Scientia Africana, Vol. 16 (No. 1), June 2017. Pp 67-81
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FOLIAGE ACCUMULATION OF PETROLEUM-BASED POLLUTANTS BY *Tallinium triangulare* GROWN ON POST-REMEDIATED SOIL AND ITS TOXICOLOGICAL POTENTIALS ON WISTAR RATS.

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Received:
Accepted:

ABSTRACT

A study of the toxicity of foliage accumulated petroleum-based pollutants by Tallinium triangulare grown on crude oil polluted and remediated agricultural soil was carried out using wistar rats. Three lots (A, B, C) of 4 m^2 farmland with 2 m spaces between them had lots B and C polluted and bioremediated for 16 weeks. Viable seedlings of T. triangulare were planted on each lot and analyses of their leaves after 28 days of growth showed highest concentrations of 9.05±0.06 mg/kg and 0.07±0.01 g/100g for C_{30} and Lead for T. triangulare from naturally attenuated soil while 8.44±0.19 mg/kg of Acenaphthene was observed for those grown on bioaugmented soil. Animals fed with formulated pellets of 50% dry weight of T. triangulare from the three lots for 28 days. Assay of animal serum showed elevated toxicological markers, slightly increased amylase activity and decreased Packed Cell Volume and haemoglobin concentration.

Key words: Absorption, bioaccumulation, biotransformation, crude oil, ingestion, excretion.

INTRODUCTION

The dominance of petroleum products in the world economy creates the conditions for distributing large amounts of these toxicants into populated areas and ecosystem around the globe (Ojumu, *et al*., 2004). Industrial activities release substantial amount of crude oil and refined products into the environment as a result of accidents such as storage tank leakage, oil spills during routine transportation and shipping operations or sabotage (Gonza'Lez, *et al*., 2008). The contaminant load of soil and water is growing steadily each year in parallel with increasing industrialization and energy demand and therefore necessitate the need for remediation. Different clean-up methods have been used for the remediation of crude oil polluted soil. Ijah and Antai (2003), reported the ability of chicken drop microorganisms in petroleum hydrocarbon remediation and they identified species of *Micrococcus, Bacillus, Pseudomonas, Enterobacter, Proteus, Aspergillus, Rhizopus, Pseudomonas aeruginosa* CDB-06 and *Penicillium* CDF-10 as potential crude oil degraders. Ohiri *et al*., (2012), also reported that the application of chicken drops in bioaugmentation of crude oil polluted site raised the pH of the top soil to a range 6.89 to 7.80 which favours the growth of soil microorganisms thereby

enhancing bioremediation. Resistance of recalcitrant hydrocarbons to degradation is thought to result from the increase in complexity of the molecular structures of branched compounds e.g. Pristane and Phytane relative to linear alkanes (Pond, *et al*., 2002; Siddiqui, and Adams, 2002). Soils subjected to petroleum hydrocarbon bioremediation are used for crop growth without possible consideration to the concentration of the recalcitrant hydrocarbons in the remediated soil. These hydrocarbons can be absorbed by crops, which may accumulate and even convert them to a more or a less toxic intermediates that are stored in specialized plant cells.

A green plant is a solar-driven, pumping and effective filtering system endowed with measurable loading and degradative capacities (Salt *et al*., 1995). Accurate modelling of the uptake and cumulative behaviour of organic contaminants like petroleum hydrocarbons in plants is essential for the assessment of crop contamination and subsequent human exposure (Yang and Zhu, 2007). Though soil/water-plant pathway has been reported by Kipopoulou *et al*., (1999), foliar uptake is regarded as the most important pathway for petroleum hydrocarbon uptake (Yang and Zhu, 2007). Khan *et al*., (2008), reported root uptake as the main pathway for high molecular weight polycyclic aromatic hydrocarbon accumulation in lettuce (*Lactuca satuva L.*) grown on waste water-contaminated soil, while Tao *et al*., (2004), reported a six and half times higher concentration of polycyclic aromatic hydrocarbons in the aerial part of the vegetables than the roots, suggesting foliar uptake and accumulation as the primary transfer pathway and storage of polycyclic aromatic hydrocarbon from soil to vegetables. Most humans in developing countries depend on carbohydrate-based foods as staple food for the supply of energy and protein (Akubugwo, *et al*., 2007). In the rural parts Nigeria and in most other tropical countries of Africa where the daily diet is dominated by starchy foods, vegetables are the most natural, cheapest and most readily available source of vitamins, proteins, minerals and essential amino acid (Okafor, 1983). The level of photosynthesis in leafy vegetables greatly contributed to their high protein, minerals and carbohydrate content (Leaf, 2011). Notable among these leafy vegetables is *T. triangulare.*

T. triangulare commonly called Waterleaf was long considered a vegetable for the poor and was thus not highly valued (Schippers, 2000). Since the increased popularity of eru (*Gnetum* leaves) in Cameroon, the Niger delta and the eastern part of Nigeria, the demand for *T. triangulare* has steadily risen (Akachuchu, and Fawusi, 1995). It is now a common produce in local markets. The leaves are used in the preparation of slightly slimy soups and stews to complement the starchy main dish (Akachuchu, and Fawusi, 1995). In Cameroon, where it is called 'bolki' or 'belok-sup', *T. triangulare* combined with eru (*Gnetum* leaves) and fufu (starchy dish) is considered a delicacy (Akachuchu, and Fawusi, 1995). In southern Nigeria, where it is called 'gbure', it is commonly mixed in soups with Jew's mallow (*Corchorus olitorius* L.), pepper and some dry fish and meat are often added to improve the taste and nutritional qualities of the sauce (Akachuchu, and Fawusi, 1995). *T. triangulare* sauce may also be a mixture of tomatoes, onion and *T. triangulare* leaves, to which palm oil and salt are added (Akachuchu, and Fawusi, 1995). The leaves are also eaten raw in salads and also used as a colouring agent in okra soup (Burkill, 1997). This vegetable has been used in Cameroon as a treatment for measles, whereas in Assam (India), it is used in the treatment of diabetes (Rifai, 1993). In Indonesia a tonic is made from the fleshy root. *T. triangulare* has been used in

Scientia Africana, Vol. 16 (No. 1), June 2017. Pp 67-81
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agriculture as a fodder for raising giant snails (Rifai, 1993). The aim of this study is to evaluated the toxicological indices of Wistar albino rats (*Ratus ratus*) fed with *T. triangulare* grown on crude oil polluted and remediated agricultural soil.

