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SOME LIVER ENZYMES AND BIOMARKERS OF RABBITS FED ON GROUNDNUTS GROWN IN KUTCHALLI WASTE-PIT MATERIALS (BORNO STATE) NIGERIA.

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ABSTRACT

Kutchalli drilling waste-pit materials (kwpm) in Nigerian National Petroleum Corporation (NNPC) exploration sites in Borno State were evaluated for systemic toxicity to inhabitants (man, animasl and plants) via food chain. The biochemical parameters used to assess the liver (AST, ALT, AP, TB, CB, TP and Alb) of the laboratory rabbits were evaluated at intervals for 14 weeks using standard procedures.Results revealed disparity in the parameters in the control animals when compared with those fed with groundnut produced in kwpm (AST 12±0.6, ALT 12±0.5, ALK phos 26±2.0, TB 0.8±0.2, DB 0.6±0.2, TP 6.1±0.8, alb 3.4±0.5 and Glb 2.7±0.4 for control rabbits and (AST 36±2.0, ALT 25±2.2, Alk.phos 38±1.2, TB 2.9±0.4, DB 2.5±0.4, TP 5.6±1.1, Alb2.8±1.0 and Glb 2.8±0.t) It was significant at $P<0.05$. In the first one week, the parameters evaluated except TP, Alb and Glb were significantly elevated compared to the control $P<0.05$ in the following weeks 4, 8 and 14 in the groups (5 and 7). The livers of rabbits fed with groundnuts grown in kwpm showed abnormal patterns of liver biochemical parameters investigated in this Kutchalli area of Borno State, where this research was carried out. The inhabitants are mainly farmers, fishers and cattle rearers due to green vegetation. Therefore, there is need for proper waste disposal system probably via bioremediation to avoid harmful carry over to the generation of the inhabitants yet unborn.

