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[1] Zoology Department, Faculty of Science, Mansoura University, Mansoura, Egypt.
[2] Urology and Nephrology Center, Mansoura University, Mansoura, Egypt.

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Original Article

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1 Zoology Department, Faculty of Science, Mansoura University, Mansoura, Egypt.

2 Urology and Nephrology Center, Mansoura University, Mansoura, Egypt.

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Abstract

The renoprotective effect of Peanut oil in rats suffered from chronic renal failure with adenine was evaluated. Adult male albino rats were classified as follow: normal group, Peanut oil group (0.75ml/kg), adenine fed group (0.75% w/w), the 4th group received both adenine in concomitant with peanut oil by the same dose and route as in the above groups, for 4 weeks. The antioxidant activity, total phenolic and flavonoid content of Peanut oil was estimated. Adenine administration reduced body weight, exceed the relative kidney weight as well as excessive serum creatinine, uric acid and BUN. In addition, exceed urine volume, appearance of urinary protein associated with significant increased urine NAG activity, while urinary Creatinine and creatinine clearance were decreased. It raised serum concentrations of inflammatory markers (C-RP, TNF-α, IL-6 and IL-10) relative to control group. Histopathological changes were noticed including tubulointerstitial atrophy and fibrosis. These kidney functions abnormalities were associated with increased renal oxidative stress markers (MDA and XO), decreased renal antioxidants markers (SOD, CAT, GSH and TAC). Administration of Peanut oil with adenine suppress the hazardous effect of adenine, where they keep most of the estimated parameters within normal. In conclusion, these results indicate that the use of Peanut oil has efficient protective effect against renal injury in adenine fed rats.

1. Introduction

Chronic kidney disease (CKD) is associated with gradual missed kidney functions, decrease in glomerular filtration rate (GFR) accompanied with exceed nitrogen metabolites, in addition to renal structural abnormalities (Baracho *et al*., 2016), loss of nephron, renal fibrosis was also noticed (López-Novoa *et al*., 2011).

CKD seriously cause decrease in health quality or length of life (Abdelrahman et al., 2019). CKD is associated with oxidative stress, inflammation, and apoptosis through the effect on cytokines, like C-reactive protein (C-RP) and tumor necrosis factor-α (TNF-α) (Al Za'abi et al., 2018). Adenine (AD) administered rats showed symptoms like that of human CKD through its metabolic abnormalities affecting renal structure and functions (Zhao et al., 2014). Ali et al. (2010) reported that, rats fed on AD affect the level of serum creatinine (Scr), uric acid (UA) and blood urea nitrogen (BUN). The imbalance between oxidant and antioxidant mechanism lead to oxidative stress, enhanced reactive oxygen species (ROS) (Nuhu and Bhandari, 2018). These oxidative stress lead to formation of interleukin-6 (IL-6), TNF-α and activate macrophages. The inflammation lead also to tissues oxidative stress (Bulbul et al., 2018).

Natural plants nowadays may compete most physiological alterations caused by many drugs by its potency, lower toxicity and lower expensive. Peanut (Arachis hypogaea L.) have polyunsaturated fatty acids protect health and decline the ischemic heart diseases risk (Naz et al., 2016). Peanut oil (PO) is a main source of many stilbenes, flavonoids, and antioxidant components (Arya et al., 2016). Smithson et al. (2018) recorded that, PO contains polyunsaturated fatty acids, fiber, antioxidants, vitamins, phytochemicals, proteins and polyphenols. These compounds perform effective anti-inflammatory activity by inhibiting tumor necrosis factor-α (TNF-α) and IL-6 (Kang et al., 2010). Akhtar et al. (2014) reported that, PO has the ability to protect body health against many diseases, including heart disease and cancer and may decline inflammation. The present work was design to examine the efficacy of PO to block the kidney dysfunctions induced by AD in male rats, suppressing its dangerous effect on the kidney structure and function.

2. Materials and Methods

2.1. Chemicals

AD was purchased from Loba Chemie Pvt. Ltd., from India. PO of high purity was obtained from Alpha Chemika, Mumbai, from India. All other chemicals were of analytical grade.

2.2. Animals

Male Albino rats weighing 165 ± 10g were used. Rats were purchased from the Egyptian Organization for Biological Products and Vaccines (VACSERA) at Helwan city, Egypt. They were housed in stainless steel cages kept in standard `temperature, 25±2°C and relative humidity of, 58±2%, with a light/dark cycle of 12 hours. They were fed on normal rodent diet and water ad libitum. All experiments were carried out according to the guidelines of the National Research Council (NRC, 1995).

2.3. Experimental protocol

Rats were acclimized for one week, pre randomly classified into 4 groups (n=6).

1) Control (CON): Rats without any treatment.
2) Adenine (AD): Rats administered with AD (0.75 % w/w) (Ali et al., 2013).
3) Peanut oil (PO): Rats fed on normal diet and orally received Peanut oil at a dose (0.75 ml/kg) (Edrees et al., 2008).
4) AD + PO: Rats fed adenine containing diet (0.75 % w/w) and orally received of peanut oil (0.75 ml/kg).