MATERIALS AND METHODS

Study Area

Bonny light crude oil was obtained from Shell Petroleum Development Company (SPDC) flow station at Egbema, Imo State, Nigeria. Chicken drops (40kg) was purchased from Godvine, Poultry Farm, Eliozu, Obio Akpor, Rivers State, Nigeria. Viable seedlings of *T. triangulare* were purchased from Rumuokoro market, Obio Akpor, Rivers State, Nigeria. While thirty six (36) healthy wistar albino rats (*Ratus ratus*) made up of 9 adult males, 9 adult females, 9 weanling males and 9 weanling females were obtained from the Animal House of Department of Biochemistry, University of Port Harcourt, Choba, River State, Nigeria. Alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, urea and creatinine kits were obtained from Randox lab Ltd, Antrim U.K., while alpha amylase kit was obtained from Giesse Diagnostics, Rome Italy.

The study area was located along Eneka-Oyigbo new link road (longitude 7^0 10″ E and latitude 4^0 40″ N) in Obio Akpor, Rivers State, Nigeria. The soil of this area belongs to the ultisols. The entire area consisted of deep uniform sand and clay sand, with slightly humus topsoil and a topsoil pH of approximately 4.86$\pm$0.12. There was no record of oil spillage or pipeline vandalization in the area.

Pollution, amendment and growing and collection of vegetable samples

Eighteen square metre farmland was cleared and divided into three sites (A, B, C) of 4 m^2 each with 2 m spaces in between them. Two sites were polluted, amended and labelled as follows: A = Unpolluted 4 m^2 farmland (control soil); B = 4 m^2 farmland with 40 dm^3 of bonny light crude oil (Natural attenuated soil) and C = 4 m^2 farmland with 40 dm^3 of bonny light crude oil to which 40kg of chicken drops was added (Bioaugmented soil). Viable seedlings of *T. triangulare* (water leaf), were planted on the three sites and allowed to grow for 4 weeks. Plant leaves were collected at fourth week after planting with sterilized razor blade into sterilized plastic bags sealed with rubber bands. All sample bags were labelled with a permanent marker and were taken to the laboratory within 1 hour of collection for hydrocarbon and heavy metal analysis.

Determination of Aliphatic hydrocarbons, polycyclic aromatic hydrocarbons (PAHs) and heavy metals content

Aliphatic and polycyclic aromatic hydrocarbon concentrations in *T. triangulare* were extracted and determined using the method of Association of Official Analytical Chemist (AOAC), (2006), while Heavy metal content was determined by the emission spectroscopy method of Jones Jr. (1975).

Formulation of animal feed, acclimatization, treatment and sacrificing of experimental animals and determination of toxicological parameters

Dried *T. triangulare* leaves was weighed on a Mettler Toledo AB204 electronic weighing balance, ground in a Thomas Scientific Model 4 Wiley's mill, formed into vegetable pellets and integrated into normal rat feed. A total of thirty six (36) wistar rats were grouped into 9 rats and sub-grouped into 3 rats each. All the test and control animals were housed at room temperature in stainless steel animal cages with free access to drinking water and standard laboratory animal diets (vital feed) for 1 week of acclimatization. Each sub-

group was fed with formulated feed pellet of 50% dry weight of *T. triangulare* from the control, bioaugmented and natural attenuated sites each for a period of 28 days. The test and control animals were sacrificed under chloroform anaesthesia and blood was collected through cardiac puncture. The blood was stored in ethylene diamine tetraacetic acid (EDTA) sample bottles for analysis of urea, creatinine, alkaline phosphate (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), amylase, packed cell volume (PCV) and haemoglobin concentrations. The liver, kidney and pancreas were quickly dissected out and placed into containers with 10% physiological saline as preservative. These samples were taken to the laboratory within 1 hour of collection for some toxicological analysis.

Determination of some toxicological parameters of rats fed with vegetables grown on amended crude oil polluted soil

Serum urea concentration was determined by the diacetyl monoxine method (DAM) of Marsh *et al.*, (1961), Creatinine concentration was determined by the Alkaline Picric acid method of Slot, (1965),while Serum amylase activity and Alkaline phosphatase activity were determined by the 2-chloro-p-nitrophenyl-α-D-maltotrioside (CNPG3) kinetic method of Hohenwallner *et al*., (1979) and the method of King and King (1954) respectively. However, Aspartate aminotransferase and Alanine aminotransferase activities were determined by the Colourimetric end-point method of Reitman and Frankel, (1957). Packed cell volume (haematocrit) and haemoglobin concentration was determined by the method of Corash, 1995, while histological analysis of rat tissues was carried out using the haematoxylin and eosin method of Conn, (1969) as modified by Kumar and Gill, (2010).

Statistical analysis

The mean $\pm$ standard deviations were calculated for each group of observations and One Way Analysis of Variance (ANOVA) were calculated electronically using International Business Machine (IBM) Statistical Program for Social Sciences (SPSS) 19 statistics software (SPSS Inc Chicago) at 95% confidence level, using post hoc Duncan (1955), multiple range test of significance.

