THE PREVALENCE OF *ANGIOSTRONGYLUS CANTONENSIS* (Chen) IN *ARCHACHATINA MARGINATA* (Swainson) IN ILE-IFE, NIGERIA

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Abstract

The study was carried out between April 1997 and May 1998 to provide information on the prevalence of helminth infection in the giant land snail, *A.marginata*. Specimens of *Archachatina marginata* collected from Ile-Ife were examined for infection with parasitic helminths. The length and weight of the snails were taken. The snails were caught in the wild and dissected and examined in the laboratory. Various organs which include the oesophagus, crop, stomach, intestine, hepatopancreas, lung, heart, rectum, and common hermaphroditic duct were examined for helminth parasites. Out of 218 specimens of *A. marginata* examined, 86.7% were infected with the larvae of a nematode *Angiostrongylus cantonensis*. The mean intensity of infection was 22.68 + 3.5. Seasonal variation in the intensity level of *A. cantonensis* during the year was more pronounced. This is probably due mainly to the moderately high rainfall recorded in September, which probably favour higher infestation of *A. marginata*. The prevalence however exhibited much less seasonal variation, although a trend towards higher value in September was also noticeable. This study has revealed that *A. marginata* serves as intermediate host of the rat lungworm *Angiostrongylus cantonensis*.

Keywords: *Archachatina marginata*, prevalence, *Angiostrongylus cantonensis*.

1. Introduction

Snails serve as sources of protein and a number of gastropod species are used for food by people in many parts of the world (Barnes, 1987). Snail meat is highly relished and considered a delicacy for the peasant population living in the rural rainforest zone of West Africa, especially in Nigeria. Snail meat have been reported to be high in protein (14.32%) (Mead, 1961) and also that some organs of the snails are rich in vitamins (Raffy and Ricant, 1943). Snails have also become an important source of income for some farmers who dwell in the rainforest areas.

Archachatina marginata, the African giant land snail is the largest among the terrestrial gastropods living in Africa (Segun, 1998). In south West of Nigeria, *A. marginata* is very important as food and this giant land snail forms an important source of animal protein and they are served as delicacies on the table (Segun, 1975). In addition to its nutritive value, it is also used for medicinal purposes. Agbelusi and Ejidike (1992) reported that the fluid from *A. marginata* could be used to cure headache and malaria. They further reported that the shells of the giant land snail when burnt to a colourless condition are ground and mixed with other ingredients could be used in preparing a concoction for pregnant women during labour or as fertility drug for women experiencing difficulty with conception. However, in spite of the wide usage of this snail species for food, information on the parasitic fauna of the giant land snail *A. marginata* is generally lacking. The present study was carried out to provide information on the prevalence of helminth infection in this snail species.

2. Materials and Methods

218 specimens of *A. marginata* examined in this study were bought from markets in and around Ile-Ife. The examination of the snail was carried out between April 1997 and May 1998. The snails were brought into the laboratory in well aerated cages and examined for helminth parasites. Each snail was given an identification number. The length and weight of the snails were taken. The snail was dissected and the various organs removed into saline solution (0.85%) in separate Petri-dishes and then examined for helminth parasites. The shell was broken and the heamolymph collected into a clean beaker was later examined for parasites under the microscope. Various organs which include the oesophagus, crop, stomach, intestine hepatopancreas, common hermaphroditic duct, lung (vascularised mantle), heart and the rectum were also examined for helminth parasites. Each organ was carefully opened by a longitudinal cut and the content expressed in saline (0.85%). The content was then examined on a dark background under the dissecting microscope.

The foot of the snail was chopped, macerated in saline (0.85%) and left for 24 hours at room temperature. The chopped pieces were then removed and the liquid centrifuged at 1,500rpm for 3mins. After decanting the supernatant, the sediment was collected and examined under a dissecting microscope for helminth parasites. The

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nematodes were counted and their numbers recorded. Some of the worms were fixed in A.F.A. (alcohol formo acetic), cleared and mounted in Lactophenol on clean glass slides and covered with cover slips. The parasite was later identified using the method of Bhaibulaya (1968).

3. Results

Among the 218 specimens of *A. marginata* investigated, 189 (86.7%) snails were found to be infected with a nematode larva. The nematode larva was identified to be *Angiostrongylus cantonensis*. A total of 4286 larvae were recovered representing mean of 22.68±3.5 larvae per infected snail. Table 1 shows the distribution and number of *A. cantonensis* recovered from various organs of *A. marginata.*

The specimens of *A. marginata* examined in this study varied in length from 10.4 – 17.0cm. As shown in Fig. 1a prevalence is high across different size classes starting from 50% in the 10.0cm size class. This shows snails acquire infection early in life. The rate rose to 100% in the next length class of 10.6cm after which the rate varied between 75% and 100%. However, this pattern in the intensity appears different. Smaller snails carry less worm burden with the intensity rising to a peak in the medium size snails of 12.6 - 13.0cm length size. The intensity fell progressively thereafter (Fig. 1b).

The prevalence and intensity of *A. cantonensis* in *A. marginata* in different months for a period of one year are shown in Table 2. The lowest prevalent rate was recorded in August 1997 while the highest was recorded in September 1997. The period when the prevalence was lowest corresponds to the period of lower rainfall while the period of higher prevalence corresponds to the period when the rainfall is moderately high (Fig. 2). The pattern of intensity was similar with the highest intensity recorded in September 1997 and the lowest in May 1997.

4. Discussion

This study has confirmed the findings of earlier workers that the snail *Archachatina marginata* is one of the intermediate hosts of *Angiostrongylus cantonensis* (Alicata, 1965). Mackerras and Sandars (1955) reported that the first-stage larvae of *A. cantonensis* may enter the body of the mollusc effectively either through the digestive tract or by active penetration of its cuticle. They further revealed that the first -stage larvae escape from the faeces of infected rats (definitive host) thereby contaminating the soil. From the present study, the foot of the snail was found to harbour the greatest number of the larvae of *A. cantonensis*. The preference for the foot of the snails by the larvae of *A. cantonensis* apparently suggests that the foot being in constant touch with the soil may be the entry route for the larvae. Field studies have shown clearly that the prevalence of infection with parasites varies among different age classes within snail population (Sturrock *et al.*, 1975). Anderson *et al.* (1982) reported that snail susceptibility to parasitic infection declines as host size and age increased and that this has important implications for the interpretation of age-prevalence patterns observed in natural snail population. Further they reported a decline in the prevalence of infection of *Biomphalaria glabrata* with *Schistosoma mansoni* in older age classes of snails (when compared with snails of intermediate age) in a variety of natural habitat. The case was however different in this study where the prevalence of *A. cantonensis* was higher in snails of larger sizes. However, it was observed that the mean intensity of *A. cantonensis* rose to a peak value in medium size snail decreasing in the large size snail. This is in agreement with the findings of Anderson and Gordon (1982) who also observed that the maximum mean parasite burden occurs in host of intermediary age classes (medium size), as a result of the more rapid death of heavily infected host. They further emphasized that the decrease in mean intensity in older hosts may be as a result of either changes in feeding habit, changes in habitat utilization or the development of acquired immunity.

There was a pronounced seasonal variation in the intensity of *A. cantonensis* during the middle of the rainy season with a maximum intensity recorded in September 1997. There was a higher infestation of *A. cantonensis* in *A. marginata* observed during this season. This may be due to the rain, which favours the survival of the nematode in the soil during this season. It was observed that lower infection rate was recorded in the middle of the dry season and this may be as a result of the dry condition. Such inhibits the survival of the larvae, and also during this period, the snails were in a state of aestivation. Dinnik and Dinnik (1963) reported that high proportion of *Lymnaea natalensis* were infected with *Fasciola gigantica* during the wet season but there was a decline in the infection rate during the dry season. They suggested that dry conditions are detrimental to the hatching of eggs of the parasite (which plays an important role of lowering the infection rate) during the dry season. The seasonal variation in the level of prevalence is much less pronounced than the seasonal variation in intensity level. Nevertheless a small peak was also observed in September 1997 which corresponded with the peak in intensity level. This minor increase may be also due in part to the higher rainfall recorded during this month and could also be as a result of the migratory activities of this snail which is at the peak during the rainy season.

5. Conclusion

It can therefore be concluded that this study has revealed that at Ile-Ife and its environs, the giant land snail *A. marginata* is parasitised by the nematode *Angiostrongylus cantonensis.* The discovery of this parasite however suggests that people eating this snail in this area stand the risk of contracting Angiostrongyliasis. *(Eosinophilic meningitis)*. However, since the disease is contracted by eating uncooked snail the chances of people contracting the infection in this area are thereby reduced because snails are well cooked before they are consumed. Other sources of infection as reported include eating uncooked vegetables, prawns and also drinking water from open sources (Alicata, 1991). These findings therefore suggest that to avoid infection with this parasite snails, prawns and crabs should be cooked properly before they are consumed. People should avoid drinking

Table 1: The distribution and number of *A. cantonensis* recovered from various organs of *A. marginata*

Organs	Number of worm larvae recovered
Foot	2190
Lung	1835
Rectum	209
Intestine	37
Heart	15
TOTAL	4286

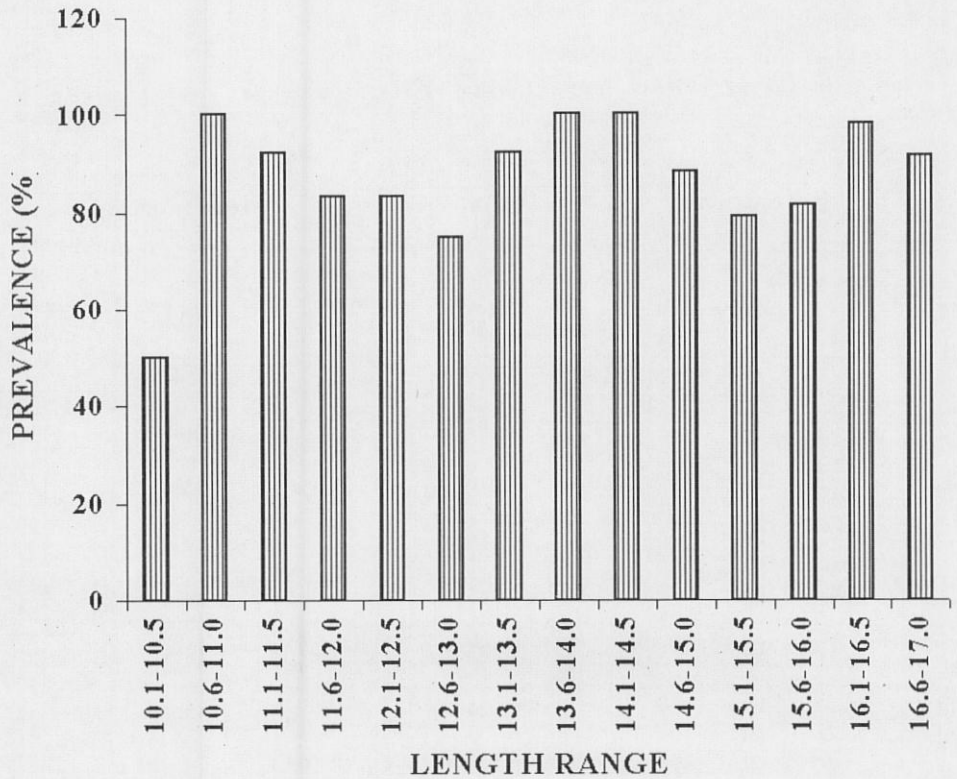

Fig. 1a: Prevalence of *Angiostrongylus cantonensis* infection relative to host length in the snail *Archachatina marginata*

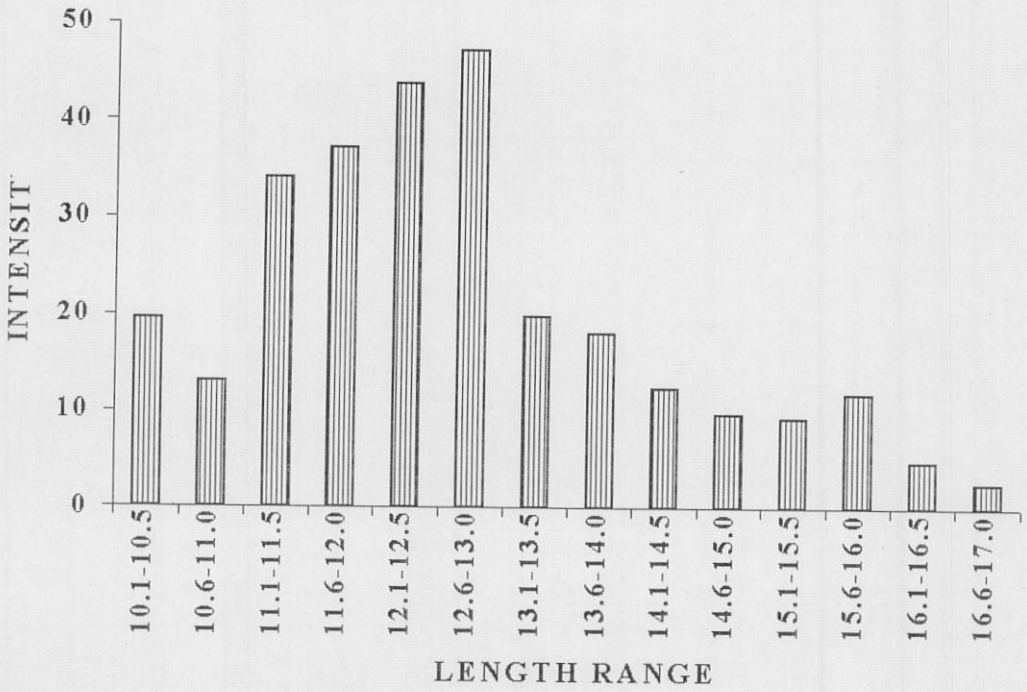

Fig. 1b: Intensity of infection with *Angiostrongylus cantonensis* relative to host length in the snail *Archachatina marginata*

Table 2: Seasonal variation in the prevalence and intensity of *Angiostrongylus cantonensis* in *Archachatina marginata*