2.4. Sample collection and tissue preparation

After the end of experimental period, 4 weeks, animals were weighed, 24 hrs urine were collected, blood samples were taken in non-heparinized tubes. Sera were separated after centrifugation at 860 Xg for 20 min. Sera and urine samples were kept at -20°C.

Rats were dissected, the two kidneys were removed, washed with normal saline. The right kidney was wiped with tissue paper and weighed. Relative kidney weight (%) was evaluated. Known weight of the right kidney was homogenized in ice–cold distilled water to give a 10% w/v homogenate. The obtained renal homogenates were spinned with 860 Xg for 20 min and the supernatants were stored to further analysis at -20°C. For histopathological observation, the left kidney placed in 10% neutral formalin.

2.5. Biochemical determinations

2.5.1. Phytochemical analysis of PO

The antioxidant activity of PO was determined against 2, 2 diphenyl-1-picrylhydrazil (DPPH) free radical as showed by Pratap et al. (2013). Total PO phenolic content was determined by using Folin-Ciocalteu reagent method according to Lin and Tang (2007). Total PO flavonoid content was determined after Zhishen et al. (1999).

2.5.2. Renal biomarkers

Serum creatinine (Scr), urine creatinine (Ucr) concentrations and blood urea nitrogen (BUN) were determined using kits purchased from Diamond Diagnostic, Cairo, Egypt, after Murray (1984) and Kaplan (1984) respectively. Serum uric acid (UA) level and urinary protein were estimated using kit purchased from Spinreact Company, Spain, based on the method of Schultz (1984) and Orsonneau et al. (1989). The activity of Urine N-acetyl-beta-D-glucosaminidase (NAG) was estimated using Rat N-Acetyl-β-D Glucosaminidase (NAG) Kit Instructions, catalog # 80390, Crystal Chem, Inc. Creatinine clearance (Crcl) was calculated after the equation of Bowers and Wong (1980):

Crcl = (Ucr x Uv)/Scr (ml/min).

2.5.3. Antioxidant & oxidative stress biomarkers

Renal glutathione (GSH) content, malondialdehe (MDA) concentration, superoxide dismutase (SOD) activity, catalase (CAT) activity and total antioxidant capacity (TAC) were estimated according to Beutler et al. (1963), Ohkawa et al. (1979), Niskikimi et al. (1972), Aebi (1984) and Koracevic et al. (2001) respectively. Renal xanthine oxidase (XO) activity was estimated using Rat xanthine oxidase (XO) ELISA kit, Catalog Number, CSB-E13614r purchased from Cusabio.

2.5.4. Inflammatory Biomarkers

Serum C-reactive protein (C-RP) concentration was estimated using Sigma Diagnostic kit after Schultz and Arnold (1990). Serum tumor necrosis factors- α (TNF- α) and interleukin-6 (IL-6) levels were estimated using rat TNF-α ELISA Kit, Catalog K1052-100 and rat IL-6 ELISA Kit, Catalog K4145-100 respectively purchased from BioVision, Milpitas Blvd, Milpitas, CA 95035 USA. Serum interleukin-10 (IL-10) was determined using Rat Interleukin 10 (IL10) ELISA Kit, Catalog Number, KT-18968 purchased from Kamiya Biomedical Company, Gateway drive, Seattle, WA 98168, USA.

2.5.5. Histopathological examination

The fixed left kidney was dehydrated, cleared in xylene and embedded in paraffin wax. Thin sections (4μm) of each kidney was cut and stained with Hematoxylin & Eosin (H&E) according to

Gamble (2008) then examined under light microscope.

2.6. Statistical analysis:

Statistical analysis of the obtained data was evaluated using SPSS, version 20.0 software. Comparisons between animal groups were performed by ANOVA. Results were represented as means±SE. The values were considered significant at p≤0.05 (Snedecor and Cochran, 1980).

3. Results

The antioxidant activity of PO, showing an IC50 value = 1.06564 µg/ml, phenolic content of PO = 0.27598 mg of gallic acid/g and its flavonoid content = 0.134519 mg of catechin/g, table (1).

Table (1): Antioxidant activity, phenolic and flavonoid contents of peanut oil.

Parameter	Antioxidant activity IC50 value (µg/ml)	Phenolic content (mg of gallic acid /g)	Flavonoid content (mg of catechin /g)
Peanut oil	1.06564	0.27598	0.134519

Concerning the renal function markers, the observed data from table (2) showed a significant decrease in B.wt with significantly exceed of absolute and relative kidney weight in AD-treated rats compared with control group. However, the results also recorded protective effect in these parameters in the AD+PO treated group compared to the AD treated group.

Table (2): Starting and last body weight (B.wt) and (absolute & relative) kidney weight in animal groups.