RESULTS

The mean concentration of aliphatic hydrocarbons, polycyclic aromatic hydrocarbons and heavy metals in *T. triangulare* grown on the control, natural attenuated and bioaugmented soil are presented in Tables 1 - 3. Aliphatic hydrocarbon content increased significantly ($P<0.05$) in *T. triangulare* grown on the natural attenuated and bioaugmented soils except for C_{11}, C_{19}, C_{34} and C_{39} where higher concentrations were observed in *T. triangulare* grown on the control soil than in those grown on both the natural attenuated and bioaugmented soils. Polycyclic aromatic hydrocarbon content also increased in *T. triangulare* grown on the natural attenuated and bioaugmented soil except for phenanthrene where a value of 2.71 mg kg^{-1} was observed in *T. triangulare* grown on the control soil, while those grown on the bioaugmented soil had 2.49 mg kg^{-1}. Although the heavy metal concentration of *T. triangulare* grown on the natural attenuated soil was higher than those grown on the bioaugmented soil, lead and cadmium were absent in those grown on the bioaugmented soil, while arsenic was not observed in all the *T. triangulare* grown on both the control, natural attenuated and bioaugmented soils.

Scientia Africana, Vol. 16 (No. 1), June 2017. Pp 67-81
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Table 1: Aliphatic hydrocarbon content (mg kg^{-1}) of *T. triangulare* grown on amended crude oil polluted soil.

No. of Carbon atoms	Control soil	Natural attenuated soil	Bioaugmented soil
C_8	1.33 ± 0.05^{a}	1.87 ± 0.00^{bc}	1.79 ±0.03^{bc}
C_9	2.57 ± 0.05^{a}	7.06 ± 0.02^{b}	4.10 ±0.06^{c}
C_{10}	1.37 ± 0.04^{ac}	2.70 ± 0.07^{b}	1.93 ±0.10^{ac}
C_{11}	1.76 ± 0.07^{a}	1.42 ± 0.08^{b}	1.08 ±0.03^{c}
C_{12}	1.23 ± 0.08^{a}	3.09 ± 0.02^{b}	2.70 ±0.10^{c}
C_{13}	2.41 ± 0.01^{a}	1.11 ± 0.03^{b}	3.79 ±0.02^{c}
C_{14}	2.34 ± 0.13^{a}	3.71 ± 0.02^{b}	7.73 ±0.05^{c}
C_{15}	4.95 ± 0.07^{a}	1.60 ± 0.13^{b}	8.09 ±0.01^{c}
C_{16}	3.63 ± 0.18^{a}	7.52 ± 0.23^{b}	2.34 ±0.00^{c}
C_{17}	1.09 ± 0.05^{a}	2.05 ± 0.06^{b}	6.09 ±0.03^{c}
Pristane	1.19 ± 0.03^{a}	5.21 ± 0.19^{bc}	5.17 ±0.01^{bc}
C_{18}	1.47 ± 0.04^{ab}	1.38 ± 0.94^{ab}	4.53 ±0.03^{c}
Phytane	2.53 ± 0.05^{a}	3.85 ± 0.20^{b}	6.70 ±0.03^{c}
C_{19}	5.85 ± 0.06^{a}	3.47 ± 0.13^{b}	4.08 ±0.02^{c}
C_{20}	3.15 ± 0.03^{ac}	2.96 ± 0.10^{b}	3.20 ±0.04^{ac}
C_{21}	1.23 ± 0.10^{a}	1.74 ± 0.09^{b}	1.01 ± 0.01^{c}
C_{22}	1.06 ± 0.04^{ab}	1.11 ± 0.10^{ab}	3.45 ± 0.19^{c}
C_{23}	1.61 ± 0.01^{a}	4.11 ± 0.03^{b}	2.66 ± 0.06^{c}
C_{24}	1.28 ± 0.03^{ab}	1.38 ± 0.03^{ab}	2.37 ± 0.07^{c}
C_{25}	3.81 ± 0.05^{a}	1.95 ± 0.09^{b}	5.66 ± 0.16^{c}
C_{26}	2.08 ± 0.08^{ac}	3.36 ± 0.20^{b}	2.25 ± 0.02^{ac}
C_{27}	2.49 ± 0.16^{a}	3.56 ± 0.05^{b}	2.18 ± 0.06^{c}
C_{28}	1.17 ± 0.03^{a}	4.06 ± 0.04^{b}	6.26 ± 0.20^{c}
C_{29}	1.74 ± 0.05^{a}	1.47 ± 0.09^{b}	1.93 ± 0.03^{c}
C_{30}	2.80 ± 0.14^{a}	9.05 ± 0.06^{b}	6.04 ± 0.21^{c}
C_{31}	5.28 ± 0.14^{a}	6.91 ± 0.11^{b}	2.43 ± 0.20^{c}
C_{32}	1.47 ± 0.19^{a}	3.90 ± 0.07^{b}	3.20 ± 0.01^{c}
C_{33}	1.07 ± 0.06^{a}	2.78 ± 0.14^{b}	1.83 ± 0.03^{c}
C_{34}	2.08 ± 0.04^{a}	1.78 ± 0.03^{bc}	1.86 ± 0.10^{bc}
C_{35}	4.77 ± 0.21^{a}	6.27 ± 0.20^{b}	2.51 ± 0.02^{c}
C_{36}	2.08 ± 0.05^{a}	1.69 ± 0.03^{b}	3.44 ± 0.14^{c}
C_{37}	4.25 ± 0.11^{ac}	7.39 ± 0.04^{b}	3.94 ± 0.30^{ac}
C_{38}	1.36 ± 0.02^{ab}	1.22 ± 0.04^{ab}	1.77 ± 0.08^{c}
C_{39}	4.97 ± 0.27^{a}	1.48 ± 0.09^{b}	2.82 ± 0.25^{c}
C_{40}	1.25 ± 0.03^{a}	7.36 ± 0.14^{b}	4.15 ± 0.07^{c}

Values are Means ± standard deviations of triplicate determinations.

Superscript "a" shows significant difference ($P < 0.05$) when *T. triangulare* from the control soil is compared to the other groups.

Superscript "b" shows significant difference ($P < 0.05$) when *T. triangulare* from the bioaugmented soil is compared to the other groups.

Superscript "c" shows significant difference ($P < 0.05$) when *T. triangulare* from the natural attenuated soil is compared to the other groups.

Superscript "ab" shows no significant difference ($P < 0.05$) when *T. triangulare* from the control and bioaugmented soil are compared to each other, but statistically significant when both are compared to those from the natural attenuated soil.