Months	No. Examined	% Infected	Mean Intensity
MAY '97	15	80.0	5.7
JUN. '97	20	85.0	10.0
JUL. '97	20	90.0	7.9
AUG. '97	23	78.3	9.4
SEP. '97	10	100.0	53.6
OCT. '97	23	86.9	22.6
NOV. '97	16	93.7	24.4
DEC. '97	18	83.3	37.5.
JAN. '98	18	94.4	45.4
FEB. '98	20	90.0	26.3
MAR. '98	15	86.7	26.4
APR. '98	20	80.0	14.2
TOTAL	218	86.7	22.7

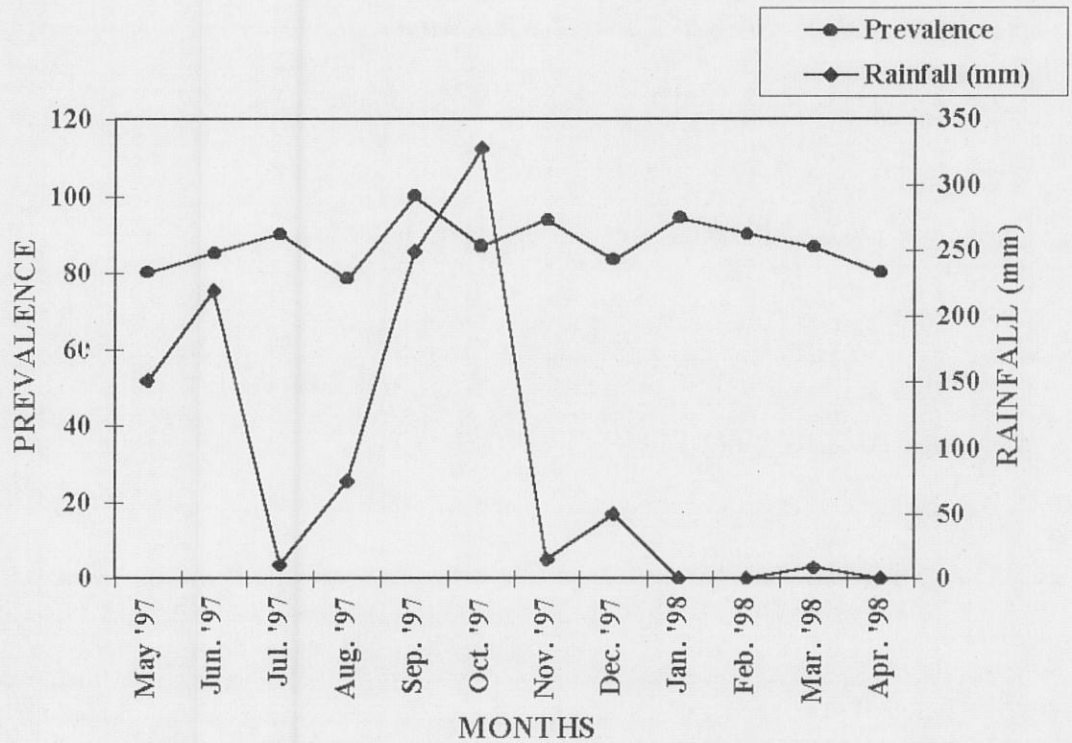

Fig.2: The Prevalence and total rainfall against the different seasons of the year.

water from open sources and finally children should be protected from playing with snails.

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FIELD CONTROL OF PERIDOMESTIC MOSQUITOES OF MEDICAL IMPORTANCE WITH EXTRACTS OF *Petiveria alliacea L.*

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Abstract

The toxicity of the aqueous extract and turbid distillate of roots of *Petiveria alliacea L.* to larvae of *Aedes aegypti. Culex pipiens fatigans* and *Anopheles gambiae* were investigated in the laboratory and in the field. Both extracts were larvicidal to second instars. In laboratory tests, a 10% (v/v) aqueous extract caused 100% mortality in all three species. The turbid distillate was less potent than the aqueous extract under field conditions. The significance of the present findings for mosquito control using locally – an available natural resource is discussed.

Keywords: *Petiveria alliacea*, larvicidal, *Aedes aegypti*, *Culex pipens fatigans*, *Anopheles gambiae*.

1. Introduction

Many plants in the family Phytollacaceae are known to be toxic to mammals when consumed fresh. The toxicity of the Australian species *Phytolacca octandra* to cattle and pigs has been reported by Duncan, (1962). *P. Americana* gives rise to haemorrhagic gastritis, nausea, depression and prostration (Kingsburry, 1964) *P. dodecandra* is lethal to sheep and cattle (Mugera, 1970).

The guinea hen weed *Petiveria alliacea L.* (Phytollacaceae), common in central America (Clarke and Clarke, 1975), is planted in residential areas in Southwestern Nigeria to repel snakes, scorpions and insects (Olaifa and Akingbohungbe, 1987). The plant imparts a garlic-like taint to milk, contains a volatile carbamate-like substance (Blohm, 1962; Ruiz, 1972), antimicrobial trisulphide (Szozcepanski *et al.*, 1972) and has insecticidal properties (Olaifa and Akingbohungbe, 1987). The aqueous extract and turbid distillate have been found to deter oviposition by gravid females of *Anopheles gambiae, Aedes aegypti* and *Culex pipiens fatigans* (Adebayo, 1992).

The current study was undertaken to further explore the bioactive properties of the aqueous extract and turbid distillate of the plant against three species of mosquitoes that are endemic in Nigeria: *Anopheles gambiae* (vector of malaria parasites), *Aedes aegypti* (vector of yellow fever viruses) and *C. pipiens fatigans* the intermediate host of filaria worms *Wuchereria bancrofti* and *Dirofilaria immitis*.

2. Materials and Methods

Preparation of Extracts

Fresh roots of *P. alliacea* were obtained from the medicinal plant reserve of the Department of Agronomy, Ladoke Akintola University of Technology, Ogbomoso, Nigeria. They were chopped into small pieces and placed in a round bottom flask fitted to a glass extractor. Distilled water was added at ratio 1: 2 (w/v) (weight of roots: volume of water) and heated to boiling on a heating mantle. The first 60ml of the condensed steam (turbid distillate) were collected and stored at 4°C until needed. This served as a stock solution which was later diluted to different concentrations for the bioassays.

In another set up, fresh roots of *P. alliacea* were cut into small pieces and distilled water added at a 1:2 (w/v) ratio. The water plus the pieces of roots were heated for 2hrs at 100°C and then cooled to room temperature. The liquid was later decanted and kept refrigerated until use (i.e. 24h later). This served as a stock solution of the aqueous extract and was diluted in subsequent bioassays.

Culture of insects

Individual colonies of each mosquito species, i.e. *A. aegypti, A. gambiae and C. pipiens fatigans* were obtained as larvae from the malaria and vector control unit of the National Institute for Medical Research, Yaba, Lagos, Nigeria. These larvae were fed with ground dog biscuits until they became pupae and later adults. Both sexes were raised together in the same cage and allowed to mate freely. Adult female mosquitoes have hypodermic mouthparts which enable them to pierce the skin and suck the blood of mammals, birds, reptiles, and other arthropods while adult males which have reduced mouthparts feed on nectar and water. In this study, females were allowed to take a blood meal every morning from a restrained chicken whose feathers had been removed and males were fed on a 10% sugar solution according to methods described earlier (Adebayo and Olaifa, 1994). Larvae hatched from eggs laid, were also fed as described earlier and used for bioassay at the second instar stage.

Bioassay

Laboratory Test: The larvicidal activity of each extract (aqueous extract or turbid distillate) was tested by preparing the following range of dilutions from the respective stock solutions: 0, 1, 3, 5, 7.5 and 10% (v/v) using distilled water to give a final test volume of 200ml in a 250ml beaker. Fifty second instar larvae of *C. pipiens fatigans, A. aegypti or A. gambiae* were placed in each beaker and fed with dog

+ corresponding author

biscuits. Each test was replicated three times. The test containers were placed on tables and held under ambient conditions (25 –30°C, (Relative humidity) 70%). Larval mortality was assessed 12 and 24h after treatment and every 24h thereafter for 5 days (120 h). Mortality data by treatment were corrected according to the control mortality using Abbott's formula (Abbott, 1925) and then subjected to probit analysis (SAS Institute, 1985) and the means ranked using Duncan's multiple range test (Duncan, 1955).

Larvicidal activity of the aqueous extract of P. alliaceae in the field

Tests were conducted in concrete tanks (96 x 60x 50 cm) each containing 30 liters of aqueous extract at the following concentrations: 1, 3, 5 and 10% (v/v). Untreated water served as the control. One hundred and thirty 2nd instar *A. Aegypti* were introduced into each pond and fed with dog biscuit. *C. pipiens fatigans and A. gambiae* larvae were similarly exposed to the extracts. All treatments were replicated three times.

Mosquito net was spread over each pond to prevent emerging adults from escaping and to shield the ponds from feral mosquitoes and predators. The effects of the aqueous extracts on the test mosquito larvae were evaluated by counting the number of adults emerging from each pond from 3 to 11 days after treatment (DAT) at 24 h intervals. All these were done without adjusting water level. Dead adults on the surface of the ponds were also counted as emerged adults. Percentage emergence was determined according to Rathburn *et al.* (1980) as follows:

$$\%(Emergence) = \frac{CS - DA}{CS + PE + DP}$$

where,

CS = Cast pupal skin

DA = Dead adults on water surface

PE = Partially emerged adults

DP = Dead pupae

The % control was calculated by comparison with the 'check' treatment with mortality levels adjusted accordingly, using Abbott's (1925) formula.

Test of the larvicidal activity of the turbid distillate of *P. alliacea*

The experiment was carried out in 15 liter plastic bowls, each containing 4 liters of the turbid distillate at the following concentrations: 0, 1, 3, and 7.5 and 10% (v/v). Ninety, 2nd instar C. *pipiens fatigans* were introduced into each bowl. Each bowl was placed inside a cage (to prevent the escape of emerged adults and to shield the bowl from feral mosquitoes) and set out doors. The treatments were similarly evaluated against *A. aegypti* and *A. gambiae* larvae. Each treatment was replicated 3 times during a single experiment. The percentage emergence and corrected percent control were determined as outlined above.

4. Results

Laboratory assays

A. egypti, C. pipiens fatigans and *A. gambiae* exhibited different levels of susceptibility to the extracts of *P. alliacea* in the laboratory (Fig. 1). After 96h, *A. aegypti* was the most susceptible to the aqueous extract with an LC_{50} of 4.5% (v/v) followed by *C. pipens fatigans* with an LC_{50} 4. 7%. *A. gambiae* was the least susceptible with an LC_{50} of 5.5 %. However, a dose of 10% caused 100% mortality in all of the test larvae.

Experiments with different doses of turbid distillate (Fig 2), revealed that, the LC_{50} for *A. aegypti* and *C. pipiens fatigans* was 4.5% and 5.9% respectively, and 7.1% for *A. gambiae.* A 10% (v/v) concentration of turbid distillate was required to cause 100% mortality in *An. Gambiae* after 96h (Fig. 2). Suggesting that this species is less sensitive to the distillate and that the aqueous extract is more potent than the turbid distillate.

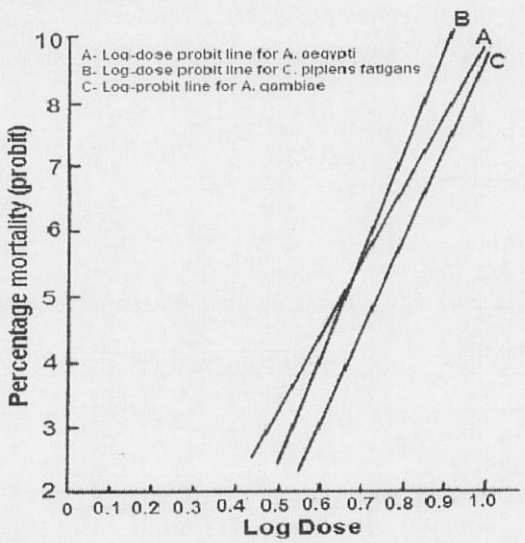

Figure 1: The dose-mortality response of second instar *A. aegypti, C. pipiens fatigans* and *A. gambiae* exposed to the extract of *P. alliacea.*

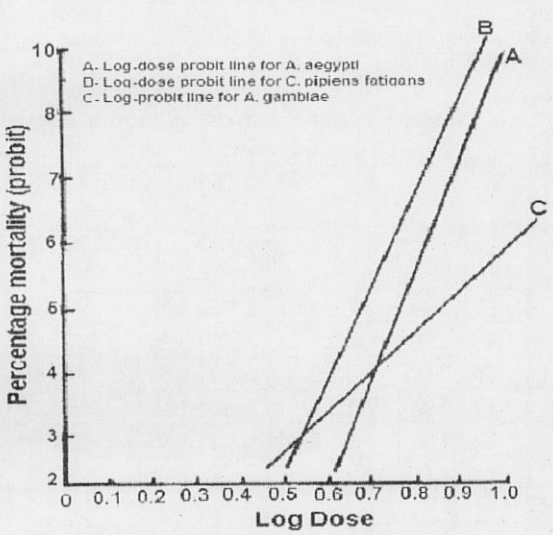

Figure 2: The dose-mortality response of second instar *A. aegypti, C. pipiens fatigans* and *A. gambiae* 96hr after exposure to turbid distillate extract of *P. alliacea.*

Field activity of aqueous extracts and turbid distillate

In all the field tests, adult emergence in all the ponds occurred mostly between 7 and 8 DAT. However, in the ponds containing 7.5% and 10% (v/v) aqueous extracts, foam and a thin layer of colloidal substances covered the surface of the ponds from 3 DAT. No *A. aegypti* adults emerged from ponds containing 10% (v/v) extract (Table1). Larvae and pupae sampled from the ponds 48h after exposure revealed that 2nd instars died while molting to the 3rd instars or during pupal – adult ecdysis. A dose of 7.5 % (v/v), which gave 100% control in the laboratory, caused only 52.8% mortality in the field. Results in Table 2 show that, compared to the untreated check, the 5, 7.5 and 10% (v/v) aqueous extracts reduced *C. Pipiens fatigans* adult emergence by 62.7, 73.1 and 100% respectively.

When larvae of *A. gambiae* were exposed to the aqueous extract or turbid distillate of *P. alliacea* in the field, the aqueous extract provided superior levels of control (Table 3). A 10% (v/v) extract caused 95. 5 % mortality, compared to 47.9% for the turbid distillate. All of the larvae that pupated emerged as adults. Larval mortality caused by the distillate against *C. pipens fatigans and A. aegypti* was not significantly different from the untreated check at the doses tested.

