germany through Bristol Scientific Company Limited, Apapa, Lagos State, Nigeria.

2.1.2 Procurement of experimental animals

Twenty one (21) adult female wistar albino rats weighing 170-200g were obtained from the animal house of the department of biochemistry, University of Port Harcourt, Rivers State, Nigeria. They were randomly sorted into seven groups and three rats each. The animals were housed in plastic cages and allowed normal feed (Port Harcourt Flour Mills, Port Harcourt, Nigeria) and *water ad libitum.*

2.2 Methods

2.2.1 Experimental design for soil amendment

Seven experimental cells (20cm diameter x 20cm high polypropylene bags) labelled A-G, each containing 2kg of polluted soil were used. The amendment schedule is shown in the Table 1.

After amendment/inoculation, each of the polypropylene bags were thoroughly mixed and tied with masking tape. All the bags were

Table 1. Experimental cells for soil amendment

Cell	Amendment
A	Crude oil Impacted soil sample (CISS) with no amendment
B	CISS + 147 g of *Pleurotus ostreatus* spawn
C	CISS + 200 g of stem of *Costus afer* fresh whole mixture
D	CISS + 3 ml of Triton x-100
E	CISS + 147 g of *Pleurotus ostreatus* spawn + 200 g of stem of *Costus afer* fresh whole mixture
F	CISS + 147 g of *Pleurotus ostreatus* spawn + 3 ml of Triton x-100
G	CISS + 200 g of stem of *Costus afer* fresh whole mixture + 3 ml of Triton x-100

incubated at 28 ± 2°C for 60 days. After 60 days of incubation, 100g of soil was weighed out from each of the cells for soil analysis and fluted pumpkin (*T.occidentalis*) seeds were planted deeply on each of the amended cells for 6 weeks. The morphological characterization of the plant was determined after 6 weeks of planting and the leaves and stems were harvested and used to prepare aqueous extract which was administered to wistar albino rats for another 6 weeks after which they were sacrificed and the effect of the crop grown on the contaminated and amended soil samples on electrolytes, urea and creatinine levels were ascertained.

2.2.2 Determination of plant morphological characterization/Growth performance

After a growth period of six weeks, *Telfairia occidentalis* leaves were assessed and harvested. Morphological characterization/ Growth performance (number of leaves, plant height, fresh weight and dry weight) were determined per plant in each of the cells using a measuring tape for the plant height and electronic weighing balance for the fresh weight. For the dry weight, samples were left in a dessicator for 48 hours until constant weight was obtained.

2.2.3 Preparation of the *T. occidentalis* leaf aqueous extract

The leaves of *T.ocidentalis* were harvest from each of the cells and sun dried for 5 days. The dried samples were ground into powder and stored in labelled air-tight container. Two thousand milligram (2000mg) of the resultant powder was soaked in 100ml hot water for 24 hours, after which the resultant mixture was filtered and the filtrate (aqueous extract) was stored for subsequent use. Ten milliliters of this extract was evaporated to dryness by oven-drying and the weight of the residue used to determine the concentration of the filtrate, which was in turn used to determine the dose of administration of the extract to the test animals.

Table 2. Experimental groups for post remediation study

Group	Description
A	Animals were administered 1 ml of aqueous leaf extract of *T. occidentalis* grown on Crude oil impacted soil sample (PSS) with no amendment
B	Animals were administered 1ml of leaf aqueous extract of *T. occidentalis* grown on CISS + 147 g of *Pleurotus ostreatus* spawn
C	Animals were administered 1 ml of leaf aqueous extract of *T. occidentalis* grown on CISS + 200 g of stem of *Costus afer* fresh whole mixture
D	Animals were administered 1ml of leaf aqueous extract of *T. occidentalis* grown on CISS + 3 ml of Triton x-100
E	Animals were administered 1 ml of leaf aqueous extract of *T. occidentalis* grown on CISS + 147 g of *Pleurotus ostreatus* spawn + 200 g of stem of *Costus afer* fresh whole mixture
F	Animals were administered 1 ml of leaf aqueous extract of *T. occidentalis* grown on CISS + 147 g of *Pleurotus ostreatus* spawn + 3 ml of Triton x-100
G	Animals were administered 1 ml of leaf aqueous extract of *T. occidentalis* grown on CISS + 200 g of stem of *Costus afer* fresh whole mixture + 3 ml of Triton x-100

2.2.4 Animal grouping for post remediation study

The animals procured were randomly sorted into seven groups and three rats each. The animals were housed in plastic cages and allowed one week of acclimatization on normal feed (Port Harcourt Flour Mills, Port Harcourt, Nigeria) and *water ad libitum.*

After one week of acclimatization, the animals were administered the aqueous extract of *T.occidentalis* planted on the polluted soil and amended soil for a period of 6 weeks. After 6 weeks of administration, they were anaesthetized by exposure to chloroform. While under anesthesia, they were painlessly sacrificed and blood was collected from each rat into heparin and EDTA sample bottles. The heparin anti-coagulated blood samples were centrifuged at 1000 x g for 10 minutes after which there plasma was collected and stored for subsequent analysis.