Superscript "ac" shows no significant difference ($P < 0.05$) when *T. triangulare* from the control and natural attenuated soil are compared to each other, but statistically significant when both are compared to those from the bioaugmented soil.

Superscript "bc" shows no significant difference ($P < 0.05$) when *T. triangulare* from the bioaugmented and natural attenuated soil are compared to each other, but statistically significant when both are compared to those from the control soil.

Table 2: Polycyclic aromatic hydrocarbon (PAH) content (mg kg^{-1}) of *T. triangulare* grown on amended crude oil polluted soil.

Polycyclic aromatic hydrocarbon	Control soil	Natural attenuated soil	Bioaugmented soil
Naphthalene	0.85 ± 0.11^{a}	7.07 ± 0.10^{b}	1.05 ± 0.02^{c}
Acenaphthylene	1.12 ± 0.06^{a}	6.82 ± 0.27^{b}	2.74 ± 0.11^{c}
Acenaphthene	2.13 ± 0.32^{a}	4.57 ± 0.35^{b}	8.44 ± 0.19^{c}
Fluorene	1.84 ± 0.14^{a}	2.92 ± 0.11^{b}	4.18 ± 0.20^{c}
Phenanthrene	2.71 ± 0.40^{ac}	5.92 ± 0.19^{b}	2.49 ± 0.06^{ac}
Anthracene	1.66 ± 0.07^{a}	2.74 ± 0.05^{b}	2.28 ± 0.06^{c}
Fluoranthene	1.45 ± 0.04^{a}	2.00 ± 0.16^{b}	2.43 ± 0.06^{c}
Pyrene	ND	ND	ND
Benzo(α)anthracene	3.67 ± 0.03^{a}	7.16 ± 0.02^{b}	8.38 ± 0.07^{c}
Chrysene	2.12 ± 0.01^{a}	3.05 ± 0.04^{b}	2.22 ± 0.04^{c}
Benzo(β)fluoranthene	0.54 ± 0.03^{a}	5.22 ± 0.06^{b}	1.21 ± 0.01^{c}
Benzo(k)fluoranthene	ND	N	ND
Benzo(α)pyrene	1.75 ± 0.06^{a}	2.94 ± 0.10^{bc}	2.82 ± 0.02^{bc}
Indeno(1,2,3)pyrene	1.37 ± 0.05^{a}	1.96 ± 0.11^{b}	3.67 ± 0.04^{c}
Indeno(1,2,3-cd)pyrene	2.89 ± 0.06^{a}	4.86 ± 0.09^{b}	7.24 ± 0.07^{c}
Dibenz(a,h)ant racene	2.05 ± 0.10^{a}	5.26 ± 0.45^{b}	2.17 ± 0.03^{c}
B nzo(g,h,i)perylene	ND	ND	ND

Values are Means ± standard deviations of triplicate determinations.

Superscript "a" shows significant difference (P < 0.05) when *T. triangulare* from the control soil is compared to the other groups.

Superscript "b" shows significant difference (P < 0.05) when *T. triangulare* from the bioaugmented soil is compared to the other groups.

Superscript "c" shows significant difference (P < 0.05) when *T. triangulare* from the natural attenuated soil is compared to the other groups.

Superscript "ac" shows no significant difference (P < 0.05) when *T. triangulare* from the control and natural attenuated soil are compared to each other, but statistically significant when both are compared to those from the bioaugmented soil.

Superscript "bc" shows no significant difference (P < 0.05) when *T. triangulare* from the bioaugmented and natural attenuated soil are compared to each other, but statistically significant when both are compared to those from the control soil.

Table 3: Heavy metal content (g/100g) of *T. triangulare* grown on amended crude oil polluted soil.

Heavy metal	Control soil	Natural attenuated soil	Bioaugmented soil
Copper	ND	0.01±0.00^{b}	0.02±0.00^{c}
Lead	ND	0.07±0.01^{b}	ND
Chromium	ND	0.04±0.00^{b}	0.05±0.00^{c}
Cadmium	ND	0.01±0.00^{b}	ND
Arsenic	ND	ND	ND

Values are Means ± standard deviations of triplicate determinations.
Note: ND = Not detected.
Superscript "b" shows significant difference (P < 0.05) when *T. triangulare* from the bioaugmented soil is compared to the other groups.
Superscript "c" shows significant difference (P < 0.05) when *T. triangulare* from the natural attenuated soil is compared to the other groups.

Weight of rats fed with *T. triangulare* grown on amended crude oil polluted soil are presented in Tables 4 and 5. In all the three vegetables, the rats showed pronounced decrease in weight after 28 days of feeding. The percentage decrease in

Scientia Africana, Vol. 16 (No. 1), June 2017. Pp 67-81
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weight was more pronounced in those fed with *T. triangulare* grown on the natural attenuated soil (see Tables 4. and 5). However, rats fed with *T. triangulare* from the control soil for 28 days showed significant increase in weight. Percentage increase in weight was more pronounced in the weanling females followed by the weanling males.

Table 4: Weight of rats (g) before (day zero) and after (28 days) of feeding with *T. triangulare* grown on amended crude oil polluted soil.

	Before feeding (at day 0)			After 28 days of feeding		
	Control soil	**Natural attenuated soil**	**Bioaugmented soil**	**Control soil**	**Natural attenuated soil**	**Bioaugmented soil**
Weanling male	49.64 ± 4.23^{a}	48.27 ± 2.63^{a}	48.93 ± 3.18^{a}	52.77 ± 5.93^{b}	39.61 ± 3.67^{cd}	40.29 ± 1.86^{cd}
Weanling female	44.02 ± 2.87^{a}	43.87 ± 4.77^{a}	45.68 ± 6.23^{a}	48.95 ± 4.65^{b}	35.12 ± 7.06^{cd}	39.66 ± 5.37^{cd}
Adult male	170.45 ± 6.74^{a}	169.27 ± 7.93^{a}	170.01 ± 5.14^{a}	181.06 ± 2.74^{b}	147.38 ± 8.12^{cd}	152.44 ± 4.86^{cd}
Adult female	165.23 ± 5.93^{a}	164.89 ± 5.02^{a}	164.92 ± 4.43^{a}	169.23 ± 8.63^{b}	145.79 ± 10.04^{cd}	148.39 ± 9.21^{cd}

Values are Means ± standard deviations of triplicate determinations.