	Animal groups			
Estimated parameter	Control	Peanut oil	Adenine	Adenine + Peanut oil
Starting B.wt (g)	167.40 ± 2.65	169.40 ± 1.17	169.17 ± 2.54	168.33 ± 1.94
Last B.wt (g)	206.40 ± 2.14	209.0 ± 1.06	124.00 ± 2.87 [a]	132.0 ± 2.78 [a]
Absolute kidney weight (g)	0.64 ± 0.02	0.65 ± 0.02	1.02 ± 0.11 [a]	0.78 ± 0.02 [a]
Relative kidney weight (%)	0.31 ± 0.01	0.31 ± 0.01	0.82 ± 0.08 [a]	0.59 ± 0.02 [ab]

Data expressed as means ± SE (n=6).
[a] significant compared to control group.
[b] significant compared to adenine group.

Rat group fed with AD, showed significant exceed in Scr, UA and BUN relative to that of normal rats. Meanwhile, Scr, UA and BUN in the AD+PO treated group significantly decrease compared to that fed AD. In addition, AD administration declines urinary creatinine while urine volume exceed associated with significant decrease in Crcl relative to that of control. On the other hand, the AD+PO group revealed a significant elevation in urinary creatinine and exceed in Crcl level when compared with AD-treated group.

Table (3): Serum creatinine (Scr), uric acid (UA) and blood urea nitrogen (BUN) levels in animal groups.

	Animal groups			
Estimated parameter	Control	Peanut oil	Adenine	Adenine + Peanut oil
Scr (mg/dl)	0.55 ± 0.03	0.54 ± 0.03	1.79 ± 0.12 [a]	1.24 ± 0.07 [ab]
UA (mg/dl)	1.33 ± 0.12	1.30 ± 0.04	3.06 ± 0.13 [a]	2.30 ± 0.07 [ab]
BUN (mg/dl)	14.01 ± 0.79	12.04 ± 0.72	71.79 ± 3.63 [a]	55.22 ± 1.28 [ab]

Data expressed as means ± SE (n=6).
[a] significant compared to control group.
[b] significant compared to adenine group

Regarding, urinary protein level and NAG activity they are significantly exceed in AD-fed group compared with control. But they decline in AD+PO treated group relative to the AD-treated group. The AD-treated group showed deteriorations of renal oxidative stress markers (MDA level and XO activity) and the antioxidant markers (GSH level, TAC and SOD, CAT activities) relative to control. While AD+PO administered rats showed a significant protection in these parameters in comparison with AD-fed group.

Table (4): Urinary creatinine (Ucr) level, urine volume, creatinine clearance (Crcl), urinary protein level and N-acetyl-beta-D-glucosaminidase (NAG) activity in animal groups.

	Animal groups			
Estimated parameter	Control	Peanut oil	Adenine	Adenine + Peanut oil
Ucr (mg/dl)	97.05 ± 4.99	97.79 ± 4.09	21.75 ± 1.95 [a]	40.05 ± 1.23 [ab]
Urine volume (ml/24h)	2.10 ± 0.24	2.0 ± 0.22	6.9 ± 0.44 [a]	4.33 ± 0.25 [ab]
Crcl (ml/min)	0.25 ± 0.04	0.26 ± 0.03	0.06 ± 0.01 [a]	0.10 ± 0.01 [ab]
Urinary protein (mg/24h)	2.82 ± 0.32	2.80 ± 0.38	18.38 ± 1.22 [a]	8.13 ± 0.47 [ab]
NAG (IU/l)	14.89 ± 0.70	13.15 ± 0.74	96.56 ± 1.97 [a]	81.08 ± 1.45 [ab]

Data expressed as means ± SE (n=6).
[a] significant compared to control group.
[b] significant compared to adenine group.

Table (5): Renal Malondialdehyde (MDA) level, Xanthine oxidase (XO) activity, Glutathione (GSH) level, Superoxide dismutase (SOD) activity, Catalase (CAT) activity and Total antioxidant capacity (TAC) in animal groups.

	Animal groups			
Estimated parameter	Control	Peanut oil	Adenine	Adenine + Peanut oil
MDA (nmol/g)	140.62 ± 1.63	133.90 ± 1.02	286.90 ± 9.34 [a]	179.08 ± 1.16 [ab]
XO (ng/100mg)	0.56 ± 0.15	0.47 ± 0.03	1.33 ± 0.02 [a]	1.12 ± 0.02 [ab]
GSH (mg/g)	283.20 ± 20.18	324.80 ± 6.46	156.80 ± 7.33 [a]	200.0 ± 5.36 [ab]
SOD (U/g)	346.83 ± 3.89	360.0 ± 3.11	188.33 ± 5.47 [a]	273.17 ± 3.07 [ab]
CAT (U/g)	385.20 ± 9.47	408.80 ± 9.16	109.73 ± 4.97 [a]	244.07 ± 1.58 [ab]
TAC (mM/g)	413.10 ± 9.93	427.37 ± 5.94	187.07 ± 21.06 [a]	323.53 ± 1.07 [ab]

Data expressed as means ± SE (n=6).
[a] significant compared to control group.
[b] significant compared to adenine group.

Significant increase in the serum inflammatory markers concentrations (C-RP, TNF-α, IL-6 and IL-10) of AD-fed rats accompanied by decline in these parameters in AD+PO treated groups (Table 6).

Table (6): Serum C-reactive protein (C-RP), Tumor Necrosis Factor-α (TNF-α), Interleukin 6 (IL-6) and Interleukin 10 (IL-10) levels in animal groups.