KEY WORDS

Kutchalli; waste-pit materials. AST, ALT, AP

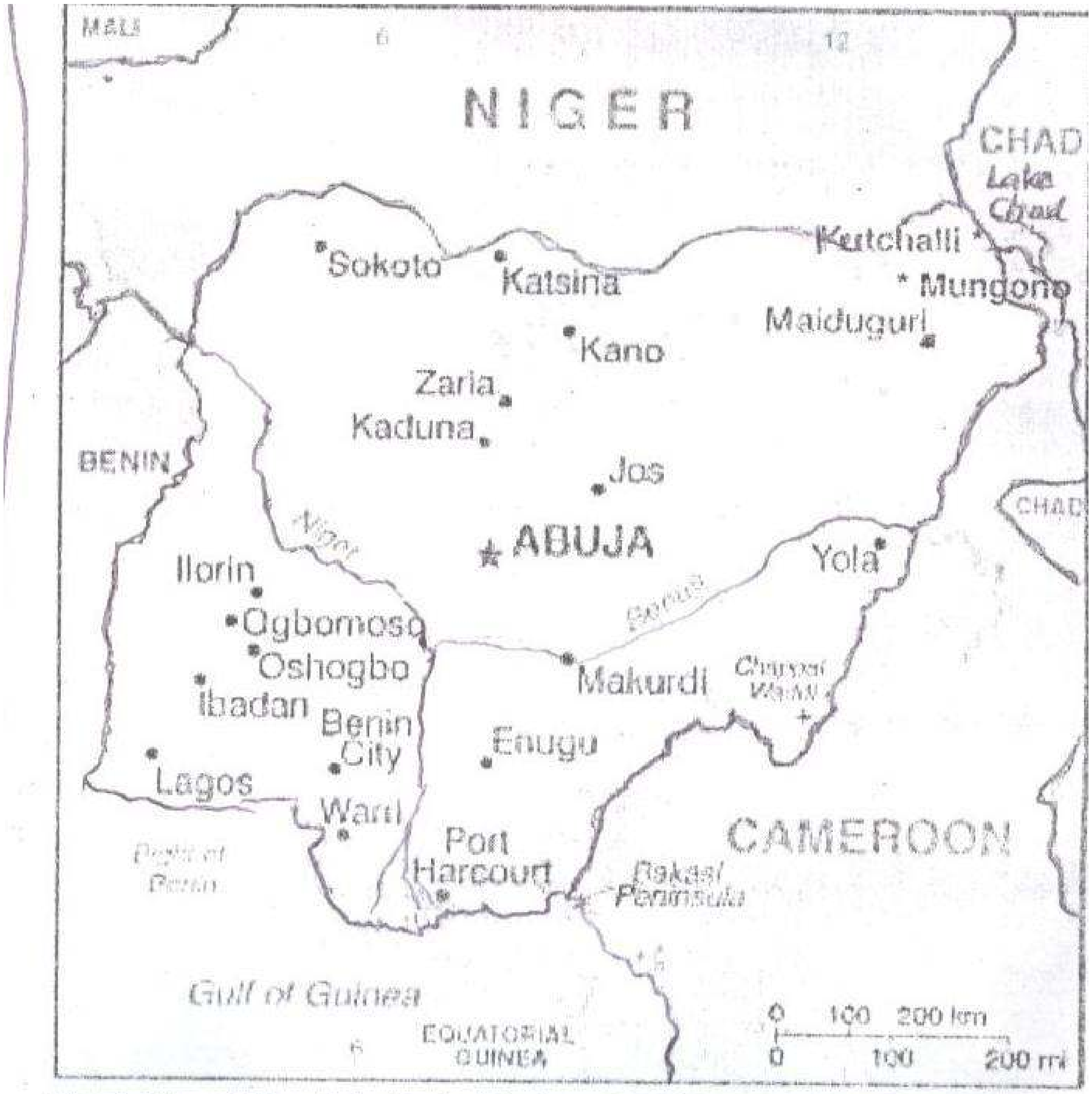

Fig. 1: Map of Nigeria indicating the location of Kutchalli between Maiduguri and Lake Chad. Source: Modified from http://www.cia.gov/cia/publications/factbook/geos/ni.html. date 29 June 2005

INTRODUCTION

Nigeria is major oil producing country with a production total of about 2.29 million barrels/day (OPEC bulletin, Jan. 2005) and 1.88 million barrels/day (OPEC. Dec. 2008).

The economy and industrial growth that Nigeria has enjoyed as a result of petroleum exploration is not without environmental cost. A realization that chemical contamination of the environment is extensive and significant has emerged, largely as a result of awareness and improved methods of evaluation (Anoliefo, 1991). Osuno (1989) reported that Nigeria had 20 drilling rigs, more than 4500 oil wells, 140 flow stations and production platforms. However, Ifeadi, *et al* (1985) reported that between 1960 and 1985, 1581 wells (45%) were drilled on land as against 1196 wells (36%) offshore and 748 (21%) in swamps. This shows that more oil wells are drilled on land than offshore.

Reduced growth may also be due to reduction in the level of available plants nutrients or toxic level of certain elements such as maganesse (Udo & Fayemi, 1975, Amadi, et *al* 1996) and interference with the uptake of soil water by the root system (Anoliefo&Vwioko 1995, Anoliefo&Isikhuemhen 2002).

Crude and refined petroleum, and oil field chemicals and emissions are highly complex chemical mixtures. Crude petroleum contains hundreds of compounds and the chemical composition varies between geologic formations (Coppock *et al*, 1995).

A lot of literatures abound on the percentages of pollution due to leakages arising from burst pipelines, high pressure or corrosion, tanks overflow, tank loading failure etc. (Otitoju *et al*,2007;Anoliefo *et al*,2006&Osuji,2006). The pollutants generated from these explorative procedures have been implicated in the cause of many biochemical and toxicological effects on plants, aquatic and terrestrial animals (Ovuru *et al*, 2004)

Oil spills on agricultural land generally lead to reduced plant growth, although, low levels of oil contaminations may stimulate growth. Udo and Fayemi (1975) suggested that reasons for reduced plant growth in oil contaminated soils may range from direct toxic effect of oil on plants (Amadi *et al* 1996), lack of germination due to lack of viable seeds (Osadolor, *et al* 2009), or interference with the water uptake by the root system due to unsatisfactory soil conditions such as presence of heavy metals (Anoliefo and Vwioko 1995, Atuanya 1987). Ekundayo and Benka Coker (1994) and Ifeadi *et al* (1985) reported that during routine drilling operations in the extraction of oil and gas, drilling mud cuttings and other waste materials generated in the process must be properly disposed. The paucity of information on the toxicity of waste pit materials to the soil prompted the carrying out of bioassays on selected abundantly occurring indigenous and economically important plants (*Arachis hypogaea*).