Superscript "a" shows no significant difference ($P < 0.05$) when rats to be fed with *T. triangulare* from the control are compared to the other groups.
Superscript "b" shows significant difference ($P < 0.05$) when rats fed with *T. triangulare* from the control are compared to the other groups .
Superscript "cd" shows no significant difference ($P < 0.05$) when rats fed with *T. triangulare* from the natural attenuated soil are compared to that of the bioaugmented soil but significant when both are compared to those fed with *T. triangulare* from the control.

Table 5: Change in weight (%) of rats fed with *T. triangulare* grown on amended crude oil polluted soil.

	Control soil	Natural attenuated soil	Bioaugmented soil
Weanling male	$\uparrow 5.93\pm0.29^{a}$	$\downarrow 21.87\pm0.29^{bc}$	$\downarrow 21.42\pm0.71^{bc}$
Weanling female	$\uparrow 10.08\pm0.38^{a}$	$\downarrow 24.92\pm0.34^{b}$	$\downarrow 15.19\pm0.16^{c}$
Adult male	$\uparrow 5.83\pm0.59^{a}$	$\downarrow 14.85\pm0.02^{b}$	$\downarrow 11.52\pm0.06^{c}$
Adult female	$\uparrow 2.33\pm0.31^{a}$	$\downarrow 13.10\pm0.50^{b}$	$\downarrow 11.14\pm0.52^{c}$

Values are Means ± standard deviations of triplicate determinations.
↑ = Increase.
↓ = decrease.
Superscript "a" shows significant difference ($P < 0.05$) when rats fed with *T. triangulare* from the control are compared to the other groups.
Superscript"b" shows significant difference ($P< 0.05$) when rats fed with *T.triangulare* from the bioaugmented soil are compared to the other groups.
Superscript "c" shows significant difference ($P < 0.05$) when fed with *T. triangulare* from the natural attenuated soil are compared to the other groups.
Superscript "bc" shows no significant difference ($P < 0.05$) when fed with *T. triangulare* from the bioaugmented and natural attenuated soil are compared to each other, but statistically significant when both are compared to those fed with *T. triangulare* from the control.

Haematological parameters of rats fed with *T. triangulare* grown on amended crude oil polluted soil are presented in Tables 6 - 8. The urea, creatinine, amylase, alkaline phosphatase, alanine and aspartate aminotransferases increased in the rats fed with *T. triangulare* grown of natural attenuated and bioaugmented soil. The packed cell volume (PCV) and haemoglobin concentration decreased in all the rats fed with *T. triangulare* grown of the natural attenuated and bioaugmented soils. The decrease in packed cell volume and haemoglobin concentration were more pronounced in rats fed with *T. triangulare* grown on bioaugmented soil than in those fed with *T. triangulare* grown on the natural attenuated soil (see Table 8).

Table 6: Haematological parameters of rats fed with *T. triangulare* grown on amended crude oil polluted soil.

	Control soil	Natural attenuated soil	Bioaugmented soil
	Urea (mg dl^{-1})		
Weanling male	25.47±1.17^{a}	52.21±1.59^{bc}	51.20±0.21^{bc}
Weanling female	26.60±0.42^{a}	50.96±0.72^{bc}	51.74±1.00^{bc}
Adult male	34.60±2.30^{a}	55.72±0.91^{bc}	52.90±2.10^{bc}
Adult female	32.90±0.93^{a}	51.40±0.51^{bc}	51.41±0.28^{bc}
	Creatinine (mg dl^{-1})		
Weanling male	0.62±0.10^{a}	1.80±0.06^{bc}	1.74±0.03^{bc}
Weanling female	0.71±0.09^{a}	1.79±0.08^{bc}	l.71±0.11^{bc}
Adult male	0.67±0.04^{a}	1.81±0.11^{bc}	1.77±0.05^{bc}
Adult female	0.54±0.08^{a}	1.80±0.03^{bc}	1.83±0.06^{bc}
	Alkaline phosphatase (I.U.l^{-1})		
Weanling male	211.00±0.86^{a}	487.40±1.80^{b}	568.30±2.40^{c}
Weanling female	210.30±l.90^{a}	463.70±1.90^{b}	514.47±5.64^{c}
Adult male	286.00±2.10^{a}	438.20±1.50^{b}	535.60±2.80^{c}
Adult female	284.00±2.20^{a}	378.50±2.60^{b}	447.40±2.01^{c}
	Aspartate aminotransferase (I.U.l^{-1})		
Weanling male	25.01±0.79^{a}	55.68±2.16^{b}	62.00±0.14^{c}
Weanling female	27.02±0.11^{a}	54.72±2.48^{bc}	58.07±2.38^{bc}
Adult male	23.04±1.60^{a}	55.38±3.08^{b}	70.47±1.79^{c}
Adult female	21.01±0.51^{a}	55.00±0.38^{b}	57.02±0.40^{c}
	Alanine aminotransferase (I.U.l^{-1})		
Weanling male	26.02±0.60^{a}	38.40±1.30^{b}	51.70±0.44^{c}
Weanling female	24.30±1.49^{a}	38.70±0.68^{b}	49.32±0.60^{c}
Adult male	21.61±0.99^{a}	36.67±1.25^{b}	46.01±0.19^{c}
Adult female	21.00±0.26^{a}	37.33±0.74^{b}	44.02±0.90^{c}
	Amylase (I.U.l^{-1})		
Weanling male	27.98±0.21^{ab}	28.70±0.85^{abc}	30.14±1.06^{bc}
Weanling female	26.42±0.45^{a}	34.53±0.29^{b}	31.08±0.36^{c}
Adult male	34.10±0.41^{abc}	36.07±1.07^{abc}	36.97±1.52^{abc}
Adult female	33.65±0.29^{ac}	37.04±0.77^{bc}	35.10±1.28^{abc}
	Packed cell volume (%)		
Weanling male	30.67 ± 0.58^{a}	25.68 ± 1.53^{bc}	22.33 ± 2.52^{bc}
Weanling female	32.32 ± 1.53^{a}	24.35 ± 2.52^{bc}	24.00 ± 4.03^{bc}
Adult male	33.68 ± 0.58^{a}	26.67 ± 1.54^{b}	22.33 ± 1.16^{c}
Adult female	31.33 ± 1.60^{a}	22.34 ± 2.51^{bc}	23.00 ± 3.00^{bc}
	Haemoglobin concentration (g dl^{-1})		
Weanling male	10.22 ± 0.19^{a}	8.56 ± 0.51^{bc}	7.44 ± 0.84^{bc}
Weanling female	10.78 ± 0.51^{a}	8.11 ± 0.84^{bc}	8.00 ± 1.33^{bc}
Adult male	11.22 ± 0.17^{a}	8.89 ± 0.51^{b}	7.45 ± 0.39^{c}
Adult female	10.44 ± 0.52^{a}	7.44 ± 0.84^{bc}	7.67 ± 1.00^{bc}