	Animal groups			
Estimated parameter	Control	Peanut oil	Adenine	Adenine + Peanut oil
C-RP (mg/dl)	0.12 ± 0.03	0.10 ± 0.04	2.65 ± 0.34 [a]	0.75 ± 0.03 [ab]
TNF-α (Pg/ml)	58.72 ± 0.93	61.0 ± 1.09	291.01 ± 17.03 [a]	203.59 ± 2.29 [ab]
IL-6 (Pg/ml)	107.58 ± 1.64	116.36 ± 2.51	173.63 ± 2.03 [a]	156.63 ± 0.91 [ab]
IL-10 (Pg/ml)	25.71 ± 1.01	26.25 ± 0.86	65.89 ± 1.62 [a]	52.52 ± 0.76 [ab]

Data expressed as means ± SE (n=6).
[a] significant compared to control group.
[b] significant compared to adenine group

Histopathological examination in AD-fed group showed moderate tubular dilation, tubular necrosis, diffuse interstitial fibrosis, and inflammation. This pathological alteration was nearly abolished in AD+PO treated group (Plate 1).

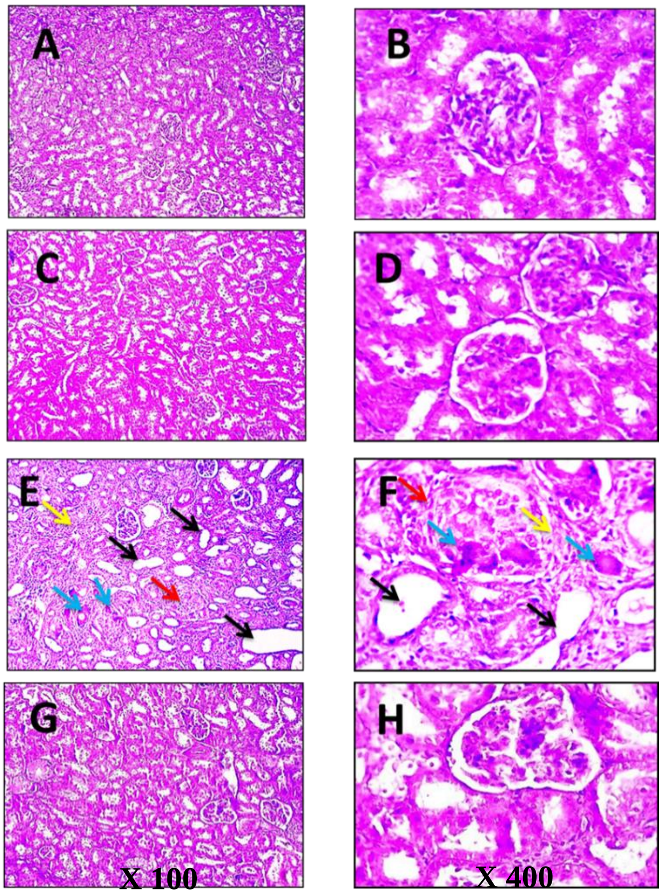

Plate (1): Kidney structure in different experimental groups stained with H&E. (A&B): Control group, (C&D): Peanut oil group, (E&F): Adenine group, (G&H): Adenine + Peanut oil group. Black arrows: tubular dilation, blue arrows: tubular necrosis, red arrows: interstitial fibrosis, yellow arrow: inflammation.

4. Discussion

The occurrence of hydrocarbon induced changes in spent oil polluted soil are imminent in areas with diverse anthropogenic activities as recorded in several studies (Milala *et al*., 2015; Vwioko *et al*., 2018; Nwite and Alu, 2015; Edwin-Wosu and Nkang, 2019a). The physicochemical properties of soil in parts of Eastern Niger Delta are persistently exposed to induced changes due to anthropogenic automobile sources of untreated and unregulated waste oil discharge to the environmental supporting system. This corroborates similar assertion of waste oil soil pollution (Uquetan *et al*., 2017; Edwin-Wosu and Nkang 2019a; Beckley and Mathew, 2020). Variation and changes in the status of polluted soil due to diverse waste oil hydrocarbon inducement has recorded variant levels of physicochemical properties (Table 1), besides the correlating pattern of the various properties as exemplified in Table 2.

The non-significant ($P<0.05$) decrease across polluted condition when compared to increase in the phytoremediated soil condition of chloride (Cl^-) and sulphate (SO_4^{-2}) content implies the sensitivity of these anions despite their maximum baseline content in the waste oil (Table 3). This corroborate the assertion that sensitivity might be due to the fact that hydrocarbon has affected the soil of the area (Edwin-Wosu and Nkang, 2019b). This must have as well decreased the Cl^- and SO_4^{-2} in waste oil polluted soil as exemplified in a positive correlation ($r = 0.25$; $r = 0.33$; $P<0.05$) between THC and Cl^- and SO_4^{-2} respectively, indicating dependent variable decrease and independent variable increase though with a non-significant variation. Decreased Cl^- and SO_4^{-2} content in soil polluted with crude hydrocarbon has similarly been reported (Edwin-Wosu and Nkang, 2020). However study has also shown elevated Cl^- content of soil under hydrocarbon pollution (Seifi *et al*., 2010).