5. Discussion

The results clearly demonstrate that both the aqueous extracts and the turbid distillate of *P alliacea* are toxic to larvae of *A. aegypti, C. pipiens fatigans* and *A. gambiae*. The results of the study corroborated earlier findings on the toxicity of the turbid distillate of *P. alliaceae to C. albiventris* (Edwards) larvae (Olaifa, *unpublished*). Ruiz (1972) reported that the plant contains a volatile carbamate-like substance, which may be the insecticidal component. Carbamates such as propoxur (2–1sopropoxyphenyl methyl carbamate) are popular insecticides for controlling mosquitoes and other household pests.

In the current investigations, the 10% (v/v) aqueous extracts provided operationally feasible levels of control (causing 100% mortality) in all of the tests. The differential response to the turbid distillate shown by *A. aegypti, Cx. pipens fatigans and An. gambiae* could be due to differences in larval physiology that are yet to be identified.

The toxins present in the extracts appear to have neuromuscular activity, as evidenced by the sluggishness and coiling of the larvae following exposure to the preparations. The general symptoms of carbamates in insects are primarily those of poisoning of the central nervous system, since the

Table 1: Field tests of an aqueous extract of *Petiveria alliacea* as a mosquito larvicide against second instar *Aedes aegypti*

	EMERGENCE OF ADULTS/PER DAY (± SD).								
Conc. (%)	1-5	6	7	8	9	10	11	Total Emergence	Corrected Control %
0	0	6.3 ± 1a	63 ± 2.6a	50 ± 1.5a	3 ± 2.1a	3 ± 1.0a	0	125a	-
1	0	3 ± 1ab	59 ± 2.0a	47 ± 2.6a	3 ± 1.5a	0 ± 00b	0	112a	10.4
3	0	5 ± 1.6a	65 ±15.5a	7 ± 2.1c	1 ± 1.0b	3 ± 0.5a	0	79b	36.8
5	0	1 ± 1b	20 ± 2.1b	36 ± 5.0b	1 ± 1.0b	0. 00	0	58c	53.7
7.5	0	0b	12 ± 2.0b	40 ± 2.0b	3 ± 1.0a	0. 00	0	59c	52.8
10	0	0b	0	0	0	-	0	0	100

(Initial larval population 130)
Within columns, means followed by a common letter are not significantly different at $P = 0.05$ Duncan's Multiple Range Test.

Table 2: Field tests of an aqueous extract of *Petiveria alliacea* as a larvicide against second instar *Culex pipiens fatigans*

	EMERGENCE OF ADULTS/PER DAY (± SD).								
Conc. (%)	1-5	6	7	8	9	10	11	Total Emergence	Corrected Control %
0	0	59 ± 5.0a	51 ± 2.6a	8± 1.5b	10a	5 ± 1a	1±1	134a	-
1	0	48 ± 2.0b	32 ± 2c	26± 3.3a	0c	1 ±1b	0	107b	20.1
3	0	26 ± 2.0c	48±2.0b	1± 1.5c	3 ± 1b	0b	1±1	85c	36.6
5	0	19 ± 5.0d	21 ± 2.5d	10± 1.5b	0c	0b	0	50d	62.7
7.5	0	16 ± 4.8d	18 ± 2.0d	1± 1.5b	0c	0b	0	36e	73.1
10	0	0e	0e	0c	0c	-	0	0.0	100

(Initial larval population 130)
Within columns, means followed by a common letter are not significantly different at $P = 0.05$ Duncan's Multiple Range Test.

Table 3: Evaluation of the larvicidal activities of different extracts of *Petiveria alliacea* against second instar *Anopheles gambiae*

	Aqueous extract		Turbid distillate	
Concentration	% Emergence	Corrected	% Emergence	Corrected
0	97.3	-	98.1	-
1	89.0	8.5	90.7	7.54
3	80.4	17.4	84.6	13.75
5	70.1	27.9	77.2	21.30
7.5	36.7	62.3	51.1	47.91
10	4.0	95.9	47.1	51.98
	LSD=25.9	LSD=26.3	LSD=15.8	LSD=16.1

insect neuromuscular junction is not cholinergic. Nerve poisoning, digestive disturbances, muscular atrophy and glomerulonephritis have been reported when cattle were fed with the plant (Clarke and Clarke, 1975). Further studies are needed to elucidate the specific chemical nature of the bioactive compound present in the plant.

The foam and colloidal substances which formed and covered the surface of the treated ponds 3 DAT with 7.5 and 10% (v/v) aqueous extracts in both laboratory and field trials might have contributed to larval mortality. Levy *et al.* (1980) pointed out that such substances can modify the physical properties of the water surface in ways which interfere with the normal behaviour and development of mosquito larvae and pupae and also with the emergence of adults. The surface film formed by the aqueous extract significantly reduced the surface tension of the water and subsequently killed larvae and pupae by inhibiting proper orientation at the air – water interface and/or by increasing the wetting of the tracheal structures. It seems unlikely that both the aqueous extracts and turbid distillate of *P. alliacea* exerted any juvenoid effects as larval development rate was not significantly different in the treated and untreated ponds.

Under field conditions, the turbid distillate was only marginally active compared to the mosquitocidal effect shown in laboratory bioassays. The reduction in activity may have been caused by photodegradation of the active ingredients, a common phenomenon in the botanicals. Similarly the potency of the aqueous extract was lower in the field than the laboratory, 5% and 7.5% (v/v) concentrations caused 100% mortality in most bioassays whereas only the 10% (v/v) preparation effected 100% mortality under field conditions. The lower activity may have been due to the photo – instability of the extracts but in addition many biotic and abiotic factors can influence activity in the field. Further studies are necessary to identify the most important of these in order to refine formulations that will maintain the larvicidal activity of the active compound after application, or keep the active ingredient in the feeding zones of the mosquito larvae.

Both the aqueous extract and turbid distillate of *P. alliacea* are inexpensive, easy to prepare, handle and apply. Furthermore these extract are excellent oviposition deterrents to gravid females of *C. pipens fatigans* and *A. aegypti* at a 10% (v/v) concentration (Adebayo, 1992). Extracts of indigenous plant have significant potential for use in mosquito abatement programmes in the tropics, where they will not only be more economical but more environmentally acceptable than synthetic insecticides. Extracts of *P. alliacea* may thus be one of the first options in response to Gratz's (1985) demand for mosquito control measures that can be "undertaken by individuals and families in and around their homes".

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THE INSECTICIDAL ACTIVITY OF EXTRACTS AND OILS OF SOME TROPICAL PLANTS AGAINST THE YAM MOTH *EUZOPHERODES VAPIDELLA* MANN (LEPIDOPTERA: PYRALIDAE)

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Abstract

The ethanolic extracts and oils of eight tropical plants were evaluated under laboratory conditions for their relative toxicities to the eggs and larvae of the yam moth, *Euzopherodes vapidella Mann.*

Contact toxicity of the extract and oil on the eggs and larvae of the yam moth was tested by treating 15g of *Dioscorea alata* measuring (4cmx4cmx1.5cm) with 0.5ml and 0.1ml of ethanol and oil extract in separate Petri dishes. This represents 3.3 and 0.6% concentration respectively. Each treatment was replicated three times. Each Petri dish containing the treated yam slice was infested with freshly laid eggs of *E. vapidella* on one hand and 15 third instar larvae on the other hand. Petri dishes containing the eggs and the larvae were left inside the insect-breeding cage until adult emergence.

Ethanolic extract of *Aframonmum melegueta Schum* and *Thonn*; *Eugenia aromatica Baillion*; and *Zingiber officinales Rosco* were able to inhibit egg hatch and adult emergence. *E. aromatica* and *Z. officinales* were also able to prevent adult emergence when treated 4 days before eggs were introduced. The effect of ethanolic extract on larvae of *E. vapidella* at an application rate of 0.6 and 3.3% were low since there was no significant difference ($P > 0.05$) when compared with the control. The effect of oil extract was greater in the slices protected with *Monodora tenuifolia* and there was no egg hatch and adult emergence. The oil extracts from *Arachis hypogaea* and *Elaeis guineensis* had 20.0% and 13.3% adult emergence and there were significant differences ($P < 0.05$) when compared with the control which had 66.7% adult emergence. The oil can be applied on bruises or wound in yam tubers.

Keywords: Ethanolic extract, tropical plants, *Euzopherodes vapidella,* toxicity, hatchability

1. Introduction

Yams are members of the genus *Dioscorea*, which produce bulbils, tubers, or rhizomes that are of economic importance. Yams are staple food for millions of people in the tropical region of the world including those of West Africa being indeed the preferred source of carbohydrate among many ethnic groups (Ashamo, 2000). In spite of the great economic impotence of this food item in the diet of the people, 20-30% are lost during storage (FAO, 1985). Storage losses can result from physical, physiological or pathological factors or a combination of all three (Booth, 1974). *Euzopherodes vapidella* Mann is one of the moths which attack stored yam tubers. Dina (1976) studied the biology of *E. vapidella* and reported that only mechanically damaged tubers were susceptible to attack by the moth.

Chemical method still remains the most effective means of controlling both field and stored products pests. Despite the dramatic successes recorded in the control of insect pests by the use of insecticides, it has a number of disadvantages. For example, high toxicity in mammalian tissues, high level of persistence in the environment, health hazards, residual effect of synthetic insecticides on mammals, adverse effect on non-target organisms and pest resistance among pests (Sighamony *et al.*, 1986). These have necessitated the use of other control measures which have little or no negative impact on the environment.

One way is to replace the insecticides with compounds, which occur naturally in plants (Olaifa *et al.*, 1987). This involves the use of plant products in the form of powders, extracts, edible and non-edible vegetable oils and essential oils (Adedire and Lajide, 1999). Many reports on toxicity and deterrent activity of plant products on pests of stored products exist only for beetle pests (Lale, 1992; Adedire and Lajide, 1999) there is little or no report on the use of plant materials to control moths especially those affecting stored yam tubers.

This work investigated the toxicity of ethanolic and oil extracts of some tropical plants on the eggs and larvae of the yam moth, *E. vapidella.*

2. Materials and Methods

Culture of *Euzopherodes vapidella*

The initial source of culture was obtained from infested water yam tubers, *Dioscorea alata L.* collected from markets and farms. Signs of moth infestation included pres-

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ence of black granules of larval faecal matter held together by silken threads and the presence of empty pupal cases on surfaces of the tubers. These infested tubers were kept in Kilner jars. The openings of the Kilner jars were covered with muslin cloth held in place by rubber bands to prevent the escape of emerged adult moths. The Kilner jars were then kept inside insect breeding cages made of wood and ½ inch wire mesh. A culture of *Euzopherodes vapidella* was established and maintained with fresh water yam tubers as old ones deteriorated. The culture and the experiments were kept at a temperature and relative humidity of 26±2°C and 75±5% respectively.

Effect of Ethanolic Extracts and Oil Extracts on Development of *E. vapidella* eggs and larvae

The ethanolic extracts of *P. guineense* (seed), *A. melegueta* (seed), *Z. officinales* (corm), *E. aromatica* (fruit) and *H. sauvolens* (leaves) were obtained by weighing 20g of each powdered material into round bottom flasks. The plant parts had previously been cleaned, sundried and then pulverised into fine powder using electric blender. They were soaked in 100ml of absolute ethanol for 24 hr. and then boiled at 60°C for 30 minutes on a heating mantle (Adedire and Lajide, 1999). The solution was then filtered through Whatman No 12 filter paper. The filterate was kept in a brown bottle until needed. Table 1 shows the list of plants evaluated for their insecticidal activity.

Oil was extracted from the seeds of the plants using soxhlet extractor and tested as insecticides against the eggs and larvae of *E. vapidella*. The plants whose seeds were used are *A. hypogaea*, *E. guineensis* and *M. tenuifolia*. The different seeds were cleaned, sun dried and pulverized into fine powder using electric blender. 20g of each powdered materials was weighed into a thimble and extracted with ethanol in a soxhlet extractor. The extraction was carried out for about four hours. Thereafter, the thimble was removed from the units and the ethanol was recovered by redistilling the content of the soxhlet extractor at 40-60°C. The resulting extract was air dried in order to remove traces of the solvent. A slice of yam (*D. alata*) measuring (4cm x4cmx1.5cm) and weighing 15g was kept in individual Petri dishes. 0. 5ml of ethanolic extract and oil extract (representing 3.3%) from different materials was spread evenly on top of the yam slice. Freshly laid eggs (30 eggs) of *E. vapidella* were introduced on top of each of the treated yam slices. The development was observed from the treated slice and the number of adults emerging was verified. In another experiment, thirty freshly laid eggs of *E. vapidella* were placed on top of the slice of *D. alata* 4 days after treatment with ethanol and oil extracts. Observation was made daily until adult emergence and the number reaching adult stage was recorded. In the same manner as described above, fifteen third instar larvae were introduced on top of treated yam slice. The number of larvae reaching adult stage was recorded. Each experiment was replicated thrice. A control experiment without ethanol and oil extract was also set up in triplicate.

Analysis of Data

All the data were subjected to analysis of variance and where significant differences existed, treatment means were compared at 0.05 significant level using the New Duncan's Multiple Range Test (Zar, 1984).

3. Results

Effect of ethanolic extract on egg survival

Table 2 shows the effect of ethanolic extract on survival of *E. vapidella* egg treated and left for 20 minutes before eggs were introduced. The extract was effective in inhibiting hatchability and adult emergence. No eggs hatched and consequently no adult emerged from those slices treated with *A. melegueta, E. aromatica* and *Z. officinales*. However, 36.7% eggs hatched in slices treated with *H. sauvolens* and *P. guineense* and 80% for control. There was no significant difference $P>0.05$ in percentage adult emergence from the slices treated with *H. sauvolens* (30.0%) and *P. guineense* (33.3%) but significantly different $P<0.05$ from the control (70.0%).

The effect of ethanolic extract on development of *E. vapidella* eggs treated and left for 4 days before eggs were introduced is shown in Table 3. Ethanolic extract of *E. aromatica* and *Z. officinales* prevented adult emergence. This shows that they were still effective in preventing adult emergence when applied 4 days before eggs were introduced. The other extracts were not effective since there was no significant difference $P>0.05$ when compared with the control.

Effect of Ethanolic Extract on larva survival

Table 4 shows the effect of ethanolic extract on third instar larvae of *E. vapidella*. There was no significant difference ($P > 0.05$) in adult emergence in the treated samples and control.