Values are Means ± standard deviations of triplicate determinations.

Superscript "a" shows significant difference ($P < 0.05$) when rats fed with *T. triangulare* from the control are compared to the other groups.

Superscript "b" shows significant difference ($P < 0.05$) when rats fed with *T. triangulare* from the bioaugmented soil are compared to the other groups.

Superscript "c"shows significant difference($P< 0.05$) when rats fed with *T.triangulare* from the natural attenuated soil are compared to the other groups.

Superscript "ab" shows no significant difference ($P < 0.05$) when rats fed with *T. triangulare* from the control and bioaugmented soil are compared to each other, but statistically significant when both are compared to those fed with *T. triangulare* from the natural attenuated soil.

Superscript "ac" shows no significant difference ($P < 0.05$) when rats fed with *T. triangulare* from the control and natural attenuated soil are compared to each other, but statistically significant when both are compared to those fed with *T. triangulare* from the bioaugmented soil.

Superscript "bc" shows no significant difference ($P < 0.05$) when rats fed with *T. triangulare* from the bioaugmented and natural attenuated soil are compared to each other, but statistically significant when both are compared to those fed with *T. triangulare* from the control.

Superscript "abc" shows no significant difference ($P < 0.05$) when rats fed with *T. triangulare* from the control, bioaugmented and natural attenuated soil are compared to each other.

Scientia Africana, Vol. 16 (No. 1), June 2017. Pp 67-81
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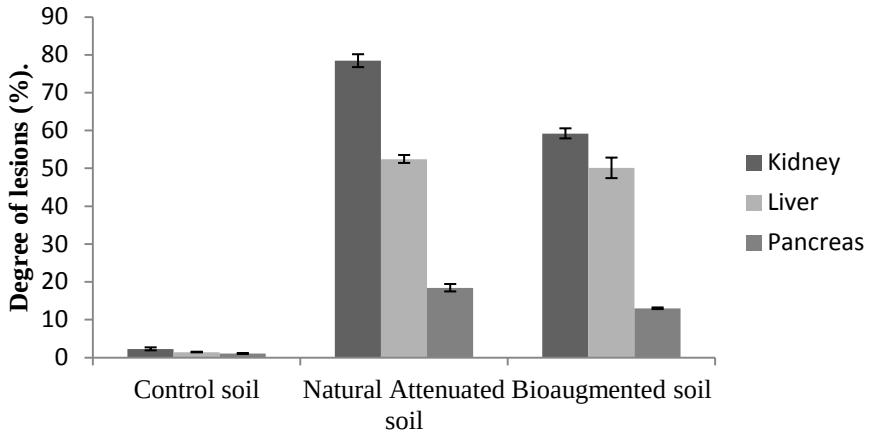
Histological scores of sections of rats fed with *T. triangulare* grown on amended crude oil polluted soil are shown in fig.1. The percentage degree of lesions were highest in the kidney, liver and pancreas sections of rats fed with *T. triangulare* grown on natural attenuated and bioaugmented soil respectively, while sections of rats fed with *T. triangulare* grown on the control soil showed healthier sections with non-significant degrees of lesions.



Values plotted are means ± standard deviations of three determinations.

Fig.1. Histological scores of sections of rat organs fed with *T. triangulare* grown on amended crude oil polluted soil.

Histological sections of rats fed with *T. triangulare* grown on amended crude oil polluted soil are presented in figures 2a – 2i. Kidney sections of rats fed with *T. triangulare* grown on the control soil showed healthy organs with perfect glomeruli and tissue architecture, while those fed with *T. .triangulare* grown on the natural attenuated and bioaugmented soil had unhealthy necrotic kidney with shrunken glomeruli and multicystic spaces (see figs. 2a – 2c).

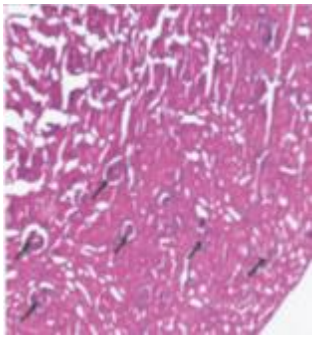


Fig. 2a

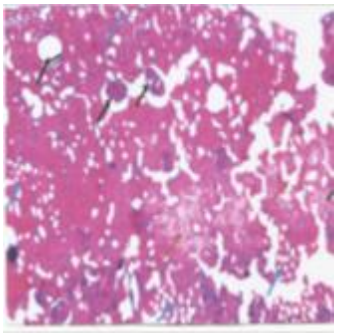


Fig. 2b

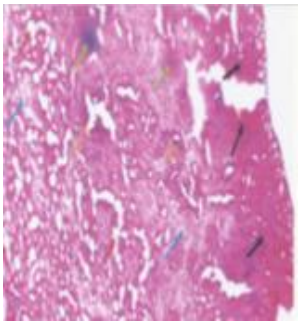


Fig. 2c

(2a). Representative section of the kidney of rat fed with *T. triangulare* grown on the control soil. (Magnification: X400). [Observations: Glomeruli perfectly in order (Black arrows), No observable change]. (2b). Representative section of the kidney of rat fed with *triangulare* grown on the natural attenuated soil (Magnification: X400). [Observations: Glomeruli shrunken (Black arrows), Multicystic spaces with distorted stroma (Blue arrows), Necrosis (Red arrows)].