In like manner such ionic elevation due to hydrocarbon pollution has been recorded in NO_3^- and PO_4^{-3} content of the waste oil polluted soil as exemplified in a weak positive correlation ($r = 0.04$; $r = 0.02$; $P<0.05$) with THC respectively indicating dependent variable increase with independent variable increase. This corroborate the study on hydrocarbon-induced changes including nitrate increase in a crude oil polluted tropical Niger Delta soils (Nwite and Alu, 2015; Edwin-Wosu and Nkang, 2020). It has been revealed that soil salinity can be determined by the concentration of dissolved salt. This is in tandem with the fact that low level salinity in soil of most humid regions are link to ionic salt concentration and when elevated due to pollution has consequently increased salinity (Abdulfattah *et al*., 2016, Edwin-Wosu and Nkang, 2020). This can also be reflected in the present research in which salinity increased in relation to NO_3^- and PO_4^{-3} increase beside Cl^- and SO_4^{-2} decrease across pollution as exemplified in a positive correlation (r = 0.16; *P<0.05*) between salinity and THC as well as positive correlation (r = 0.28; r = 0.09; r = 0.18; *P<0.05*) between salinity and Cl^-, SO_4^{-2} and PO_4^{-3} respectively beside a weak negative correlation (r = - 0.04; *P<0.05*) with NO_3^-. The phytoremediated soils had variation in the trend of anionic salinity content among the macrophytes with non-significant difference, despite *Peltophorum* soil being significant in NO_3^- content at 1.5% level. The trend observed among the phytoremediated soil condition can imply changes in the hydrocarbon soil condition as suggested in Edwin-Wosu and Nkang (2019b). This can be exemplified in negative correlation (r = - 0.65; r = - 0.35; r = - 0.25; *P<0.05*) between THC and *Pp, Ll* and *Cr* respectively (Table 2) with a corresponding weak negative correlation (r = -0.30; r = -0.18; r = - 0.08) with OG. Generally the slight increase in SO_4^{-2} and PO_4^{-3} across species remediated soils and with 1.5% levels of *Peltophorum* soil for Cl^- and NO_3^- was observed in *Peltophorum* treated soils with higher content among the macrophytes and with a corresponding positive correlation (r = 0.65; r = 0.45; r = 0.30; *P<0.05*) in the order *Pp> Cr>Ll* for Cl^-; positive correlation (r = 0.75; r = 0.55; r = 0.40; *P<0.05*) in the order *Pp>Cr>Ll* for NO_3^-; with positive correlation (r = 0.55; r = 0.46; r = 0.35; P<0.05) in the order *Pp>Ll>Cr* for SO_4^{-2}, while a positive correlation (r = 0.05; r = 0.38; r = 0.25; P<0.05) order of *Pp>Ll>Cr* was recorded for PO_4^{-3} content. The dynamics of the anions either in a decreasing or increasing order among the phytoremediated soils have also been discussed in Odunze *et al*. (2015), Anna *et al*. (2020); Edwin-Wosu and Nkang (2019b). The salinity of the phytoremediated soil has recorded increased variation at increasing levels of remediated soils with *C. retusa* treated soil recording higher salinity across the species soil in the order *Cr>Pp>Ll* with corresponding positive correlation (r = 0.38; r = 0.20; r = 0.10; *P<0.05*) respectively.

The decrease in cationic content across the waste polluted condition as exemplified in positive correlation (r = 0.14; r = 0.22; r = 0.07; r = 0.04, *P<0.05*) between THC and Mg^{2+}, Ca^{2+}, Na^+ and K^+ respectively with a corresponding positive correlation (r = 0.07; r = 0.25; r = 0.11; r = 0.44) between OG and Mg^{2+}, Ca^{2+}, Na^+ and K^+ (Table 2) indicating dependent variable decrease and independent variable increase, agrees with the earlier assertion on the effect of spent oil on soil properties (Uquetan *et al*., 2017; Bassey and Ebele, 2016; Milala *et al*., 2015; Nwite and Alu, 2015). The phytoremediated soil though non-significantly varying with decreasing trend of Mg^{2+} and Ca^{2+} across species treated soil as compared to the polluted condition, their was increasing trend in K^+ content of the remediated soils (Table1) with significant difference (*P<0.05*) at 0.8% and 1.5% levels of *Peltophorum* and *Leucaena* soils and 1.5% level of *Crotolaria* soil besides the non-significant increase in Na^+ across the species treated soils. Generally, the non-significant decrease in Mg^{2+} and Ca^{2+} among the macrophyte was observed in *Crotolaria* soil with higher content in the order *Cr>Pp>Ll* as exemplified in a strong negative correlation (r = -0.30; r = -0.45; r = -0.48) between Mg^{2+} and *Cr, Pp* and *Ll* respectively. Similarly was a higher content of the decreased Ca^{2+} in *Peltophorum* treated soil in the order *Pp>Cr>Ll* represented in a strong negative correlation (r = - 0.25; r = -0.32; r = -0.40) between Ca^{2+} and *Pp, Cr* and *Ll* respectively. The increased Na^+ and K^+ content of species treated soils were higher in *Leucaena* soil (*Ll>Cr>Pp*) and *Crotolaria* soil (*Cr>Ll>Pp*) respectively and exemplified in positive correlation (r = 0.32; r = 0.25; r = 0.15) between Na^+ and *Ll, Cr* and *Pp* respectively as well as positive correlation (r = 0.56; r = 0.55; r = 0.47) between K^+ and *Cr, Ll* and *Pp* respectively.