Although various research works have been done on crude oil contamination, very little or no work has actually been carried out on the impact waste pit materials emanating from crude oil exploration on plants and animals lives, (Osadolor *et al,* 2009)

The impact of waste-pit materials on the physiology of plants has rarely been studied and therefore, little or no information is available. It is therefore the aim of this research work to provide experimental evidence on the impact of oil prospecting on the physiology of groundnut and invariably on the animals in the environment via food chain,by looking at the impact on some liver enzymes and some other liver biomarkers. This is of particular interest because currently, waste –pit materials (WPM) generated from exploration activities are not properly disposed of and therefore usually get washed into various water bodies and viable farmlands through leaching sometimes.

MATERIALS AND METHODS

Plant (groundnut) Arachis hypogaea:

The plant material used was *A. hypogaea* which was confirmed by Kutchalli villagers as those grown within the village and its environs. The seeds were purchased at Kangarowa, being the only market near Kuchalli village. In one purchase, enough seeds (15Kg bag) was purchased for this study. Seeds were tested for viability by floatation technique before being put to use. Planting was done on triplicates. Germination was monitored till the time of harvest.

Animals; Only males of New Zealand inbred rabbits from the same colony were purchased from the rabbitory of the Edo state Ministry of Agriculture at the age of 2 weeks. They were dewormed by subcutaneous injection of 0.1ml invomec super. Also, anticoccoidal drug (Embazin) was administered along with vialyte during the period of acclimatization which lasted for 3 weeks in the laboratory.

Diets, Grower Mash and Feeding: One hundred and fifty (150) inbred rabbits were used for the experiment comprising of two stages; I and II.

Stage I: Sixty (60) rabbits were fed with groundnuts harvested from Kutchalli waste-pit material (KWPM) and control normal soil for 14 weeks. In this group, there were 5 rabbits/group. In this stage I, experimental feeding trials consisted of three groups- Group 1 (control), group 5 (KWPM) and group 7 (KWPM). Groups 2,3,4,6 and 8 planting did not favour growth, so no produce.

Each group had five rabbits of equal weight. The groundnut harvested from KWPM 5 and KWPM 7 were milled and used to compound the feed at a ratio of 1:20. This was done by taking 1g of milled groundnut seeds from

KWPM and adding 19g of growers mash to form the feeding mixture. This mixture was given to the respective groups at a dose of 100g/day for 14 weeks. The animals were sacrificed at intervals of 4 weeks each up to the 14th week. Biochemical fuctional status of the liver was evaluated using standard kits (Randox Laboratory, UK).

Biochemical Assays: The assays done to assess the fuctional status of the liver were the enzymes (Aspatate transaninase (AST), Alanine transaninase (ALT), Alkaline Phosphatase (AP), Others include Total Bilirubin (TB), Conjugated bilirubin (CB), Total Protein (TP), Albumin (Alb) and Globulin (Glb). All biochemical analysis were done using colorimetric kits supplied by Randox Laboratories, U K.

Statistical Analysis: The groups mean ± S.D was calculated for eachanalyte and significant difference betweenmeans evaluated using the student t-test, withP<0.05 considered as statistically significant

RESULTS AND DISCUSSIONS

The results reported in this study are the first part of results of a two stage experimental feeding trials carried out for 14 weeks on laboratory animal model.
The groundnuts harvested and fed to New Zealand rabbits for weeks 1, 4, 8 and 14 displayed the followings when their liver biochemical parameters were examined. The results are shown in the tables below.