(2c.). Representative section of the kidney of rat fed *T. triangulare* grown on the bioaugmented soil. (Magnification: X400). [Observations: Oedema (Black arrows). Necrosis (Blue arrows). Multicystic spaces (Red arrows). Hyalinization (Green arrows). Glomeruli shrunken (Yellow arrows)].

As shown in fig. 2d, healthy liver sections with clearly identified hepatocytes were observed in rats fed with *T. triangulare* grown on the control soil, while those fed with *T. triangulare* grown on the natural attenuated and bioaugmented soils showed unhealthy proliferating liver with few hepatocytes and completely distorted tissue architecture (see figs. 2e and 2f).

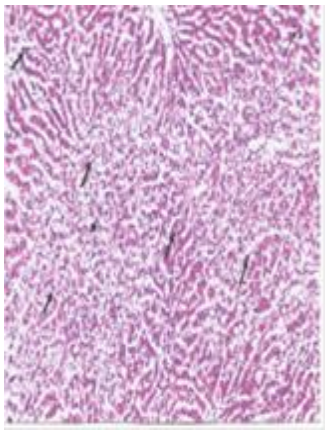


Fig. 2d

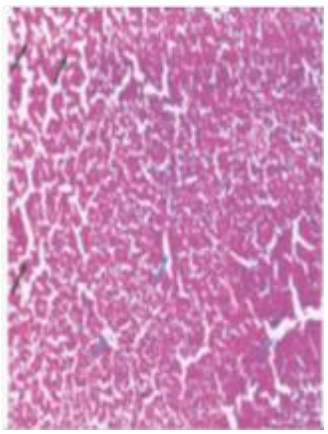


Fig. 2e

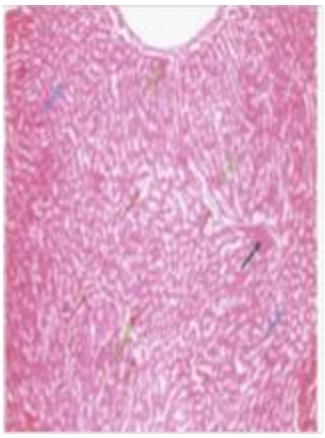


Fig. 2f

(2d). Representative section of the liver of rat fed with *T. triangulare* grown on the control soil (Magnification: X400). **[**Observations: Hepatocytes clearly identified (Black arrows), No observable change]. (2e). Representative sectionof the liver of rat fed with *T. triangulare* grown on the natural attenuated soil (Magnification: X400). **[**Observations: Tissue stromal proliferation (Black arrows), Distorted tissue architecture (Blue arrows)]. (2f) Representative section of the liver of rat fed with *T. triangulare* grown on the bioaugmented soil (Magnification: X400). [Observations: Central vein blocked (Black arrow), Proliferation of tissue stroma (Blue arrows), Laminae of hepatic cell not well arranged (Red arrows), Few hepatocytes identified (Green arrows)]

Pancreas sections of rats fed with *T. triangulare* grown on the control soil showed a healthy pancreas with undistorted tissue architecture (see fig. 2g), while scanty stroma and slight distorted tissue architecture was observed in those fed with *T. triangulare* grown on the natural attenuated and bioaugmented soils. (see figs 2h and 2i).

Scientia Africana, Vol. 16 (No. 1), June 2017. Pp 67-81
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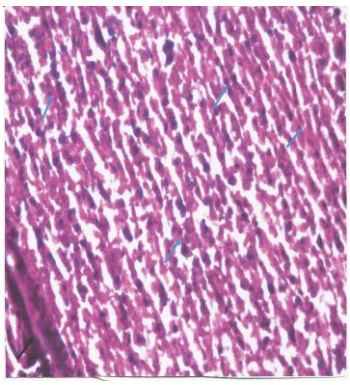

Fig. 2g

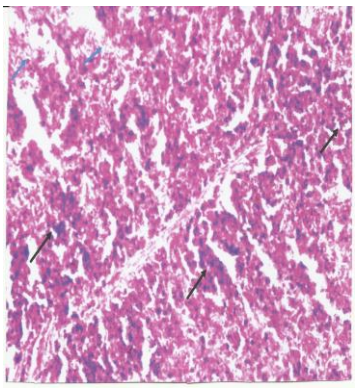

Fig. 2h

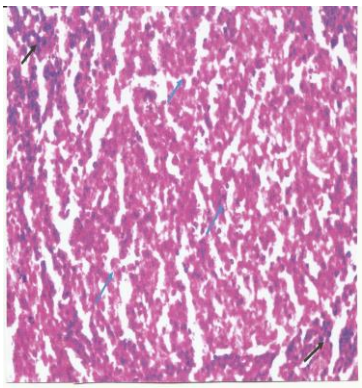

Fig. 2i

(2g). Representative section of the Pancreas of rat fed with *T. triangulare* grown on the control soil (Magnification: X400). **[**Observations: Mild oedema (Black arrows), Pancreatic acini clearly observed (Blue arrows]. (2h). Representative section of the Pancreas of rat fed with *T. triangulare* grown on the natural attenuated soil (Magnification: X400). [Observation: Scanty stroma (Blue arrows)]. (2i). Representative section of the Pancreas of rat fed with *T. triangulare* grown on the bioaugmented soil (Magnification: X400). [Observations: Mild oedema (Black arrow), Mild tissue proliferation (Blue arrows)].