The variation and changes in the physicochemical properties of the polluted and phytoremediated soils as presented in Table 1 showed non-significant decrease in pH across polluted soils; exemplified in the strong positive correlation (r = 0.53; *P<0.05*) with THC and a corresponding positive correlation (r = 0.18; *P<0.05*) with OG, indicating dependent variable decrease and independent variable increase. This corroborates similar study on pH reduction due to hydrocarbon pollution (Milala *et al*., 2015; Edwin-Wosu and Nkang, 2020) beside an increase earlier reported by Nwite and Alu (2015). The decrease in soil pH can be attributed to organic matter as well hydrocarbon degradation in tandem with a weak positive correlation (r = 0.19; *P< 0.05*) between pH and OM and a corresponding positive correlation (r = 0.53; *P< 0.05*) between pH and THC. This may have resulted in the release of acidic metabolite and final product that possibly lowered the pH. This corroborate the assertion by Bassey and Ebele (2016) who has revealed a decrease in soil pH in parts of Niger Delta under pollution on types of soil properties. Similarly low pH can be attributed to loss of exchangeable bases as earlier exemplified in the positive correlation between THC and cations (Mg^{2+} Ca^{2+}, Na^{2+}, K^+) and consequently a positive correlation (r = 0.41; r = 0.26; r = 0.02; r = 0.18; *P<0.05*) between pH and Mg^{2+} Ca^{2+}, Na^+, and K^+ respectively, due to displacement reactions in the soil. Colloidal complex following the watering regime as exemplified in positive correlation (r = 0.34, r = 0.38; r = 0.12; r = 0.05) between moisture and Mg^{2+} , Ca^{2+}, Na^+, and K^+ could lead to eluviations and leaching loses respectively. This corroborate similar findings of Ngobri *et al*. (2007) and Ezeaku and Egbemba (2014) on organic acid metabolism and release of acidic intermediates and product as well as displacement reaction due to excessive rainfall. There has being significant (*P<0.05*) variation in restoration of the decreased pH across the species treated soil at various levels. Generally *Peltophorum* soil had greater pH among the species in the order *Pp>Cr >Ll*, with corresponding positive correlation (r = 0.45; r = 0.03; r = 0.23; *P<0.05*) between pH and *Pp, Cr* and *Ll* respectively. Study has revealed pH toward alkalinity as well as pH values of 5.20 and 6.30 for soil in the Niger Delta area under hydrocarbon inducement (Bassey and Ebele, 2016; Victoria *et al*., 2016; Edwin-Wosu and Nkang, 2020).

The decrease in soil moisture content though non-significantly different (*P<0.05*) was represented in weak positive correlation (r = 0.04; *P<0.05*) with THC across pollution. The impacts of spent oil as well as related hydrocarbon compound have been reported (Nwite and Alu, 2015; Edwin-Wosu and Nkang, 2020). The phytoremediated soils recorded varying increase in moisture content restoration across the species treated soils, with *Leucaena* soil showing significant difference (*P<0.05*) in the order *Ll>Cr>Pp*. This was exemplified in strong positive correlation (r = 0.70; r = 0.55; r = 0.40, *P<0.05*) between moisture and *Ll, Cr* and *Pp* respectively. The significant percentage moisture content in *Leucaena* soil can be related to its high clay content as exemplified in a positive moisture correlation (r = 0.35; r = 0.23; r = 0.15) with clay despite significant reduction in the order *Ll>Pp>Cr* across remediated soil when compared to polluted soil. Study has revealed that high moisture content can be associated to high clay content which has ability to retain water (Ezeaku and Egbemba, 2014). This can be exemplified in a positive correlation (r = 0.65; *P<0.05*) between clay and moisture in the present study (Table 2).

The non-significant variation from pre polluted to polluted condition as exemplified in a positive correlation (r = 0.42) between sand and THC has however recorded increased sandy component across polluted levels, reflecting a dependent variable increase across independent variable increase. The increase in the sand particle of sandy loam soil texture of the study area can corroborate the assertion by Ezeaku and Egbemba (2014) that greater sandy particle was dependent on the nature of parent materials and water regime that could favour washing away and leaching of silt and clay sized fraction. This can be exemplified in a positive correlation (r = 0.45; r = 0.30; r = 0.65; *P<0.05*) between moisture and sand, silt and clay respectively in the present study. The reduction in the silt and clay particle as exemplified in positive correlation (r = 0.23; r = 0.30) respectively with THC can also corroborate earlier assertion of enhanced distortion of structural aggregate by solvent and hydrophobic component of waste oil (Edwin-Wosu and Nkang, 2017a, 2019a). The phytoremediated condition had significant variation with increased sandy and silty and reduced clay components of the treated soils.