Effect of oil extract on egg survival

Table 5 shows the effect of oil extract on eggs of *E. vapidella*. All the oil extract were effective in preventing adult emergence of the moth since there was significant different ($P < 0.05$) when compared with the control. *M. tenuifolia* oil was the most effective because it inhibited hatchability and emergence of the adult moth.

Effect of oil extract on larva survival

When 15 third instar larvae of *E. vpidella* were introduced on the slice of yam treated with various oil extracts, the oils had significant effect on the third larvae when compared with control. The most effective of all the larvicides was *Arachis hypogea* with 26.7% adult emergence. However, there was significant difference ($P < 0.05$) in the mean number of adult emergence when compared with the control which had 100% adult emergence (Table 6).

4. Discussion

In this study, the extract of *A. melegueta*, *E. aromatica* and *Z. officinale* showed the greatest insecticidal potential on egg hatchability and adult emergence in *E. vapidella* when eggs are introduced about 20minutes after application of extracts to the yam slices. There were significant differences ($P<0.05$) when compared with the control. However, intro-

Table 1: Plants evaluated for their insecticidal activity.

Scientific Name	Common name	Family	Parts used
Arachis hypogeae L	Groundnut	Papilionaceae	Seed
Elaeis guineensis Jacq.	Oil palm kernel	Palmae	Seed
Piper guineense Schum and Thorn.	Black pepper	Piperaceae	Seed
Aframomum melegueta Schum, Roscoe	Alligator pepper	Zingiberaceae	Seed
Monodora tenuifolia Benth.	Awoo ***	Annonaceae	Seed oil
Eugenia aromatica	Kanafuru **	Myrtaceae	Fruit
Zingiber officinales Roscoe.	Ginger	Zingiberaceae	Corm
Hyptis sauvolens Poit	Curry leaves	Labiatae	Leaves

** Hausa Name
*** Yoruba Name

Table 2: Percentage hatchability and percentage adult emergence of eggs raised on yam slices which were treated with 3.3% ethanolic extracts and left for 20mins before the eggs were introduced

Plant Ethanol Extract	No of Eggs Introduced	% egg Hatchability Mean ± SD	% Adult Emergence Mean ± SD
Aframomum melegueta	30	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}
Eugenia aromatica	30	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}
Hyptis sauvolens	30	36.7 ± 0.5^{b}	30.0 ± 0.5^{b}
Piper guineense	30	36.7 ± 0.5^{b}	33.3 ± 0.3^{b}
Zingiber officinales	30	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}
Control	30	80.0 ± 1.0^{c}	70.0 ± 1.0^{c}

Means followed by the same letter are not significantly different P > 0.05 from each other using New Duncan's Multiple Range Tests.

Table 3: Percentage and percentage adult emergence of eggs raised on yam slices which were treated with 3.3% ethanolic extracts and left for 4 days before eggs were introduced

Plant Ethanol Extract	No of Eggs Introduced	% egg Hatchability Mean ± SD	% Adult Emergence Mean ± SD
Aframomum melegueta	30	46.7 ± 1.1^{b}	30.0 ± 1.1^{ab}
Eugenia aromatica	30	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}
Hyptis sauvolens	30	66.7 ± 2.1^{b}	36.7 ± 1.0^{ab}
Piper guineense	30	60.0 ± 2.0^{b}	33.3 ± 1.0^{ab}
Zingiber officinales	30	6.6 ± 0.2^{a}	0.0 ± 0.0^{a}
Control	30	73.3 ± 1.5^{b}	53.3 ± 1.1^{b}

Means followed by the same letter are not significantly different P > 0.05 from each other using New Duncan's Multiple Range Tests.

Table 4: Effects of ethanolic extract on development of larvae.

Plant Extract	No of Larval Introduced	% Adult Emergence Mean ± SD 0.6%	% Adult Emergence Mean ± SD 3.3%
Aframomum melegueta	15	93.3 ± 1.1^{a}	53.3 ± 1.1^{a}
Eugenia aromatica	15	66.7 ± 1.1^{a}	53.3 ± 1.1^{a}
Hyptis sauvolens	15	86.7 ± 1.3^{a}	60.0 ± 1.6^{a}
Piper guineense	15	73.3 ± 1.5^{a}	46.7 ± 0.5^{a}
Zingiber officinales	15	86.7 ± 1.3^{a}	46.7 ± 1.1^{a}
Control	15	93.3 ± 1.1^{a}	73.3 ± 1.1^{a}

Means followed by the same letter are not significantly different P > 0.05 from each other using New Duncan's Multiple Range Tests.

Table 5: Effects of 3.3% oils extract on eggs survival to adult

Oil Extract	No of Eggs Introduced	% Hatchability Mean ± SD	% Adult Emergence Mean ± SD
Arachis hypogaea	30	33.3 ± 2.3^{b}	20.0 ± 2.0^{a}
Elaeis guineensis	30	13.3 ± 1.1^{ab}	13.3 ± 1.1^{a}
Monodora tenuifolia	30	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}
Control	30	73.3 ± 1.1^{c}	66.7 ± 1.1^{b}

Means followed by the same letter are not significantly different P > 0.05 from each other.

Table 6: Effects of 3.3% oils extract on larval survival to adult.

Oil Extract	No of Larva Introduced	% Adult Emergence Mean ± SD
Arachis hypogaea	15	26.7 ± 1.1^{a}
Elaeis guineensis	15	33.3 ± 1.1^{a}
Monodora tenuifolia	15	60.0 ± 2.0^{b}
Control	15	100.0 ± 0.0^{c}

Means followed by the same letter are not significantly different P > 0.05 from each other.

duction of eggs of *E. vapidella* after 4 days of treatment with the extracts of *E. aromatica* and *Z. officinales* were the most effective against egg hatchability and adult emergence while *A. melegueta*, *H. sauveolens* and *P. guineense* reduced egg hatchability and adult emergence.

Aku *et al.* (1998) reported that extract from *A. senegalensis* root bark was more effective than the powder in the control of *C. maculatus.* Insecticidal activity of *A. melegueta* was attributed to the presence of paradol, an alkyl-phenol (Lale, 1992). Adedire and Lajide (1999) reported that ethanol extract of *Piper umbellatum* evoked 100% mortality at 5% and 10% concentration at 24 hours post treatment against *Callosobruchus maculatus.* The oil of *Elaeis guineensis, Arachis hypogaea* and *Monodora tenuifolia* were found effective in inhibiting egg hatch in *E. vapidella.*

Monodora tenuifolia oil was the most effective among the oils evaluated because development of egg and emergence of adult were completely inhibited. The oil was not very effective in preventing adult emergence in larvae. This performance corresponded to the findings of Don-Pedro (1989) that plant oil are toxic to young larvae of *C. maculatus*. The bioinsecticidal action of this oil could be attributed to the fact that *C. maculatus* eggs have respiratory pore with a gas– exchange function, which is blocked by the plant oil. Many vegetable oils have been assessed for use in preventing post-harvest losses due to insect (Golob and Webley, 1980). The findings of this study showed that *A. hypogaea*, *E. guneensis* and *M. tenuifolia* seed oil were highly toxic to the eggs and larvae of *E. vapidella*, thus effective in the protection of stored yam tubers from insect desprilation and damage. The toxic effect of plant oils on adult insects and immature forms is by asphyxiation as reported by Schoonhoven (1978). Based on this, it could be deduced that the eggs and larvae used in this study were suffocated as a result of the volatile components in the oil.

The toxicity of *M. tenuifolia* in this study could be ascribed to the presence of high molecular weight fatty acids in the seed oil as well as the presence of sterols and triterpene alcohol, which was earlier identified in the oils by Esuoso *et al.* (2000). Generally, the plants whose efficacies were tested against *E. vapidella* were available locally, not expensive, edible and form important part of the diet of tropical people since they are used as ingredient for soup or other medicinal purposes and can therefore be integrated with other pest control procedures. These plant products could be used among low- income farmers who store relatively small number of yam tubers for consumption and planting. The oils can be applied on bruises or wounds in yam tubers.

The extracts and oils can be applied on whole yam tubers before storage as well as spraying yam tubers on barn during storage may prevent the development of this pest of yam tubers.

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THE AGE AND GROWTH OF *TILAPIA ZILLII* (GERVAIS) IN OPA RESERVOIR, ILE-IFE, NIGERIA

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Abstract

Specimens of *Tilapia zillii* (Gervais) were collected from Opa reservoir in Ile-Ife, Nigeria, between October 1991 and February 1994. The fishing methods employed were castnetting and gillnetting. Annular rings were formed on the scales of 1310 specimens of the species between December and February of each year of study. Male fish specimens grew faster and bigger than the female fish irrespective of age. Fish length at maturity was 11.0cm (male) and 9.7cm (female). Allometric growth was observed in the species and the relationship between fish length and scale length gave a statistically significant correlation $r = 0.681$; $P < 0.001$. The species have a good condition factor which ranged between 1.40 to 3.06 with a mean of 2.86 in the reservoir.

Keywords: *Tilapia zillii*, annular ring, growth, age, condition-factor

1. Introduction

Cichlid fishes have been reported to dominate African freshwater bodies over many other species of fish (Harbott, 1975). Over 200 species of the cichlid family have been reported in inland waters of West Africa (Holden and Reed, 1978). Estimation of age and growth are fundamental to an understanding of the biology of fishes (Beamish and Mc-Farlane, 1983; Casselman, 1987). It is also of considerable importance if the fish is of commercial importance (Komolafe and Arawomo, 1998). Age data in conjunction with length and weight measurements can give information on stock composition, age at maturity, lifespan, mortality and production (Bagenal, 1978). In tropical waters, age determination is often difficult as reported by De Bont (1967) and Fagade (1974). This is because the rings on scales and hard parts of a fish may be associated with external factors such as dry season changes in food supply and stock density (Fryer and Iles, 1972). Fish scales exhibit great diversities in shape and size, yet they are veritable tools in age determination studies because their sizes and arrangement are constant (Lippitsch, 1992). Arawomo (1993) observed that the commercial importance of cichlid fishes in major rivers of West Africa has renewed interest in their age and growth determination. The objective of this study is to examine the annual growth in length, age at maturity and the length-weight relationship of a fish which is a commercially important species in Opa Reservoir.

2. Materials and Methods

Opa reservoir is located on the campus of Obafemi Awolowo University and has a catchment area covering 116 square kilometers. The reservoir (Longitudes 4° 31' E to 4° 32' E and Latitudes 7° 29' N to 7° 30' N; Fig. 1) has a surface area of 0.95 square kilometre and a maximum capacity of about 675 cubic metres. The minimum and maximum depths are 0.95m and 6.4m respectively.

The catchment area is characterized by wet and dry seasons. The dry season extends from November to March while the rainy season extends from April to October every year (Ekanade, 1980). During the rainy season, the reservoir receives high discharge of water from the catchment area making its water turbid. The substratum of the reservoir is mainly mud and sand. Shoreline vegetation is dense and identified macrophytes include *Commelina diffusa* Burm, *C. erecta* Linn, *Amarantus hibridus* Linn and *Acroceras zizaniodes* (Kunth) Dandy.

The specimens of *T. zillii* used for this study were caught between October 1991 and February 1994 in Opa reservoir. The fishing gears employed were castnetting and gillnetting. The gillnet was 250m long with five different mesh sizes of 50m each. The mesh sizes were 2.5cm, 5.1cm, 7.6cm, 10.2cm and 12.7cm with a depth of 1.32m stretched mesh. A castnet of 7.6cm and 2.5cm mesh sizes were used to catch fish. The total length, standard length and weight of fish were taken in the laboratory. Each fish specimen was slit open ventrally from the anus to the pectoral fin and its sex determined visually in line with the method of Roberts (1989). Scales were removed from just above the lateral line and below the dorsal fin of each fish specimen and kept in separate envelope. They were later washed in 10% Ammonia solution following the procedure of Rincon and Lobon-Cervia (1989).

Five clean and dried scales with good centra from each fish specimen were then mounted between two glass slides, labelled and examined under a dissecting microscope for annular rings. The radius of each scale was measured to assist in the determination of fish growth and the time when annular rings were laid down on the scales. The age of

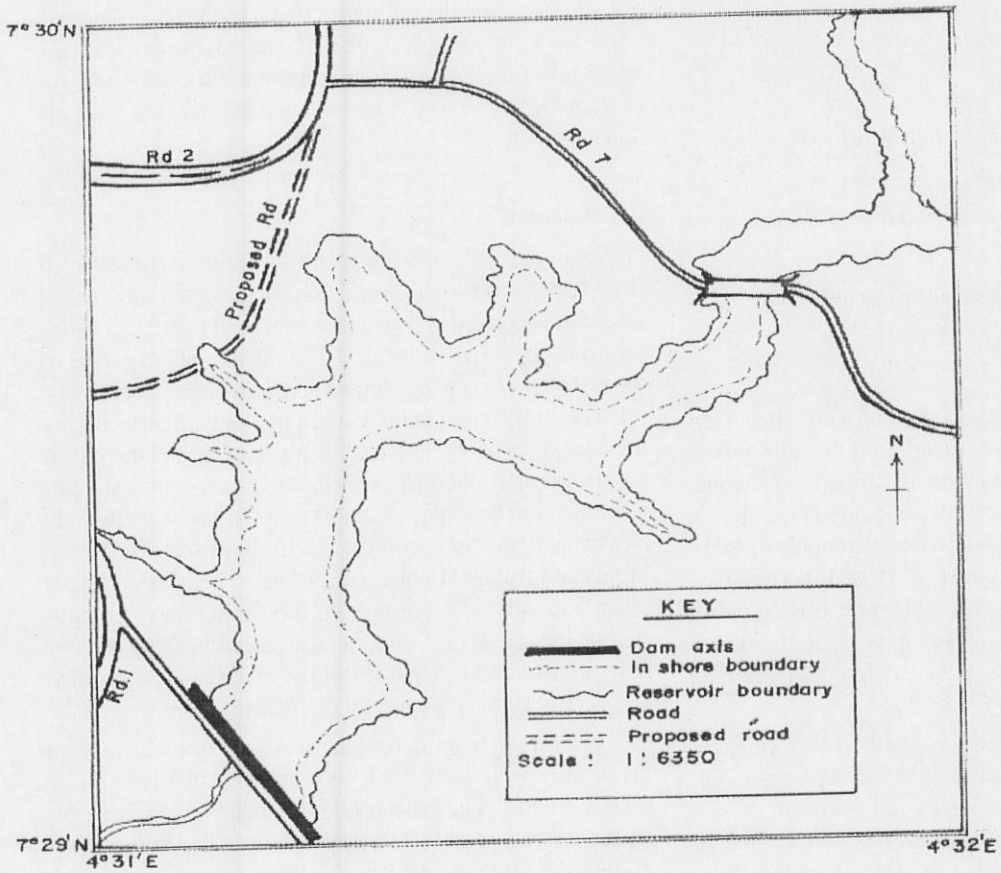

Fig. 1: Ona reservoir showing fish sampling site.