2.2.5 Estimation of electrolytes, urea and creatinine

Determination of Urea [23]

Principle

Plasma containing urea in the presence of urease (enzyme) is hydrolyzed to ammonia and the ammonia is photometrically assayed via Berthelot's reaction.

Procedure

Ten microlitres of sample was dispensed into test tube 1 (sample). Ten microlitres of standard (urea) was dispensed into test tube 2 (standard). Ten microlitres of distilled water was dispensed into test tube 3 (blank). Subsequently, to all the test tubes, fifty microlitres of the reagent labelled 1 was dispensed. Mixing and incubation was performed at 37°C for 10 min. Two point fifty millilitres of reagents labelled 2 and 3 were dispensed into all the test tubes. Incubation at 37°C for 15min and thorough mixing was carried out. For at least 8 hrs, there was the observation of a blue colour which was stable. The test tubes were inserted into a spectrophotometer and against the blank; the sample and standard's absorbance were read and recorded.

Concentration of Urea in sample was calculated as

(ΔAsample/ΔAstandard) x Standard conc. (µmol/l)

2.2.6 Determination of Creatinine [23]

Principle

Creatinine reaction with picric acid in an alkaline medium produces a coloured complex and the quantity of the complex produced is directly proportional to the creatinine concentration.

Procedure

One hundred microlitres of diluted water was dispensed into a test tube (reagent blank). One hundred microlitres of standard reagent was dispensed into a test tube (standard). Then one hundred microlitres of the specimen was dispensed into a test tube (sample) and one thousand microlitres of working reagent into all the test tubes. They were mixed and inserted into the spectrophotometer. The working reagent and sample were mixed and after 30 seconds the absorbance was read. After 2 min, the standard's absorbance as well as that of the sample were read and recorded.

Concentration of creatinine in sample was calculated as

(ΔAsample/ΔAstandard) x Standard conc. (µmol/l) = µmol/l

2.2.7 Determination of plasma electrolytes [23]

The concentration of potassium ions, sodium and chloride ions in plasma was assessed using the series electrolyte analyzers that apply ISE (Ion Selective Electrode) technology.

Principle

The analyzer utilizes Ion Selective Electrode (ISE) technology, an electrochemical sensor that translates the ion activity to the electric potential of the electrode, conforming to the NERNST equation, which states that an ion activity logarithm has a linear relation to the electrode potentials.

Procedure

The Na^+, Cl^- and K^+ activity were assessed by direct measurement procedures of Akhigbe *et al.,* 2008. The activity of the specific ion in the sample at the electrode was converted to an electrical potential which is measured with a voltmeter. The voltage is theoretically proportional to the ionic activity. The voltage is finally converted to an electrical signal and displayed as a value on the screen.

2.2.8 Determination of bicarbonate (HCO^-_3) [23]

One hundred and fifty microlitre of blood plasma and reagent were added into the sealed reaction chamber, the HCO^-_3 ions in the plasma precipitated into the reaction and released CO_2, leading to an increase in the gas pressure inside the chamber. The changes were detected by the pressure sensor and signals were sent to the microprocessor and the amount of HCO^-_3 ion in plasma was determined.

2.2.9 Histopathological analysis [24]

Procedure

The kidneys were harvested from the test and control rats and was placed in a fixative, (10% formaldehyde). The tissues were dehydrated with Isopropyl alcohol (IPA) by passing tissue through a series of solutions of increasing concentrations of isopropyl alcohol (75, 85, 95, 100%) in which the water is replaced by IPA. The tissue was infiltrated with paraffin and allowed to equilibrate for one hour in an incubator set for 58°C. The paraffin was decanted and allowed to solidify around the tissues and was trimmed out and the tissues were mounted on the microtome for sectioning. The sections were attached to microscope slides. The slides were labelled, washed with soap and water and was rinsed severally with water and allowed to dry. The slides were also dipped in an adhesive solution, Sta-On and allowed to dry overnight. The slides were then stained with hematoxylin. The sections were mounted on a cover slip after adding 2 drops of resin and left for 24 hours. The histological slides were examined under a microscope for histological changes.