The increase in particle size of sand with *P. pterocarpum* soil recording greater percentage in the order *Pp>Cr>Ll* can be represented in a positive correlation (r = 0.55; r = 0.45; r = 0.20) between sand and *Pp, Cr, Ll* respectively, while the silty particle restoration among species (*Ll>Pp>Cr*) was higher in *Leucaena* soil with a corresponding positive correlation (r = 0.30; r = 0.25; r = 0.10) respectively. This was in tandem with the assertion that plant species through dense and highly ramified fibrous root system can enhance phytoremediation hence they can penetrate impermeable layers such as hydrocarbon pollution sites ((Edwin-Wosu and Nkang, 2017b). Similar study has revealed leguminous plant improvement of aggregate sizes of degraded soils due to improved changes in physicochemical condition (April *et al*., 2020; Edwin-Wosu and Nkang, 2019a; Udom and Nuga, 2015).

A significant reduction in clay content across species treated soil when compared with the polluted condition was recorded. Though *Leucaena* soil (*Ll>Pp>Cr*) been higher in clay content there was no restoration of clay particle as exemplified in the weak negative correlation (r = -0.15; r = -0.25; r = -0.35) with the respective species. Soil texture does affect phytoremediation process due to its influence on the bioavailability of contaminant as been suggested that clay is capable of binding molecules more than silt and sand resulting in low bioavailability of contaminant (Izinyon and Seghosime, 2013).

The significant increase in organic matter (OM) content across the polluted condition is in tandem with the positive correlation (r = 0.62) with THC, indicating dependent variable increase and independent variable increase. Similar increase in OM have been reported due to exogenous carbon source in the waste oil added to the carbon present in the soil (Milala *et al*., 2015). Organic matter has the potential to bind with hydrocarbon molecules (Edwin-Wosu and Nkang 2017a, 2019a). The impact of phytoremediation has revealed significant reduction in the soil organic matter across species treated soil in the order *Pp<Cr<Ll* and supported by the positive correlation (r = 0.28; r = 0.40; r = 0.55) between organic matter and respective species (Table 2). Study has also recorded reduction in organic matter due to its use as nutrient for plant growth resulting to lesser accumulation in species treated soils than non polluted species soil (Edwin-Wosu and Nkang 2017a). This also supports organic matter mineralization in polluted and non-polluted soil under vegetated condition (Fabio *et al*., 2017; Albert, 2015; Edwin-Wosu, 2013). However study has recorded improved organic matter by the

combination of poultry manure and / or leguminous plant (Preissel *et al*., 2015; King and Blesh, 2018).

The increase in BD across the polluted condition with significant difference (*P<0.05*) indicating dependent variable increase and independent variable increase and non-significant porosity decrease indicating dependent variable decrease and independent variable increase can be justified in a positive correlation (r = 0.16) with THC and (r = 0.06) with OG for BD and (r = 0.25) with THC and (r = 0.10) with OG for porosity. Compaction among soil aggregate by increased hydrocarbon pollution, high negative charges in clay and colloidal nature of organic matter have been implicated for high adsorption capacity and ability of binding hydrocarbon molecules resulting to increased BD and reduced porosity. (Uquetan *et al*., 2017; Edwin-Wosu and Nkang 2017a, 2020). This can be represented in a positive correlation (r = 0.30; r = 0.62; *P<0.05*) of THC with clay and OM respectively and a corresponding positive correlation (r = 0.45; r = 0.55; *P<0.05*) of OG with clay and OM in this present study. Similar study has indicated that increased BD and decreased porosity across polluted levels is a function of waste oil filling the micro and macro pore spaces by hydrophobic portion, base water sediment and viscosity leading to compaction and adhesion among soil aggregate (Edwin-Wosu and Nkang 2019a). Increased bulk density suggests compaction and decreased porosity due to prevalent water regime and oil deposit that clog soil layer (Ezeaku and Egbemba, 2014). This can be supported by a positive correlation (r = 0.19) between BD and Moisture and negative correlation (r = -0.45) between porosity and moisture as well as negative correlation (r = -0.30) between porosity and BD. The phytoremediated soils have significant variation with decrease in BD and increase in porosity with *Peltophorum* soil having the least BD in the order (*Pp< Cr<Ll*) and vice versa in the order (*Pp>Cr>Ll*) with the highest porosity. This can be exemplified in a positive correlation (r = 0.35; r = 0.47; r = 0.55) of BD with the respective species indicating dependent variable decrease and independent variable increase and a corresponding positive correlation (r = 0.65; r = 0.35; r = 0.20) between porosity and respective species (Table 2) indicating dependent variable increase and independent variable increase. A higher degradation and removal of hydrocarbon in vegetated soil than non vegetated soil have been reported (Udom and Nuga, 2015); as well as enhanced root formation resulting to increase in pore spaces with grater PD (Edwin-Wosu and Nkang, 2017a). The increased BD in *Leucaena* soil could be as a result of the increased clay content that has the potential to bind water molecules as exemplified in a positive correlation (r = 0.65) with moisture as well as positive correlation (r = 0.30) with THC.