DISCUSSION

The high concentration of hydrocarbons and heavy metals observed in the *T. triangulare* grown on both the natural attenuated and bioaugmented soils can be attributed to the presence of recalcitrant hydrocarbons and incomplete degradation of crude oil hydrocarbons in the remediated soil (see Tables 1, 2 and 3). This result corroborates the work of Ziolkowskai and Wyszkowskii, (2010), which reported that petroleum contamination of soil may not weaken the vegetative development of crops but can cause accumulation of hydrocarbons in in the aerial parts of the plants. As shown in Tables 1 and 2, trans-boundary transfer of contamination caused by horizontal flow or possibly by human or animal activities on the study area may be responsible for the increased hydrocarbon concentration in the control soil. Increased heavy metal concentration of crude oil contaminated and bioremediated agricultural soil has been reported by Ohiri, *et al*., (2013). They attributed such increase in concentration to the presence of heavy metals in the crude oil. Their report also corroborates the work of Chicarelli, *et al.,* (1990), which reported that heavy metals such as Copper and Nickel are found in crude oil via their association with porphyrins. The significant decrease in weight of rats fed for 28 days with *T. triangulare* grown on the natural attenuated and bioaugmented soils (Tables 4 and 5) can be attributed to the presence of hydrocarbons and heavy metals in the vegetable. As shown in Tables 1 - 3, the presence of these hydrocarbons and heavy metals in the *T. triangulare* may induce a stress condition, thereby reducing the nutritive value of this vegetable by depleting its vitamin and protein contents. This may elicit an adverse effect on the weight of the treated animals. In small concentrations, some heavy metals can be regarded as important nutrients. For example, Anderson (1998) reported the importance of chromium in the regulation of carbohydrate and lipid metabolism. In addition to its effects on glucose, insulin, and lipid metabolism, he also reported the ability of chromium to increase lean body mass and

decrease percentage body fat, which may lead to weight loss in humans. This may be responsible for the decrease in weight of rats fed with *T. triangulare* grown on the natural attenuated and bioaugmented soil. This may also apply to other heavy metals, whose increased concentration (mainly in *T. triangulare* grown on the natural attenuated soil) as observed in this study (Table 3), may have contributed to the decrease in weight of the treated animals.

Ingestion is the most common route of exposure of bioaccumulated toxic chemicals from edible plants to humans and other animals alike. The pronounced increase in the toxicological parameters observed in this work can be attributed to the ingestion and biotransformation of plant accumulated hydrocarbons and heavy metals and subsequent renal excretion processes of the liver and kidneys respectively. Excretion of toxicants has been greatly associated with the kidney. However, in the cause of excretion, lipid soluble substances (such as aliphatic petroleum hydrocarbons and polycyclic aromatic hydrocarbons) are readily reabsorbed in the renal tubule (Koolman and Roehm, 2005). This may lead to accumulation of these toxicants in the kidneys and subsequent impairment in renal function, resulting in increased urea and creatinine concentrations and destruction of kidney cells thereby altering the kidney architecture as observed in rats fed with *T. triangulare* grown on natural attenuated and bioaugmented soil (Table 6 and Figs. 2b and 2c). Toxic substances in the liver are biotransformed or secreted into the bile. However, biliary excretion of toxicants does not tantamount to elimination of such toxicants from the body. Reabsorption of biliary metabolites results into an enterohepatic cycle that may keep the toxicants in the body, thereby subjecting the liver to more stress conditions. This can lead to destruction of hepatic cells, alteration in hepatic architecture and subsequent increase in concentration of hepatic enzymes such as alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in the serum (Table 6). Hepatic transformation of toxicants may produce potential carcinogens (Ottaway,1980), For example, DEFRA and EA,(2002), reported that degradation of benzo(a)pyrene by cytochrome P_{450} enzymes may lead to the production of metabolites such as benzo(a)pyrene 7,8 diol-9,10-epoxide, which is a reactive epoxide believed to be responsible for benzo(a)pyrene carcinogenicity. Hanninen (1985), also reported that biotransformation of vinyl chloride produces vinyl chloride epoxide, which covalently binds to DNA and RNA, a step leading to cancer of the liver. These are also applicable to other petroleum hydrocarbon, whose biotransformation in the liver may produce a more toxic compound or possibly a potential carcinogen. This may be responsible for the proliferation of cells observed in the liver of rats fed with *T. triangulare* grown on the natural attenuated and bioaugmented soil (Figs. 2e and 2f).

The non-significant increase in serum amylase concentrations observed in most of the rats fed with the *T. triangulare* shows minimal destruction of pancreatic cells. (Table 6 & Figs. 2g – 2i). The decrease in Packed Cell Volume (PCV) and haemoglobin concentration observed in this study (Table 6) may be attributed to the destruction of kidney and liver cells. This destruction may alter the release of erythropoietin thereby resulting to anaemia. Koolman and Roehm, (2005), reported a

Scientia Africana, Vol. 16 (No. 1), June 2017. Pp 67-81
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synergetic effect of erythropoietin and colony-stimulating factors (CSF) in the regulation of bone marrow stem cell differentiation. This differentiation ensures that erythrocyte precursor cells of the bone marrow are converted to erythrocytes. Apart from neurological and developmental abnormalities, exposure to high concentration of lead may result in toxic biochemical effects in humans and animals, which may affect the kidneys, gastrointestinal tract, joints and reproductive system and subsequently impairs haemoglobin synthesis (Karri *et al*., 2008). This may also contribute to the decrease in Packed Cell Volume (PCV) and Haemoglobin concentrations observed in rats fed with *T. triangulare* grown on the natural attenuated soil.

The high concentrations of heavy metals and hydrocarbons in this vegetable may have been responsible for the elevated concentrations of the toxicological markers of the treated animals. Sequel to this fact, it is obvious that concentration of toxicants in farm produce from crude oil polluted and remediated soils must be ascertained before such produce can be recommended for both human and animal consumption.

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