Subsequently, they were observed and then subjected to photomicrography. Results were analyzed and interpreted by an expert histopathologist in the Department of Anatomical pathology, University of Port Harcourt Teaching Hospital, Port Harcourt, Nigeria.

2.3 Statistical Analysis of Data

All Data for biochemical analysis were analyzed for statistical differences and in rat treatment groups, by means of one-way ANOVA and post hoc LSD, on SPSS 20. In all, $p<0.05$ was considered significant. Data are presented as mean±S.D (standard deviation).

3. RESULTS

The results are shown in Tables 3, 4 and 5. Also the Photomicrograph of Kidney of wistar albino rats fed with aqueous extract of fluted pumpkin leaves are shown in plates 1 and 2.

4. DISCUSSION

The results for the Morphological Characterization/growth performance of fluted pumpkin (*Telfaria occidentalis*) planted on crude oil polluted soil sample and the polluted soil samples inoculated with white rot fungus (*Pleurotus ostreatus*), phyto-emulsified surfactant (PSS + *Costus afer*) and non-ionic surfactant (Triton-x-100) after 6 weeks of planting are shown in Table 3.

The application of amendments to the crude oil impacted soil affected the growth of fluted pumpkin. Cell D (PSS + Triton x-100) was observed to have the highest number of leaves, highest vine length (cm), highest fresh weight (g) of leaves and highest dry weight (g) of leaves. The control cell, cell A (CISS) and cell F (CISS + *Pleurotus ostreatus* + Triton x-100) also were observed to have positive effect on the growth performance of fluted pumpkin.

The findings showed that among all the treatments used, amendment with Triton x-100 and *Pleurotus ostreatus* alone and in combination significantly resulted to better performance of fluted pumpkin. The fact remains that these two amendments enhanced microbial activity and this result agrees with the previous works reported by [25,26,27]. After the six weeks of propagation, the control cell, cell A (CISS) was observed to have an improved growth performance on the fluted pumpkin. This is because non application of remediation material increased the rate of nodulation to enhance nitrogen fixation. These findings is in conformity with the reports of some researchers [28,29,30]. [31][32] reported that the rhizobia species have the ability to adapt to the environmental stress.

Table 5 shows the Creatinine and Urea levels of wistar albino rats fed with aqueous extract of fluted pumpkin (*T.occidentalis*) leaves cultivated on crude oil polluted soil sample and the polluted soil samples inoculated with white rot fungus (*Pleurotus ostreatus*), phyto-emulsified surfactant (CISS + *Costus afer*) and non-ionic surfactant (Triton-x-100).

Table 3. Effect of amendments on morphological characterization/growth performance of fluted pumpkin (*Telfaria occidentalis*) after 6 weeks of planting

Cell	Treatment	Number of leaves	Plant Height (cm)	Fresh weight of leaves (g)	Dry weight of leaves (g)
A	Crude oil impacted soil sample (CISS)	14.00 ± 0.70	38.00 ± 1.41	14.00 ± 0.00	1.41 ± 0.72
B	CISS + *Pleurotus ostreatus*	11.00 ± 3.53	23.0 ± 7.07	7.50 ± 3.53	1.09 ± 0.69
C	CISS + *Costus afer*	6.00 ± 7.77	26.00 ± 36.76	9.50 ± 13.43	1.08 ± 1.53
D	CISS + Triton x-100	15.00 ± 0.70	45.00 ± 0.00	16.50 ± 0.70	1.35 ± 0.63
E	CISS + *Pleurotus ostreatus* + *Costus afer*	10.00 ± 2.82	19.50 ± 0.70	6.00 ± 1.41	1.08 ± 0.26
F	CISS + *Pleurotus ostreatus* + Triton x-100	13.00 ± 4.94	32.50 ± 12.02	13.00 ± 1.41	1.28 ± 0.25
G	CISS + *Costus afer* + Triton x-100	10.00 ± 0.70	38.50 ± 13.43	12.50 ± 3.53	1.36 ± 0.51

Each value is a mean of two duplicates expressed as mean ± S.D. Values in the same column with common superscript letters (a, b,…) are significantly different at p≤ 0.05 when compared with the polluted soil sample