The soil PD across polluted condition had significant reduction as exemplified in a positive correlation (r = 0.30; r = 0.23; *P<0.05*) with clay and silt respectively and a strong positive correlation (r = 0.72; *P<0.05*) between PD and THC, indicating dependent variable decrease and independent variable increase. Study has revealed the distribution of soil aggregated component by the solvent and hydrophobic component of waste oil (Jersy *et al.*, 2015, Edwin-Wosu and Nkang 2017a, 2019a). The restoration of PD in terms of particle size of sand clay and silt across the species treated soils when compared to the polluted condition has revealed improvement. There was significant variation among the species treated soil with the *Peltophorum* soil recording a higher PD in the order *Pp>Ll>Cr*. This can be exemplified in a positive correlation (r = 0.25; r = 0.15) of PD with sand and silt respectively despite the negative correlation ((r = -0.40) with clay particles. Leguminous plant does improve aggregate sizes of degraded soil due to improved changes in physicochemical condition (Nouri *et al*., 2019; King and Blesh, 2018; Lupwayi *et al*., 2017, May and Entz, 2016). This can be justified in the present study in a positive correlation (r = 0.40; r = 0.20; r = 0.15) of PD with *Pp*, *Ll* and *Cr* respectively Indicating dependent variable increase and independent variable increase

The increase in the THC and OG content across polluted condition was significantly higher than the pre-polluted soil. This observation is in tandem with Uquetan *et al*. (2017) Colloidal nature of organic matter and clay has been implicated for their potential in binding hydrocarbon molecules (Edwin-Wosu and Nkang 2019a, 2020). This can also imply a positive correlation (r = 0.62; r = 0.30) of THC with OM and clay as well as a corresponding positive correlation (r = 0.55; r = 0.45) of OG with OM and clay, indicating dependent variable increase and independent variable increase. The phytoremeмdiated soil had recorded reduction with significant variation in the decreased THC and non-significant variation in the decreased OG, indicating dependent variable decrease and independent variable increase. This has revealed that phytoremediation can enhance polluted soil attenuation in which *Peltophorum* soil had greater performance in hydrocarbon reduction in the order *Pp< Cr<Ll* and supported by a positive correlation (r = 0.65; r = 0.25; r = 0.35) of THC with *Pp*, *Cr*, and *Ll* respectively. Similarly the reduction in OG was observed with greater performance in *Leucaena* soil in the order *Ll<Pp<Cr* as exemplified in a corresponding positive correlation (r = 0.18; r = 0.30; r = 0.08) of OG with *Ll*, *Pp*, and *Cr* respectively. A similar degradation and removal of hydrocarbon compounds in vegetated soil than non vegetated bulk soil has been reported (Udom and Nuga 2015, Edwin-Wosu and Nkang 2017a, 2019a). The performance of the species can also be

attributed to its detoxifying enzymes potency as earlier observed in Edwin-Wosu and Nkang (2016). The non-significant increase in EC across the polluted condition was exemplified in a weak positive correlation (r = 0.10; $P<0.05$) with THC. Increased changes of EC in hydrocarbon polluted soils, has also been recorded by Edwin-Wosu and Nkang (2020), and Milala *et al*. (2015). The phytoremediated soil has recorded increased variation among species treated soil with significant difference in *Peltophorum* and *Crotolaria* soils and non-significant difference in *Leucaena* soil in the order *Pp>Cr>Ll*. This can be justified in a positive correlation (r = 0.55; r = 0.40; r = 0.28) of EC with the respective species. Beside the restored increase in EC research has also revealed a decreased EC in remediated hydrocarbon crude oil soil (Edwin-Wosu and Nkang 2020).

Conclusion

Based on the result of the study the following conclusions are made: Waste oil has deleterious effect with induced physicochemical changes of the soil properties. The induced changes across pollution levels caused both significant and non-significant variation in the ionic and other physicochemical properties of the soil. An ionic content had decrease in Cl^- and SO_4^{-2} and increase in NO_3^- and PO_4^{-3} as well as salinity status. Decrease in cationic content was recorded across Mg^{2+}, Ca^{2+}, Na^+ and K^+ ions respectively. Other induced changes in physicochemical properties have revealed decrease in pH, moisture content, clay and silt, particle density, and porosity while increase was revealed among sandy particle, OM, BD, OG, THC and EC. The study has also identified some degree of potency among the phytorestoration macrophytes in both significant and non-significant levels of variation. *Peltophorum* had improvement in restoration by the increase in anionic content, pH, sand, PD, porosity, EC and Ca^{2+} and decrease in THC, OG, OM, and BD of the soil among the test plants. However, the species are promising alternatives for remediation of waste oil polluted soil. They are inexpensive, efficient and environmentally compatible and may be viable choice for oil polluted soil remediation in parts of eastern Niger Delta.

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Conflict of interest

The author(s) have not declared any conflict of interest**.**

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