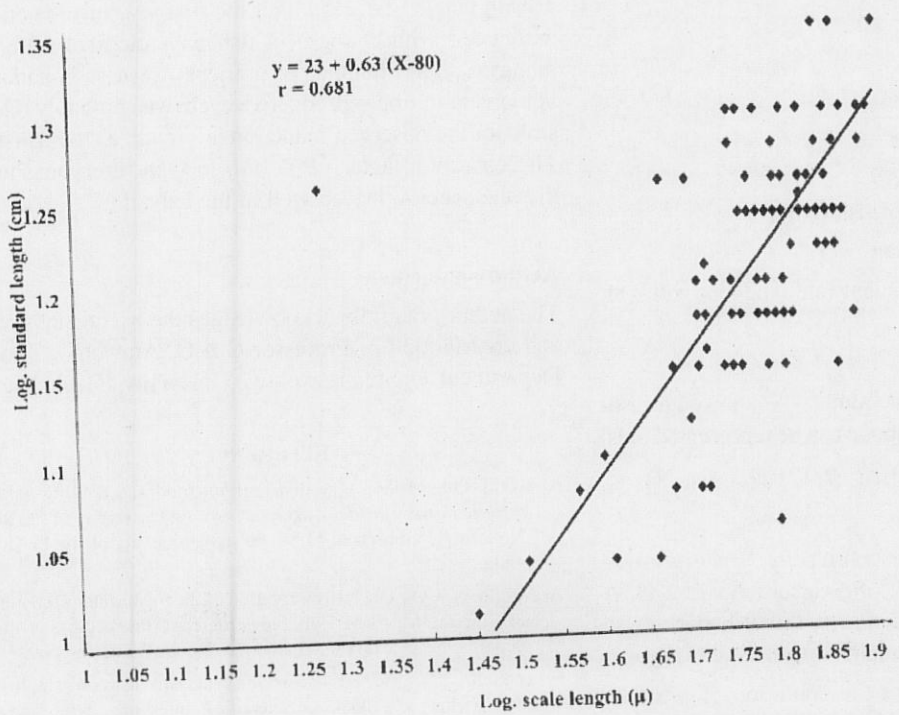

Figure 2: Graph of Log. standard length against Log. scale length.

each fish was determined by direct proportionality formula used by Bagenal (1978) viz:

$$L_n - C = \frac{S_n}{S}(L - C) \qquad (1)$$

where,

L_n = standard length when annulus 'n' was formed

C = intercept on abscissa

S_n = scale radius at annulus 'n' (at length L_n)

S = total scale radius

L = standard length when was sampled

3. Results

Annular rings formation on the scales of 1310 specimens of *T. zillii* in Opa reservoir was recognised by the characteristic crossing over of circuli which started in December and ended in February of each year of study (Plate 1). In other months, circuli were laid down regularly on the scales. A significant correlation ($r = 0.681$; $P < 0.001$) between log fish standard length and log fish scale radii was observed (Fig. 2). The result showed a steady increase in the size of fish with age. The male fish specimens grew bigger than the females in all age groups (Table 1).

However, a reduction in the rate of growth of fish with ageing process in both male and female fish was observed. There were 764 male fish specimens and the mean growth in length for the first year male fish was 11.0cm compared to 9.7cm observed in 546 female fish (Table 1). Subsequent increment in length of the male fish for the second, third and fourth year of life were 3.5cm, 2.8cm, 1.4cm compared to 3.1cm, 2.3cm, 1.2cm of the female fish of comparable age. The graph of length-weight relationship is described by the equation, (Bagenal, 1978):

$$W = al^b \qquad (2)$$

where, W = weight of fish (gm)

l = standard length of fish (cm)

a = Regression constant

b = Regression coefficient (an exponent with values between 2 and 4).

Tesch (1968) reported that the value $b = 3$ showed an isometric growth. The equation above can be represented thus;

$$\log W = \log(a) + b \,.\, \log(l) \qquad (3)$$

The graph of length-weight relationship for *T. zillii* showed allometric growth and the value of b calculated was 2.43. A significant correlation coefficient, $r = 0.960$ between fish log standard length and log weight was observed (Fig. 3).

The condition factor expresses the condition of a fish in terms of its general well being in a habitat. The values of condition factor of the male fish specimens ranged between 1.777 ± 0.150 to 1.983 ± 0.091 with a mean of 1.855 ± 0.154. In the female fish, the condition factor was between 1.799 ± 0.105 to 3.046 ± 0.160 and the mean was 1.910 ± 0.134. However, the difference between the means was not significant ($P>0.05$; df 1308). The values were quite high at all times of the year indicating that the condition of *T. zillii* in Opa reservoir is not affected by size, sex and seasonal variation.

4. Discussion

A total number of 1310 specimens of *T.* zillii were collected in Opa reservoir. The formation of annular rings on the fish scales occurred between December and February of each year of study. This coincided with the harmattan period of the dry season in the catchment area (Ekanade, 1980). The relatively low temperature caused by the harmattan during the period probably affected the water body and the physiological state of the fish in such a way as to cause annulus formation on the scales. Annulus ring formation on the species in the River Niger coincided with the onset of the floods in July and August (Banks *et al.*, 1966). The onset of floods (June and July) did not lead to the formation of annulus ring on the scales of *T. zillii* in Opa reservoir. This is probably because the water level of the reservoir is controlled and is only slightly altered by the floods.

The growth in length during the first year of life compares favourably well with the length attained in River Niger (Daget, 1956). The growth rate was relatively higher than what was observed in Egypt pond and lake Syria as reported by El-Bolock and Koura, (1960). Allometric growth was recorded for *T. zillii* in Opa reservoir. The fast growth rate recorded for the species could be attributed to the abundance of high quality natural food materials in Opa reservoir (Abayomi 1986; Komolafe and Arawomo, 1998). Decrease in growth length of *T. zillii* after the first year of life could be associated with changes in fish physiological state with maturity. The shunting of nutrients towards gonadal development during reproductive cycle was probably responsible for the observed reduction in growth as the fish aged. The condition factor of *T. zillii* in Opa reservoir showed that the species thrived well in the habitat.

Acknowledgement

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Table 1: Size range of male and female *T. zillii* at different age groups in Opa Reservoir

Age group	No. of Annuli	Designation	No of Fish	Male fish total length (cm) (Size range)	Mean total length (cm)	No of Fish	Female fish total length (cm) (Size range)	Mean total length (cm)
Less than one year old	None	0+	-	Less than 11.0	-	-	Less than 9.7	-
One year old	One	1	148	11.0 - 12.4	11.7 ± 0.44	36	9.7 - 10.5	10.1 ± 0.40
Less than two year old	One	1+	79	12.4 - 14.0	13.7 ± 0.20	28	10.5 - 12.8	11.7 ± 0.82
Two year old	Two	2	57	14.0 - 15.9	14.8 ± 0.21	151	12.8 - 13.6	13.1 ± 0.51
Less than three year old	Two	2+	25	15.9 - 16.8	16.1 ± 0.12	85	13.6 - 14.7	14.2 ± 0.49
Three year old	Three	3	111	16.8 - 18.7	17.3 ± 0.34	96	14.7 - 15.9	15.3 ± 0.55
Less than four year old	Three	3+	89	18.7 - 19.5	18.9 ± 0.16	77	15.9 - 16.6	16.1 ± 0.28
Four year old	Four	4	184	19.5 - 20.3	19.9 ± 0.28	73	16.6 - 17.1	17.0 ± 0.22
Less than five year old	Four	4+	71	Above 20.3	-	-	Above 17.1	-

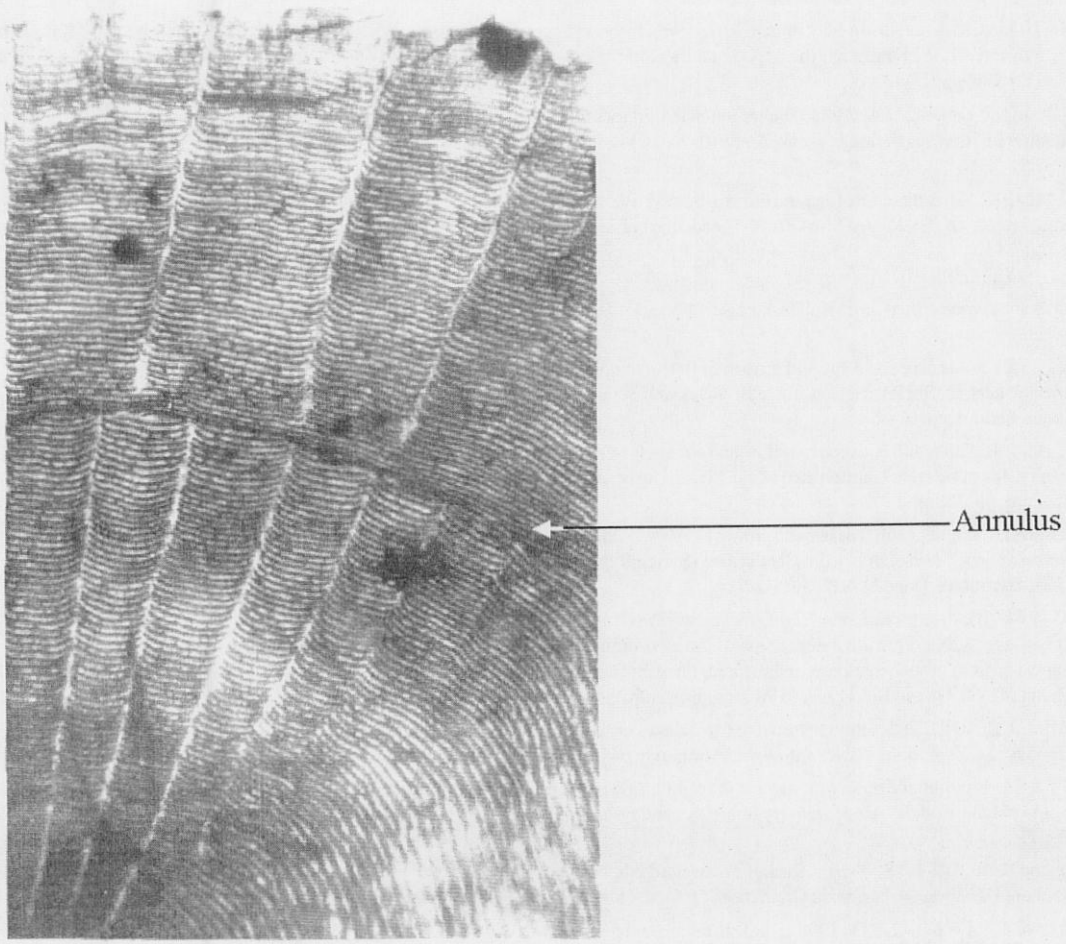

Plate 1: Fish scale showing annulus formation.

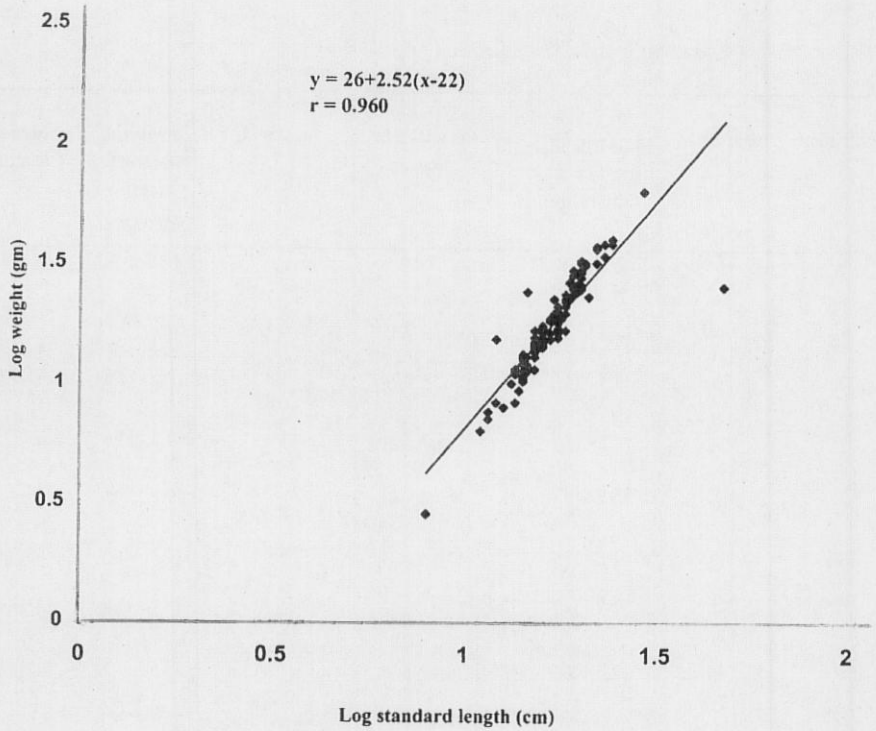

Figure 3: **Graph of Log. Weight (gm) against Log. Standard length (cm) of *Tilapia zillii* in Opa Reservoir**

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HEALTH IMPORTANCE OF FAECAL STRAINS OF *Lactobacillus acidophilus* USED AS PROBIOTICS IN RATS

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Abstract

The health promoting potential of Lactobacillus acidophilus isolated from faeces of human neonate, pig and albino rat was assessed. A set of rats were orogastrically dosed with the Lactobacillus isolates alone (safety test), while the other set was dosed with Lactobacillus isolates and infected with E. coli NCIB 86 (Challenge test). The feeding period lasted for 20 days. The live weight and the feed efficiency of the rats were recorded before they were killed. Blood samples were collected and analysed for some serum biochemical markers that can reveal toxicological effect. The study showed that Lactobacillus acidophilus from the sources above has no toxicological effect on rats. Most of the serum biochemical markers increased significantly ($P < 0.05$) in the challenge test when compared to the safety test. Furthermore, the anticholesterolaemic effect observed in the safety test was impaired in challenge test. Feed efficiency was higher in rats dosed with Lactobacillus and challenged with E. coli.