Table 4. Electrolyte levels of wistar albino rats fed with aqueous extract of fluted pumpkin (*T. occidentalis*) leaves cultivated on crude oil impacted soil sample and the impacted soil samples inoculated with white rot fungus (*Pleurotus ostreatus*), phyto-emulsified surfactant and non-ionic surfactant (Triton-x-100)

Group	Treatment	K (mmol/l)	Na(mmol/l)	Cl (mmol/l)	Ca(mmol/l)	HCO_3^-(mmol/l)
A	Crude oil impacted soil sample (CISS)	29.39±34.44	173.10±66.20	84.60±24.96	3.47 ± 4.40	17.83 ± 3.62
B	CISS + *Pleurotus ostreatus*	36.22±29.84	204.53±126.96	88.76±31.96	1.68 ± 2.35	11.85 ± 9.83
C	CISS + *Costus afer*	9.60 ± 9.71	138.16±3.17	98.33±2.38	0.96 ± 0.83	20.66 ± 2.19
D	CISS + Triton x-100	3.99 ± 0.00	140.00±0.00	100.00±0.00	0.02 ± 0.00	20.88 ± 6.81
E	CISS + *Pleurotus ostreatus* + *Costus afer*	24.62±35.22	271.96±235.53	75.50±49.70	1.56 ± 1.74	22.00 ± 2.90
F	CISS + *Pleurotus ostreatus* + Triton x-100	5.88 ± 2.84	130.63±10.05	109.43±9.55	0.76 ± 0.65	21.60 ± 6.95
G	CISS + *Costus afer* + Triton x-100	6.50 ± 4.35	135.80±7.27	108.93±15.47	0.58 ± 0.96	28.06 ± 1.19 [a]

Each value is a mean of three replicates expressed as mean ± S.D. Values in the same column with common superscript letters (a, b,…) are significantly different at p≤ 0.05 when compared with the polluted soil sample.

Table 5. Creatinine and Urea levels of wistar albino rats fed with aqueous extract of fluted pumpkin (*T. occidentalis*) leaves cultivated on crude oil impacted soil sample and the impacted soil samples inoculated with white rot fungus (*Pleurotus ostreatus*), phyto-emulsified surfactant and non-ionic surfactant (Triton-x-100)

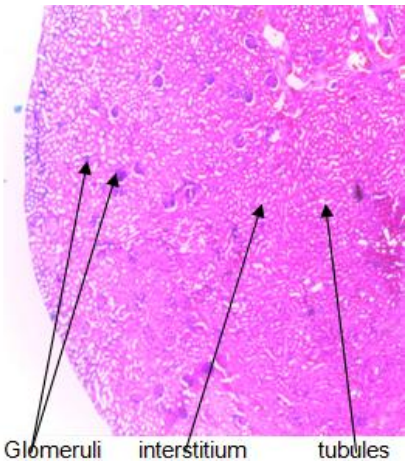
Group	Treatment	Creatinine (µmol/L)	Urea (mmol/L)
A	Polluted soil sample (PSS)	55.97 ± 5.06 [a]	3.00 ± 0.25 [a]
B	PSS + *Pleurotus ostreatus*	52.87 ± 4.80	2.83 ± 0.23
C	PSS + *Costus afer*	58.53 ± 7.50	3.13 ± 0.23
D	PSS + Triton x-100	51.10 ± 1.37	2.73 ± 0.05
E	PSS + *Pleurotus ostreatus* + *Costus afer*	55.00 ± 4.61	2.97 ± 0.20
F	PSS + *Pleurotus ostreatus* + Triton x-100	48.57 ± 1.72	2.60 ± 0.10
G	PSS + *Costus afer* + Triton x-100	46.30 ± 2.10 [a]	2.50 ± 0.10 [a]

Each value is a mean of three replicates expressed as mean ± S.D. Values in the same column with common superscript letters (a, b,…) are significantly different at p≤ 0.05 when compared with the polluted soil sample.

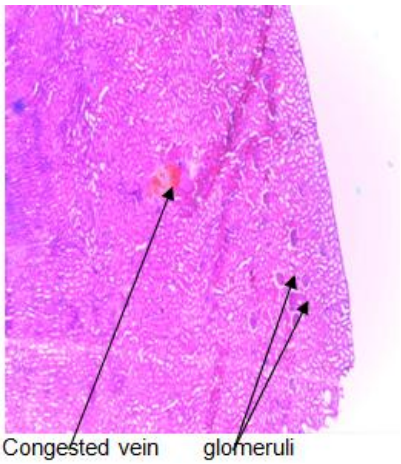
There is a significant difference ($p \leq 0.05$) when group G is compared with group A for both creatinine (µmol/L) and urea (mmol/L) levels. Also, the levels of all the amended groups are observed to be decreased except for group C (CISS + *Costus afer*) when compared with group A for both creatinine and urea.