Table 1
Assessment of Liver Function Status

GP	weeks	AST(u/l)	ALT(u/l)	AP(u/l)	TB mg/dl	CB mg/dl
1	1	12±0.6	12±0.5	26±2.0	0.8±0.2	0.6±0.2
	4	13±0.6	10±0.4	10±0.4	0.6±0.1	0.9±0.1
	8	10±0.4	10±0.5	22±2.0	0.4±0.1	0.3±0.1
	14	12±0.4	11±0.2	29±3.0	0.6±0.2	0.4±0.1
5	1	36±2.0	25±2.2	38±1.2	2.9±0.4	2.5±0.4
	4	30±2.0	27±1.6	42±2.4	4.2±0.5	2.7±0.4
	8	46±2.0	29±2.0	36±2.0	2.7±0.4	1.6±0.5
	14	32±2.0	27±2.2	39±2.1	3.3±0.3	2.3±0.5
7	1	38±2.2	27±2.0	39±2.2	6.2±0.6	4.5±0.6
	4	32±2.0	25±2.1	45±2.1	2.4±0.3	1.5±0.3
	8	28±2.0	20±2.0	40±2.0	2.2±0.3	1.3±0.5
	14	34±2.1	26±2.2	36±2.2	3.6±0.6	2.4±0.5

P<0.05

From table 1, comparing the results in group 1 (control) with those of groups 5 and 7 (KWPM), it is evidently obvious that there is statistical significant difference of at P<0.05 for the enzymes and Total Bilirubin and conjugated bilirubin.

Table 2
Assessment of Liver Function Status

GP	weeks	TP (g/dl)	Alb(g/dl)	Glb (mg/dl)	Alb/Glob
	1	6.1±0.8	3.4±0.5	2.7±0.4	1.26±0.4
1	4	6.0±1.0	3.2±0.6	2.8±0.5	1.14±0.5
	8	6.2±1.0	3.6±0.8	2.6±0.5	1.72±0.6
	14	6.1±0.8	3.4±0.6	2.7±0.5	1.26±0.6
	1	5.6±1.1	2.8±1.0	2.8±0.6	1.00±0.4
5	4	5.8±1.3	3.0±1.2	2.8±0.6	1.07±0.4
	8	5.6±1.0	3.1±1.3	2.8±0.5	1.24±0.5
	14	5.5±0.0	2.7±1.2	2.8±0.5	0.96±0.6
	1	5.8±1.0	2.8±1.0	3.0±0.5	0.93±0.6
7	4	6.0±1.0	3.1±1.2	2.9±0.6	1.06±0.6
	8	6.0±1.0	3.4±1.2	2.6±0.4	1.30±0.3
	14	5.7±1.2	3.1±1.0	2.6±0.5	1.19±0.5

$p>0.05$

From table 2, the biochemical analytes showed no significant difference when controls were compared with those of groups 5 and 7 KWPM

According to Kristensen and Horder (1996), pathological events resulting in enzyme release include shock, ischaemia, toxic and inflammatory conditions and mechanical and physical destruction of the cell. From the results in tables 1 and 2, it is evidently clear that there were membrabe destruction in the rabbits used due to toxic effects of the waste-pit materials. The levels of aspartate and alanine aminotransferase and alkaline phophatase were raised more than three times higher in animals of groups 5 and 7 compared with those of group 1 between 4-8 weeks respectively, while as from week 14, the enzymes showed slight reduction, which may be to response to acute toxicity and adaptability. Recent findings have shown that in cases of toxicity, which subsequently decreases cellular energy levels, free radicals are generated within a short period of time. These free radicals which are highly reactive molecules cause massive lipid peroxidation (Halliwell and Gutteridge 1989).

Also from tables 1 and 2, there was progressive increase in the level of Total bilirubin in groups 5 and 7 from the 4th eek of the feeding trials, which is an indication of increased rate of breakdown of red blood cells. The waste-pit materials soil was observed not to encourage plant growth, but when in combination with control soil, produce plants and probably other organisms and as such its disposal must be properly controlled. Orisakwe *et al* (2007), found that paint factory workers had reduced renal and liver functions due to the presence of heavy metals present in their blood, as found in groundnut used in this feeding trials. The impaired liver functions in this work could therefore be attributed to Kutchalli waste-pit materials used as can be seen in the table group 5 and 7 respectively. Some of the heavy metals present in KWPM can be magnify over a period of time (WHO 1989). This is particularly important because the effects not yet noticeable in the rabbits fed in the labouratory may come later, hance man needs to properly disposed off the waste-pit materials associated with petroleum exploration.

Conclusively, Kutchalli area of Borno state that was used in the study, the inhabitants are predominantly farmers, fishers, and cattle rearers due to its green vegetation. Therefore, it strongly suggest proper disposal system probably via bioremediation to avoid harmful carry effects to the generation of the inhabitants yet unborn

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