Keywords: Faecal strain, *Lactobacillus acidophillus*, health importance, probiotics, rats.

1. Introdution

Probiotics have been defined as viable microbial food supplements, which beneficially influence the health of the host (Schrezemeir and De Vrese, 2001). Metchnikoff (1907) first observed the beneficial effects of living microorganisms on human and since then much interest had been on the beneficial effects of living microorganisms on human and animals (Fuller, 1989). Probiotic organisms have been found mainly among the members of the genera *Lactobacillus* and *Bifidobacterium*. These are normal residents of the complex ecosystem of the gastrointestinal tract (Mitsuoka, 1992).

Lactobacilli have been used as biotherapeutic agents for ages and they are still the most common ingredients among those intended for consumption by farm animals (Silva *et al.*, 1999). The choice of lactobacilli as probiotic agents is appropriate since the normal gastrointestinal microbiota of man and animals is rich in this organism (Tannock, 1977). Moreover, they are non-pathogenic microorganism (Sandine, 1979).

Several beneficial effects of probiotic *lactobacilli* have been documented. Some of these health promoting effects are: prevention of gastrointestinal infection (Tannock *et al.*, 1988), enhancement of immune response (Kimura *et al.*, 1997), antimutagenic and anticarcinogenic activity (Fuller and Gibson, 1997; Zabala *et al.*, 2001), anticholesterolaemic and liver improvement functions (Bertazzoni *et al.*, 2001).

Current perspective on biotechnical applications of probiotic products require further in-vitro and in-vivo investigation to evaluate the safety of using wild type organisms or those obtained by genetic engineering (Walker and Duffy, 1998). Probiotics normally form part of the diet. The relationship between diet and disease can be revealed by biomarkers since they provide a link between the consumption of specific foods and biological outcome (Branca *et al.*, 2001). The activities of these biomarkers can therefore be used to ascertain the health promoting effect of probiotics.

The objective of the research reported here was to establish the health promoting properties of different strains of *Lactobacillus acidophilus* isolated from faeces of human neonate, pig and albino rat.

2. Materials and Methods

Lactobacillus culture

Three strains of *Lactobacillus acidophilus* were isolated from the faeces of a human neonate, a pig and an albino rat. These isolates had earlier been characterised using colonial morphological, biochemical, and RAPD-PCR. Preliminary studies reveal that these isolates can inhibit pathogenic and food spoilage bacteria, and can also adhere to the ileal cells of rats *in - vitro*.

The *Lactobacillus* isolates were cultured in MRS broth (LAB M) and incubated at 37 ^{0}C for two days in a fermentor to obtain a large cell concentration. The method described by Fujiwara *et al.* (2001) was used. The cells were washed and resuspended in rehydrated skim milk (Marvel brand) (10% w/v), lyophilised and stored at –20 ^{0}C until use. The concentration of the viable cells in the final powder was determined by serial dilution and plating on MRS agar (Taylor, 1962).

Animals and Diet

Thirty-two albino rats (Wistar strain) aged 5 – 6 weeks old were obtained from the Physiology Department, University of Ibadan. The rats were housed at the Federal University of Tech-

(email: ovofuta@yahoo.com)

nology, Akure rat house and maintained at 27±1°C. The rats were fed on basal diet - grower's mash purchased from Bendel Feeds, Edo State, Nigeria, for 1 week ad libitum before the treatment. The composition of the basal diet is shown in Table 1.

Experimental Design

Lyophilised *Lactobacillus* isolates were reconstituted by dissolving 1g in 10ml of sterile water. The safety test involve a single dose of 0.3ml of these cultures containing approximately 10^{10} cfu/g administered to 4 rats in groups 1AS, 1BS, 1PS while group C that serves as control, was given sterile skim milk only (Bertazzoni *et al.* 2001). The challenge test involves dosing with *Lactobacillus* isolates and simultaneously infecting with 10^{5} cfu/ml of *E. coli* NCIB 86 in groups 1AC, 1BC and 1PC. The same procedure was followed the second day for the safety and challenge tests. A post - ingestion period of 18 days was observed after the administration of the cultures.

The initial weight, final weight, and feed consumed were recorded before the animals were sacrificed by cervical dislocation. The blood samples of the rats were collected into EDTA bottles for analyses of major serum biomarkers.

Biochemical Assay

Reflotron M06.02<06.00 (Boehringer Mannheim Company, Germany) was used for the analyses of some major serum biochemical markers that can reveal the effects of the administered culture on the rat. The biomarkers assayed for are: Total bilirubin, Aspartate-aminotransferase (AST), Alanine-aminotransferase (ALT), Alkaline phosphatase (ALP) and total cholesterol of the serum. Standardized amounts of the sample were automatically pipetted and applied on the test zone of the appropriate test strip. The strip was inserted into the test chamber and the result was displayed after some seconds on the computer monitor. Tests were carried out at 25°C.

Statistical Analysis

Data were analysed using the one-way ANOVA method, followed by Duncan test using SPSS 10.0 package. The level of significance was taken as $P< 0.05$. The results were reported as mean of 4 rats per set and their standard deviation calculated.

3. Results and Discussion

Lactobacillus acidophilus sourced from the faeces of human neonate, pig and albino rat were selected primarily because preliminary test shows that they have two major probiotic properties of inhibiting pathogenic bacteria and being able to adhere to the ileal epithelial cell (IEC) of the rat. These probiotic strains are expected to exert a beneficial effect on the host.

The relationship between diet and diseases or good health can be ascertained by biomarkers, and major biomarkers such as plasma enzymes viz: AST, ALT, ALP etc, changes in disease and can be related in many ways to cell pathology (Baron *et al.*, 1994). The serum AST was significantly higher ($P < 0.05$) in groups 1BC (297.73 iu/l) and 1PC (564.00 iu/l) when compared with the other groups (C, 1AS, 1BS, 1PS, and 1AC) that have AST level between 85.93 and 119.67 iu/l (Table 2). The serum AST activity was not significantly different ($P<0.05$) in groups' 1AS, 1BS and 1PS when compared with the control (C). Moreover, the ALT activities was significantly higher ($P < 0.05$) in groups 1AC, 1BC and 1PC (Table 2). The AST and ALT are enzymes that are located in the liver cells and leak out into the general circulation when liver cells are injured. Alanine aminotransferase (ALT) is regarded as a more specific indicator of liver inflammation, since the AST may be elevated in diseases of other organs such as the heart and muscle (Johnston, 1999). The implication of the result above is that in the safety test, there may not be any toxicological effect but in the challenge test, there is likely to be toxicological effect because of the elevated ALT.

Alkaline phosphatase (ALP) activities of the serum also reveal a significant difference ($P < 0.05$) in the level of this enzyme in groups that were dosed with *Lactobacillus* isolates and simultaneously infected with *E. coli* (Table 2). An increase in osteoblastic activity had been linked with a rise in ALP level (Baron *et al.*, 1994). The ALP activity can also serve as an indicator of liver damaqge when there is lack of bile flow (cholestasis). In essence, feeding with *Lactobacillus* alone in the safety test has the potential of reducing the incidence of cholestasis as observed in groups 1AS, 1BS, and 1PS.

The serum cholesterol level was significantly higher ($P < 0.05$) in groups 1AC, 1BC and 1PC than the control (C) (Fig. 1). Fuller (1989) had suggested that bacterial metabolites in fermented milk inhibit cholesterol synthesis. Lactobacilli in particular had been found to have direct effect on cholesterol level by assimilation and removal from the growth medium. This has been demonstrated in pigs (Gilliland *et al.*, 1985) and in rats (Bertazzoni *et al.*, 2001). Studies have indicated that the serum cholesterol level is one risk factor in the incidence of coronary artery disease and individuals with elevated serum cholesterol values develop coronary heart disease (CHD) with greater frequency (Kannel, 1978). In essence when these *Lactobacillus* isolates were administered alone they possess anticholesterolaemic effect but this effect was impaired in the challenge test. The lower level of serum AST, ALT, and cholesterol observed in groups' 1AS, 1BS, and 1PS when compared to the other treatments shows that a high dose of the probiotic (10^{10} cfu/g), when applied twice, can persist for up to three weeks or more after administration. Pascual *et al.* (1999) had earlier suggested that more than one dose would be necessary to ensure the presence of *Lactobacillus salivarius* in the gut of birds for 21 – 28 days.

Growth performance measurements reveal that the total weight gain (TWG) of the rats in groups' 1AC, 1BC and 1PC was higher than the control (C) and the groups' fed with *Lactobacillus* alone (Table 3). The results shows that groups 1AC, 1BC and 1PC had the highest ALT activities and cholesterol level. The high level of serum ALT and cholesterol in the groups' above may be responsible for the

Table 1: Composition of Basal Diet Used In Feeding Rats.

Ingredients	Level in Diet
Crude protein	14.5%
Crude fat	4.8%
Crude fibre	7.2%
Crude ash	8.0%
Calcium	0.8%
Phosphorus	0.62%
Lysine	0.60%
Methionine	0.29%
Methionine + Cystine	0.52%
Vitamin A	8,000 i.u
Vitamin D	2,400 i.u
Vitamin E	15mg
Vitamin B_2	40mg
Vitamin C	50mg
Manganese	30mg
Zinc	30mg
Sodium	0.15%

Metabolisable energy: 2,300 kcal./kg
Source: Bendel Feeds, Edo State, Nigeria.

Table 2: Level of Biochemical Markers in the Serum of Rats After *In-vivo* Feeding.

Isolates	AST (iu/l)	ALT (iu/l)	ALP (iu/l)
C	93.47±66.66	31.43±9.47	32.90±0.06
1AS	70.97±13.77	33.03±3.89	32.93±0.06
1BS	119.67±16.44	33.87±8.29	32.90±0.06
1PS	107.03±36.02	15.87±5.18	32.93±0.06
1AC	85.93±4.00	78.80*±33.29	973.53*±92.20
1BC	297.33*±36.05	76.47*±8.60	961.67*±118.35
1PC	564.00*±29.30	85.33*±12.42	1005.33*±221.61

Results are expressed as the mean ± Std. Deviation for 4 animals per group.
*Values along the column are significantly higher (P <0.05) than the control (C).
C: Rats fed basal feed only (C)
1AS: Rats fed *Lactobacillus acidophilus* sourced from rat faeces
1BS: Rats fed *Lactobacillus acidophilus* strain sourced from human neonate faeces
1PS: Rats fed *Lactobacillus acidophilus* strain sourced from pig faeces
1AC: Rats fed *Lactobacillus acidophilus* strain sourced from rat faces and challenged with *E. coli*
1BC: Rats fed *Lactobacillus acidophilus* strain sourced from human neonate and challenged with *E. coli.*
1PC: Rats fed *Lactobacillus acidophilus* strain sourced from pig faeces and challenged with *E. coli.*
AST: Aspartate aminotransferase
ALT: Alanine aminotransferase
ALP: Alkaline phosphate

Table 3: Performance of Rats After *In-vivo* Feeding.

Strain Designation	Feed consumed	Total Weight Gain	Feed Efficiency
C	400 ± 1.21	36.50±5.29	0.091±0.06
1AS	396.63 ± 2.04	36.50±2.78	0.092±0.07
BS	397.00 ± 0.57	24.83±1.25	0.062±0.06
1PS	396.13 ± 0.55	35.50±13.59	0.089±0.06
1AC	395.4 ± 2.02	57.17*±9.27	0.144*±0.05
1BC	396.77 ± 1.25	53.50*±12.12	0.134*±0.05
1PC	396.33 ± 0.88	53.50*±7.00	0.134*±0.09

Results are expressed as the mean ±Std. Deviation for 4 animals per group.
*Values along the column are higher and significantly different (P < 0.05) from the control (C).

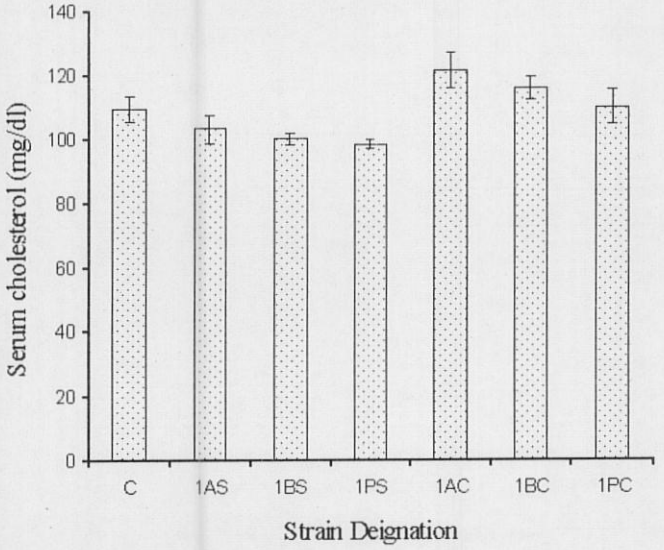

Fig 1: Serum Cholesterol of Rats after in - vivo Feeding Trials.

higher weight. The correlation between serum cholesterol, ALT, and weight had been reported (Johnston, 1999). Low cholesterol had been found to result to reduced weight and low ALT activity. Assessment of the feed efficiency also reveals that group 1AC had a better performance than the other groups. In terms feed utilisation, groups 1AC, 1BC, and 1PC would consume 56.62%, 46.57% and 46.55% respectively less feed to achieve the same liveweight as control (C). The feed utilisation in groups 1AS and 1PS were not significantly different from the control but it was significantly different in 1BS (Table 3).

These results showed that faecal strains of *Lactobacillus acidophilus* possess health-promoting activities such as anticholesterolaemic effect and liver function improvement when administered alone. The feed efficiency was however lower in rats dosed with *Lactobacillus species* alone but higher in rats dosed with *Lactobacillus species* and simultaneously challenged with *E. coli.* This may be due to the higher level of cholesterol and ALP in the serum of these rats in challenge test.