The significant decrease in creatinine and urea levels in group G and insignificant decrease observed in all other groups except for group C may be an indication that *T. occidentalis* extracts within the period of administration may or may not have affected the renal function. This result lends credence to a recent research finding which showed that acute and sub-chronic administration of an extract of *T. occidentalis* leaves on rats do not result in any severe toxicological consequences.

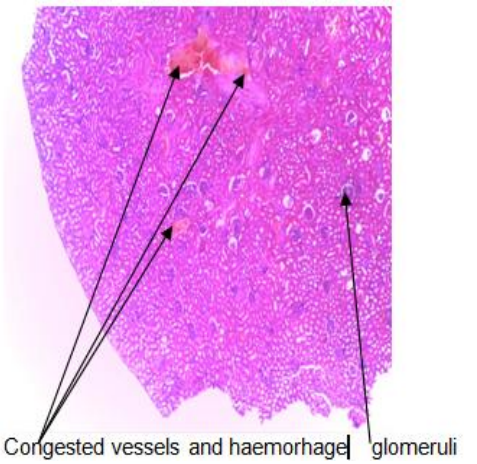
Table 3 shows the Electrolyte levels of Wistar albino rats fed with aqueous extract of fluted pumpkin (*T. occidentalis*) leaves cultivated on crude oil polluted soil sample and the contaminated soil samples inoculated with white rot fungus (*Pleurotus ostreatus*), phyto-emulsified surfactant (PSS + *Costus afer*) and non-ionic surfactant (Triton-x-100). Potassium level was decreased in groups C to G with group D having the lowest value (3.99 ± 0.00) when compared with group A. The results in the table above shows that groups C, D, F and G were decreased when compared with group A for sodium, only group E was decreased when compared with group A for Chlorine, all the groups for Calcium were significantly decreased when compared with group A. There was a significant difference when group A is compared with group G for Bicarbonate.



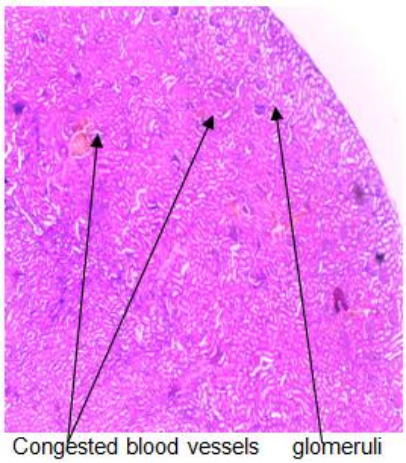
Group A: Crude oil impacted soil sample (CISS) without amendment. Slide shows normal histology



Group B: CISS + *Pleurotus ostreatus.* Slide shows congested veins.



Group C: CISS + *Costus afer*. Slide reveals the presence of congested vessels and haemorhage

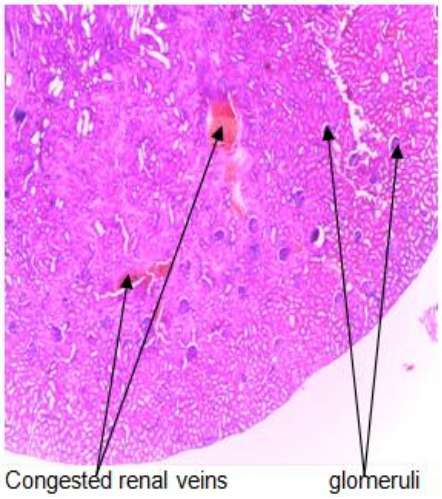


Group D: CISS + Triton x-100 Slide reveals the presence of congested renal vessels

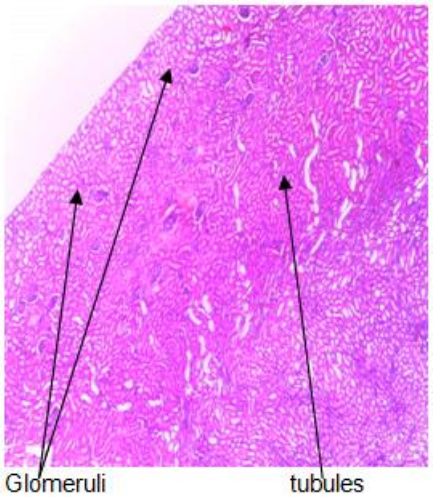
Plate 1. Photomicrograph of Kidney organs of wistar albino rats fed with aqueous extract of fluted pumpkin (*T.occidentalis*) leaves cultivated on crude oil impacted soil sample and the impacted soil samples inoculated with white rot fungus (*Pleurotus ostreatus*), phyto-emulsified surfactant and non-ionic surfactant (Triton-x-100)

These findings are consistent with studies that showed that *Telfairia occidentalis* contain unique nutritional and phytochemical constituents, which can exert varied physiological and biochemical effects. Similarly, *T. occidentalis* extract may contain some growth stimulating factors, which could be due to its well-balanced amino acid profile [33].

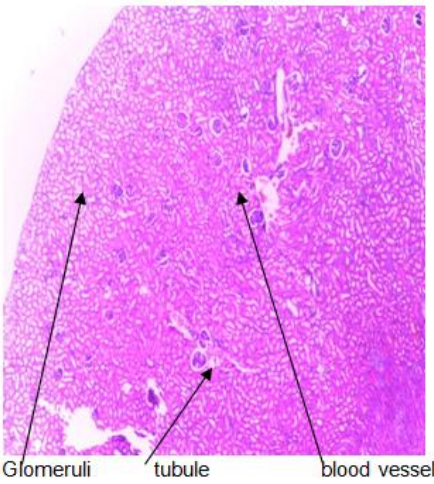
The toxicity potentials of the *T. occidentalis* observed in this study among the electrolytes may be due to the presence of some anti-



Group F: CISS + *Pleurotus ostreatus* + Triton x-100. Slide shows no histologic changes



Group E: CISS + *Pleurotus ostreatus* + *Costus afer*. Slide reveals the presence congested renal vessels.



Group G: CISS + *Costus afer* + Triton x-100. Slide shows no histologic changes.

Plate 2. Photomicrograph of Kidney organs of wistar albino rats fed with aqueous extract of fluted pumpkin (*T.occidentalis*) leaves cultivated on crude oil impacted soil sample and the impacted soil samples inoculated with white rot fungus (*Pleurotus ostreatus*), phyto-emulsified surfactant and non-ionic surfactant (Triton-x-100)

nutrients found in the extract and that *T.occidentalis* must have taken up the heavy metals and other chemical compounds that the micro-organisms could not disintegrate within the soil. [34] detected the presence of hydrogen cyanide (HCN), oxalic and tannic acids at levels of 61.2 ± 0.02, 80.7 ± 5.01 and 43 ± 0.0 mg/100g of *T. occidentalis* leaves extract respectively. [35] in a similar study reported very high levels of cyanide (60.1 mg/100 g), and tannin (40.6 mg/100 g) in young leaves of *T. occidentalis*. Oxalate (10.0 mg/100 g) and phytate (48.8 mg/100 g) were also present but at higher concentrations in the older leaves. Generally, these antinutrients are usually present in very low and nontoxic concentrations, but at high doses of the extract or if the extract is administered for a prolonged period, they accumulate to a toxic level.

For example, several other studies have indicated that chronic cyanide exposure or exposure to high concentrations for a short time may be deleterious to renal functions such as alteration in electrolyte balance, acid/base disturbances, metabolism and excretion of metabolic by-products such as urea and xenobiotics. Cyanide impairs some of these functions by inhibiting the activities of Na^+ - K^+ - ATPase [36]. Thus, it could be correct to assert that the high levels of potassium in group B, sodium in group B and E, Chlorine in all the groups except group E and bicarbonate in all the groups except B when compared with group A recorded in this study could be partly due to the effect of these anti-nutrients that could have accumulated due to the dose or prolonged administration of the extract. This assertion obtains its validity from the observation that significantly high levels of serum urea and creatinine resulted from an experimental animal group treated with added cyanide than control [37,38].

5. CONCLUSION

The application of amendments to the crude oil impacted soil affected the growth performance of fluted pumpkin. The findings showed that among all the treatments used, amendment with Triton x-100 and *Pleurotus ostreatus* alone and in combination significantly resulted to better performance of fluted pumpkin and the aqueous extract of the fluted pumpkin did not pose any significant effect on the renal function of the Wistar albino rats.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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