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SYNTHESIS OF NOVEL PYRIDO- AND PYRIMIDINO-[2',1':2,3][1,3] THIAZOLO [4,5-B]QUINOXALINES

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Abstract

New pyrido- and pyrimidino[2',1':2,3][1,3] thiazolo[4,5-b] quinoxalin-12-ium salts **7** could be synthesized from cyclizations of 2,3-dichloroquinoxalines with 2-mercaptopyridine and 2-mercaptopyrimidine, respectively. The pyrimidino- thiazoloquinoxaline **7d** reacts further with acetyl-compounds to afford the corresponding 1-(acylmethyl)-1H-pyrimidino[2',1':2,3][1,3] thiazolo[4,5-b]quinoxalines, **9**.

1. Introduction

Some thiazine derivatives are very useful drugs (Ohseng *et al.*, 1989; Kono *et al.*, 1991). Many of the thiazine derivatives, including pyridothiazines, have been reported to exhibit antiviral (Cavrini *et al.*, 1981), antibacterial (Saito *et al.*, 1990; Maeda *et al.*, 1995), insecticidal, (Reifscheider *et al.*, 1991), molluscicidal (El-Bayouki and Basyouni, 1988), potential CNS (Malinka *et al.*, 1998), antitumor (El-Subbagh *et al.*, 1999) and anticancer (Schade and Studenik, 1999) activities. Also, many quinoxaline derivatives are important commercially as pharmaceuticals and agrochemicals. They are widely used as antibiotics (Kinashi *et al.*, 1988), fungicides (Lunkenheimer and Buechel, 1975), antagonists (Sarges *et al.*, 1990), herbicides (Makino and Sakata, 1985; Hiramatsu *et al.*, 1988), dyes (Eckstein and Theidel, 1979; Ishii *et al.*, 1988; Rose *et al.*, 1990) and pigments (Sarodnick *et al.*, 1990).

Previously, we reported the stepwise nucleophilic displacement of the two chloro groups of 2, 3-dichloroquinoxalines **1**, using carbanious generated from some active methylene compounds and pyridine derivatives as the nucleophiles, to give novel, colored quinoxaline betaines **4** (Obafemi and Pfleiderer, 2004): (Scheme 1).

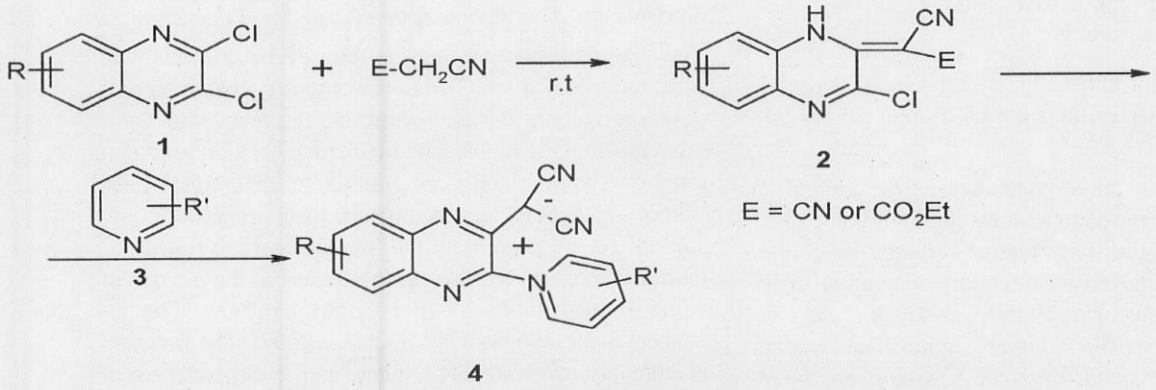
As a continuation of our desire to synthesize new quinoxaline derivatives via nucleophilic substitution, we wish to report the reaction of 2,3-dichloroquinoxalines with 2-mercaptopyridine and 2-mercatopyrimidine and the reaction of the products of 2-mercatopyrimidine with acetone and some acetyl derivatives.

2. Experimental

Infrared spectra were recorded (as KBr Pellets) on a Buck spectrometer. Melting points were determined on a Gallenkamp (variable heater) melting point apparatus; no corrections. ^{1}H–and ^{13}C–NMR were recorded as $CDCl_3$ or DMSO-d_6 solutions on a Brucker–AC-250 and JEOL-JNMGX 400-MHZ spectrometer (δ in ppm relative to Me_4Si and H_3PO_4). Mass spectra (EI-MS) were recorded on a finnigan MAT 312 machine, (in m/z (rel. %))

2,3-Dichloroquinoxalines 1a – 1c.

2,3-Dichloroquinoxalines **1a**–**1c** were prepared as described earlier (Obafemi and Pfleiderer, 1994).



Scheme 1: Synthesis of quinoxaline betaines **4**

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Pyrido[2',1':2,3][1,3]thiazolo[4,5-b]quinoxalin-12-ium chlorides.

General procedure:

The pyridothiazoloquinoxaline salts were prepared by reacting 2-mercaptopyridine with 2,3-dichloroquinoxaline derivatives in acetic acid. A typical procedure for the preparation of **7a** is as follows:

A mixture of 2,3-dichloroquinoxaline **1a**, (1.0 g, 5 mmol) and 2-mercaptopyridine (0.56 g, 5 mmol) in acetic acid (15 ml) was refluxed for 30 min. The reaction mixture was allowed to cool and the formed precipitate filtered. The solid was transferred into a flask and then washed with acetone to give yellow crystals of ***pyrido[2',1':2,3][1,3]thiazolo[4,5-b]quinoxalin-12-ium chloride*** **7a**. ^{13}C–NMR (, ppm): 155.7 (C–1), 147.2, 146.1, 144.4, 141.6, 140.4, 136.5, 135.2, 134.5, 130.7, 129.5, 126.8, 125.7.

MS 238 (100, M^+, molecular mass of the intact cation) 78 (65).

7b–**7d** were prepared in a similar manner:

9(8)-methylpyrido[2',1':2,3][1,3]thiazolo[4,5-b]quinoxalin-12-ium chloride, **7b**; Ir (cm^{-1}): 1630, 1600, 1430.

MS: 252 (81.7, M^+ intact cation), 187 (8.5), 125 (10.2), 78 (31.5), 45 (100).

9(8)-Chloropyrido[2',1':2,3][1,3]thiazolo[4,5-b]quinoxalin-12-ium chloride, **7c**: Ir (cm^{-1}): 1630, 1600, 1428. MS: 272 (100, M^+, intact cation), 237 (2.0, M^+–Cl).

^{13}C-NMR (δ, ppm): 9-isomer: 154.9 (C–1), 147.1, 145.9, 145.8, 141.1, 138.5, 136.9, 135.6, 133.5, 130.2, 127.6, 125.6, 124.1.

8-Isomer: 154.7, 147.9, 145.9, 142.5, 142.1, 138.5, 137.5, 136.8, 133.1, 130.8, 127.2, 125.6, 124.1.

pyrimidino[2',1':2,3][1,3]thiazolo[4,5-b)quinoxalin-12-ium chloride, **7d**: Ir (cm^{-1}): 1640, 1600, 1430, 1120.

Typical procedure for the synthesis of 1-(acylmethyl)-1H-pyrimidino[2',1':2,3][1,3]thiazolo[4,5-b]quinoxalines **9a**–**9d** from **1**:

To a solution of 2,3-dichloroquinoxaline (1.0 g, 5 mmol) in acetic acid (25 ml) was added 2-mercaptopyrimidine (0.57 g, 5mmol) and the resulting mixture refluxed for 5 min. The solvent was removed on a rotatory evaporator under reduced pressure. Acetone (10 ml) was added to the residue and then refluxed for another 5 min. The reaction mixture was left to stand to give ***1-(2'-oxo-propyl)-1H-pyrimidino[2',1':2,3][1,3]thiazolo[4,5-b]quinoxaline*** **9a**. Ir (cm^{-1}): 1710, 1610, 1515, 1445. ^{13}C – NMR: 205.9 (C = O), 150.3, 147.0, 142.9, 140.0, 139.3, 129.5, 127.9, 127.5, 127.2, 119.2, 110.1, 53.9, 52.3, 30.8: MS: 296 (30.5, M^+), 253 (90.2, $[M - COCH_3]^+$), 239 (100, $[M - CH_2COCH_3]^+$), 212 (3.0), 134 (5.0), 102 (13.0), 79 (31.0).

Compounds **9b**–**9d** were obtained in a similar manner:

1-(Benzoylmethyl)-1H-pyrimidino[2',1':2,3][1,3]thiazolo[4,5-b]quinoxaline **9b**: Ir (cm^{-1}): 1650, 1620, 1600, 1480. MS: 358 (11.0, M^+), 253, (33.5,$[M - COPh]^+$), 239 (55.2, $[M\text{-}CH_2COPh]^+$), 120 (38.0 $[PhCOCH_3]^+$), 105 (100, $PhCO^+$), 102 (15.0), 79 (38.0), 77 (39.0).

1-(Thenoylmethyl)-1H-pyrimidino[2',1':2,3][1,3]thiazolo[4,5-b]quinoxaline **9c**: Ir (cm^{-1}): 1660, 1450, 1360. MS: 364 (4.0, M^+), 253 (27.2, $(M\text{-}CO\text{-}C_4H_3S]^+$), 239 (33.5, $[M\text{-}CH_2CO\text{-}C_4H_3S]^+$), 202 (6.1), 160 (10.0), 126 (28.8, $ThCOCH_3^+$), 111 (100, $ThCO^+$), 102 (16.4), 83 (34.2), 79 (37.0).

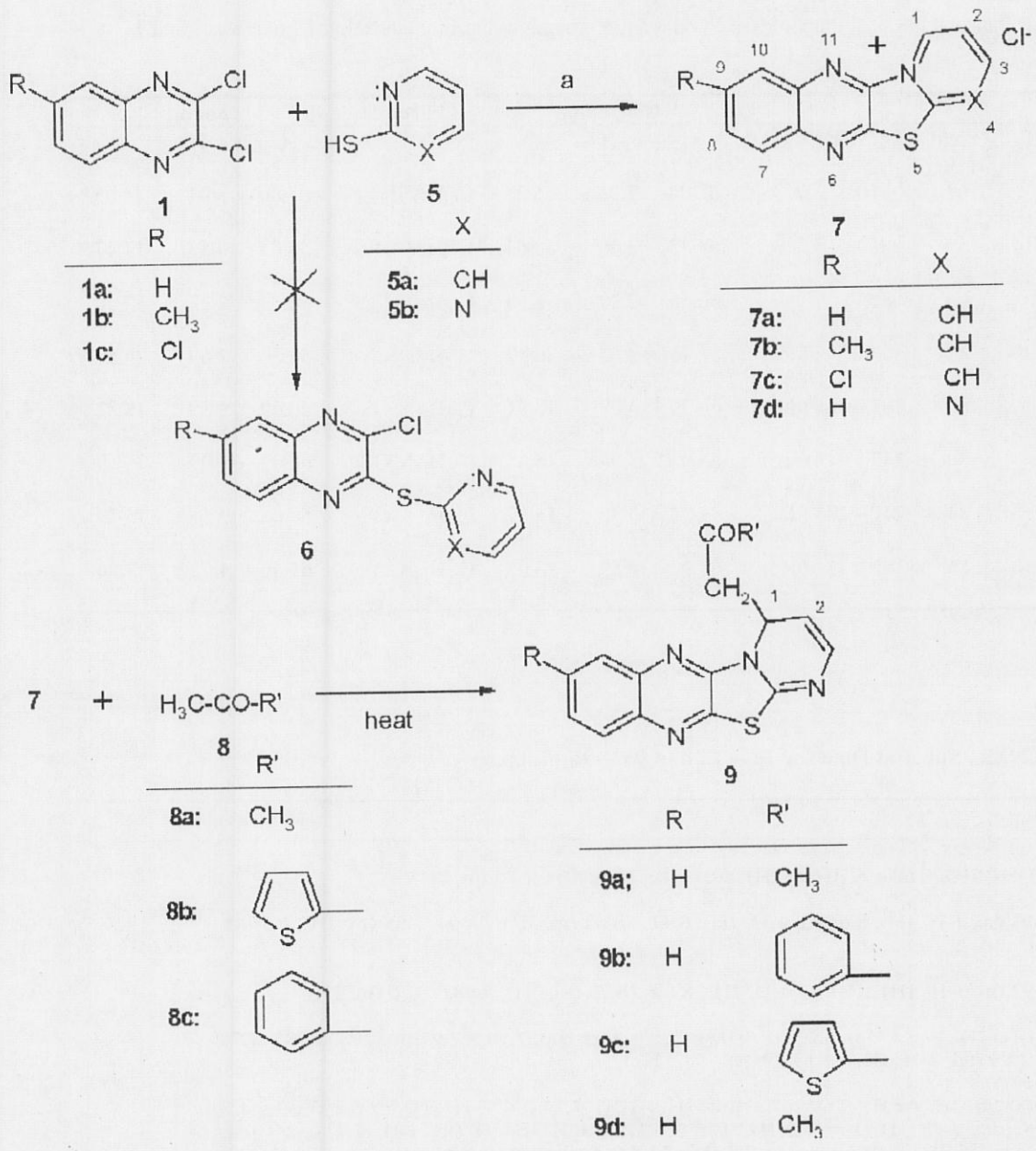
1-(2'–oxopropyl)–9(8)-methyl-1H-pyrimidino[2',1':2,3][1,3]thiazolo[4,5-b]quinoxaline **9d**: Ir (cm^{-1}): 1705. 1610, 1440. MS: 310 (32.1, M^+), 267 (85.9, $[M\text{-}COCH_3]^+$), 253 (100, $[M\text{-}CH_2COCH_3]^+$), 125 (17.8), 78 (29.9).

3. Results and Discussion

Unsubstituted–and 6-substituted–2,3-dichloroquinoxalines **1a**–**1c** were prepared as described earlier, by oxalylation of the corresponding benzene–1,2-diamines with oxalic acid dihydrate, followed by chlorination with thionyl chloride (Obafemi and Pfleiderer, 1994). We intended to synthesize 2-chloro-3-(2'-pyridylthio)quinoxaline and derivatives **6**, by reaction of **1** with 2-mercaptopyridines **5**, as potential antibacterial agents. However, **1a** – **1c** reacted with one mole equivalent each of 2-mercaptopyridine **5a** and 2-mercaptopyrimidine **5b** in acetic acid to form the salts of fused heterocyles with bridgehead nitrogen, namely, pyrido–and pyrimidino[2', 1': 2,3] [1,3]thiazolo[4,5-b]quinoxalin-12-ium chlorides **7a**–**7d**, and not the expected **6**. (Scheme 2).

The yields, physical properties and analytical data of the products are given in Table **1**. The structures of **7a**–**7d** were assigned by their elemental analyses, ^1H – and ^{13}C – nmr spectra. The ^1H-nmr data are listed in Table **2**.

The analysis of the ^1H – nmr spectra of the products with substituted R (**7b** and **7c**) indicates that cyclizations produce a mixture of 8–and 9-substituted isomers, with varying regioisomeric ratios, with their chemical shifts and patterns overlapping and very difficult to distinguish. Each isomer could not be separated but the main products are presumed to be 9-substituted–pyrido- or pyrimidinothiazoloquinoxalines **7**, in a manner similar to the reaction of some 6-substituted-2,3-dichoroquinoxalines with 2-aminopyridines (Sugita and Mitsuhashi, 1992). The first step presumably involved simple nucleophilic displacement of the C–2 chlorine atom to give the corresponding 2-heterylthioquinoxaline derivative **6**. This is followed by an intramolecular nucleophilic substitution of the C–3 chlo-



Scheme 2: Synthetic Route to 7 and 9

rine atom to form the salts of condensed heterocycles, 7 (Scheme 3).

Similar salts of condensed naphthathiazolopyridines have been prepared from 2,3-dichloro-1,4-naphthoquinone (Romanov and Lazareva, 1990).

In the ^{1}H – nmr of the salts (7), the chemical shift of the proton on C–1 is shifted downfield to δ 9.5 – 9.9 region, as has been observed for some pyridinium salts (Romanov and Lazareva, 1990; Meziane and Bazureau, 2002). Also, in the ^{13}C–nmr spectra, the chemical shift of C–1 is shifted downfield to around δ 156 ppm.

One of the work-up steps, after completion of reaction, involved washing the precipitated salts with acetone. However, washing of the pyrimidinothiazoloquinoxaline salt (**7d**) with acetone resulted in an addition reaction with the acetone to give the pseudobase 1-(2'-oxopropyl)–1H–pyrimidino[2',1':2,3][1,3]thiazolo[4,5-b]quinoxaline **9a**. Similar reactions occurred with acetophenone and 2-acetylthiophene to give the corresponding 1-substituted products **9b–9d**. The reaction mixture is probably basic enough to abstract a proton from the methyl group of the acetyl group followed by attack of the formed carbanion on the pyrimidinium ion (Scheme 4):

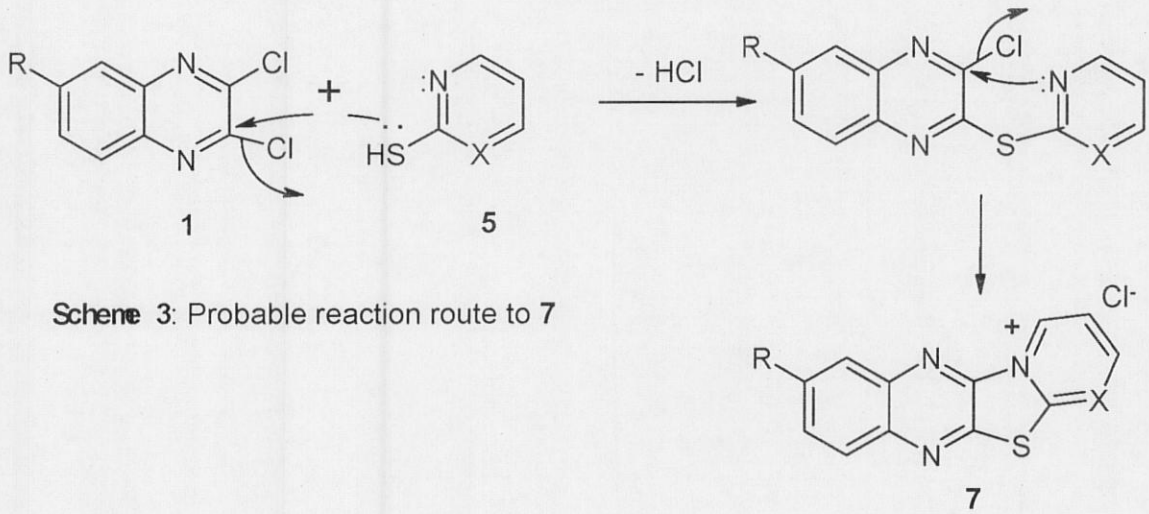
The structures of **9a – 9d** were assigned by their elemental analyses, ^{1}H– nmr, infrared and mass spectra. (See Table 2 for the ^{1}H-NMR spectra data).

Table 1: Analytical Data and Physical Properties of Pyrido- and Pyrimidino-thiazoloquinoxaline Derivatives 7 and 9.

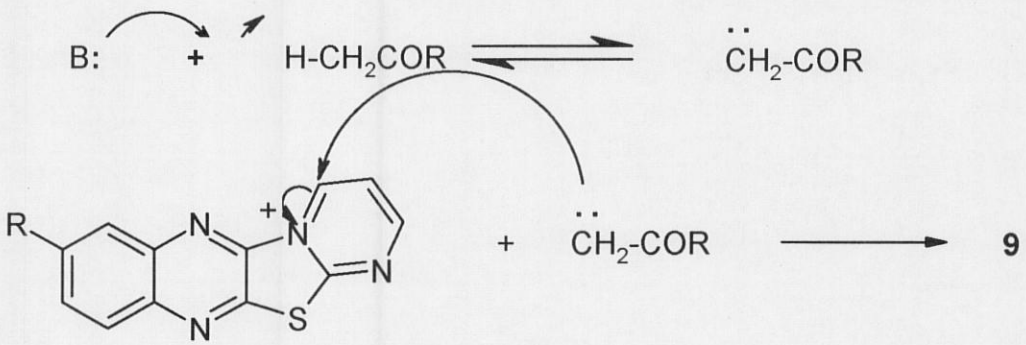
Compound				Calculated			Formula	Found		
No	R	Yield (%)	MP (°C)	C	H	N		C	H	N
7a	H	62	>310	57.04	2.95	15.35	$C_{13}H_8ClN_3S$	57.00	3.04	15.05
7b	CH_3	59	>300	58.43	3.50	14.61	$C_{14}H_{10}ClN_3S$	58.05	3.57	14.47
7c	Cl	60	>300	50.66	2.29	13.64	$C_{13}H_7Cl_2N_3S$	50.53	2.32	13.40
7d	H	72	300 – 302 (dec)	52.46	2.57	20.57	$C_{12}H_7ClN_4S$	52.36	2.69	20.50
9a	H	83	163 – 165 (dec)	60.79	4.08	18.91	$C_{15}H_{12}N_4OS$	60.59	4.19	18.82
9b	H	80	247 – 248 (dec)	67.02	3.94	15.63	$C_{20}H_{14}N_4OS$	66.93	3.99	15.59
9c	H	84	241 – 243 (dec)	59.32	3.32	15.37	$C_{18}H_{12}N_4OS_2$	59.08	3.55	15.10
9d	CH_3	85	152 – 154 (dec)	61.92	4.55	18.05	$C_{16}H_{14}N_4OS$	61.60	4.77	17.80

Table 2: Proton NMR Spectral Data for **7a – 7d** and **9a – 9d** (δ, ppm).

Compd. No	
7a	9.85 (d, 1-H-, 1H), 8.60 – 8.70 (m, 2H), 8.01 – 8.17 (m, 3H), 7.85 (br, 2H).
7b	9.88 – 9.91 (m, 1-H, 1H), 8.65 (d, 4-H, 1H), 8.41 – 8.52 (m, 3H), 7.80 – 7.95 (m, 2H), 2.50 (s, 3H, CH_3).
7c	9.89 9.92 (m, 1-H, 1H), 8.81 (d, 4-H, 1H), 8.69 8.79 (m, 3H), 7.98 8.70 (m, 2H).
7d	9.47 (d, 1-H, 1H, J– 8.5 Hz), 8.25 (d, 3-H, 1H), J – 13.1 Hz), 7.90 – 7.97 (m, 2H), 7.59 – 7.74 (m, 2H), 5.84 (dd, 2-H, 1H, J – 13.1 Hz, J – 8.5 Hz).
9a	7.91 – 8.00 (m, 2H, Ar-H), 7.69 – 7.82 (m, 2H, Ar-H), 7.24 (dd, 3-H, 1H, J – 8.2 Hz, J – 1.3 Hz), 5.58 (dd, 2-H, 1H, J = 8.2 Hz, J = 3.4 Hz), 4.91 – 4.94 (m, 1-H, 1H), 3.09 (dd, diastereotopic H, 1H, J = 17.9 Hz, J= 7.7 Hz), 2.98 (dd, diastereotopic H, 1H, J = 17.9 Hz, J = 4.8 Hz), 2.20 (s, 3H, CH_3).
9b	7.96 – 8.00 (m, 2H, Ar-H), 7.74 – 7.85 (m, 2H, Ar-H), 7.43 – 7.61 (m, 5H, Ar-H), 7.10 (dd, 3-H, 1H, J = 8.2 Hz, J = 1.2 Hz), 5.39 (dd, 2-H, 1H, J = 8.2 Hz, J = 3.0 Hz), 5.11 – 5.17 (m, 1-H, 1H), 3.53 (dd, diastereotopic H, 1H, J = 17.0 Hz, J = 5.2 Hz), 3.34 (dd, diastereotopic H, 1H, J – 17.0 Hz, J – 8.2 Hz).
9c	8.00 8.10 (m, 2H, Ar-H), 7.93 7.99 (m, 2H, Ar-H), 7.66 7.84 (m, 2H, Ar-H), 7.29 7.39 (m, 2H, Ar-H and 3-H), 5.82 (dd, 2-H, 1H, J – 8.2 Hz, J – 3.1 Hz), 5.26 (m, 1-H, 1H), 3.80 (dd, 1H, diastereotopic H of CH_2, J = 17.3 Hz, J = 7.5 Hz), 3.62 (dd, 1H, diastereotopic H of CH_2, J = 17.3 Hz, J = 4.6 Hz).
9d	9-isomer: 7.64 – 7.74 (m, 2H, Ar-H), 7.62 (s, 1H, Ar-H), 7.07 (d, 3-H, 1H, J – 8.1 Hz), 5.23 – 5.27 (m, 2-H, 1H), 4.93 (br, s, 1-H, 1H), 2.96 (dd, 1H, diastereotopic H of CH_2, J – 16.8 Hz, J – 6.3 Hz), 2.77 (dd, 1H, diastereotopic H of CH_2, J – 16.8 Hz, J – 7.4 Hz). 8-isomer: 7.55 (s, 1H, Ar-H), 7.30 – 7.54 (m, 2H, Ar-H), 6.50 (d, 3-H, 1H, J = 7.2 Hz), 5.62 (br, s, 1-H, 1H), 5.40 5.45 (m, 2-H, 1H), 3.36 (dd, 1H, diastereotopic H of CH_2, J = 17.1 Hz, J= 2.8 Hz), 3.02 (dd, 1H, diastereotopic H of CH_2, J = 17.1 Hz, J = 3.8 Hz), 2.53 (s, 3H, CH_3), 2.19 (s, 3H, CH_3).



Scheme 3: Probable reaction route to **7**



Scheme 4: Probable route to formation of **9**

In the spectra of **9a** – **9d**, the methylene protons of the acylmethyl group appeared as diastereotopic protons (doublet of doublet) because they are adjacent to a chiral centre. The ^{1}H– and ^{13}C–NMR spectra of compound **9a** are shown in Figures 1 and 2, respectively.

The antimicrobial properties of the compounds are being studied and will be reported soon.

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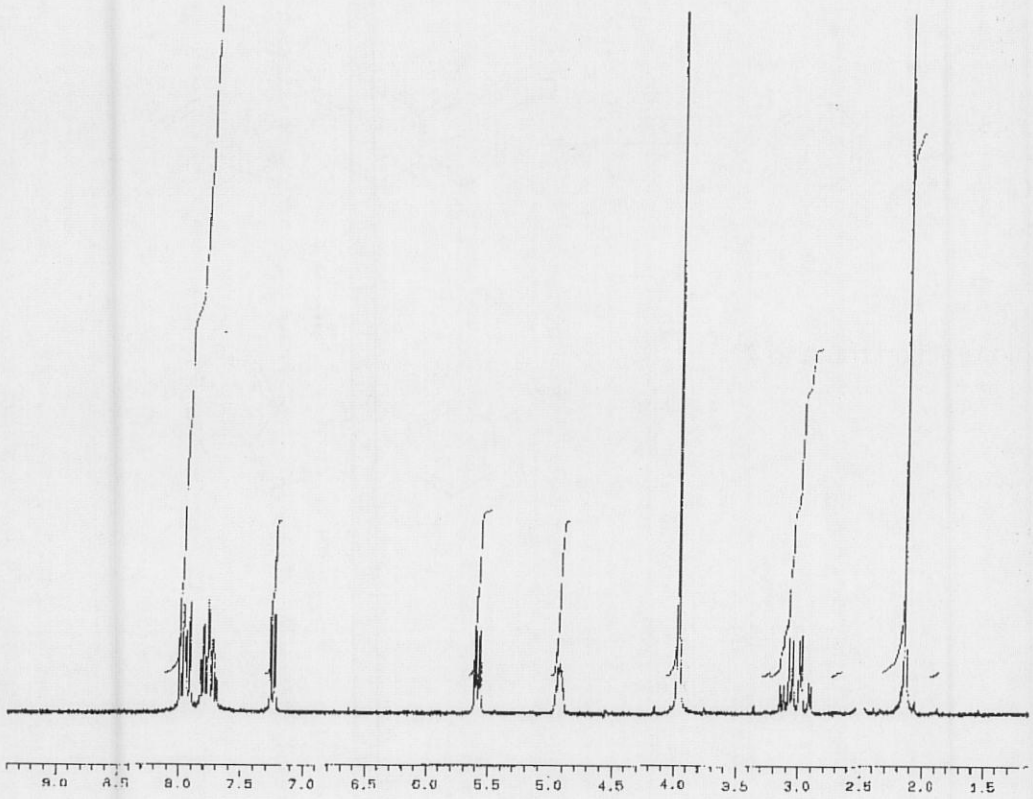

Figure 1. ^{1}H NMR Spectrum of **9a**

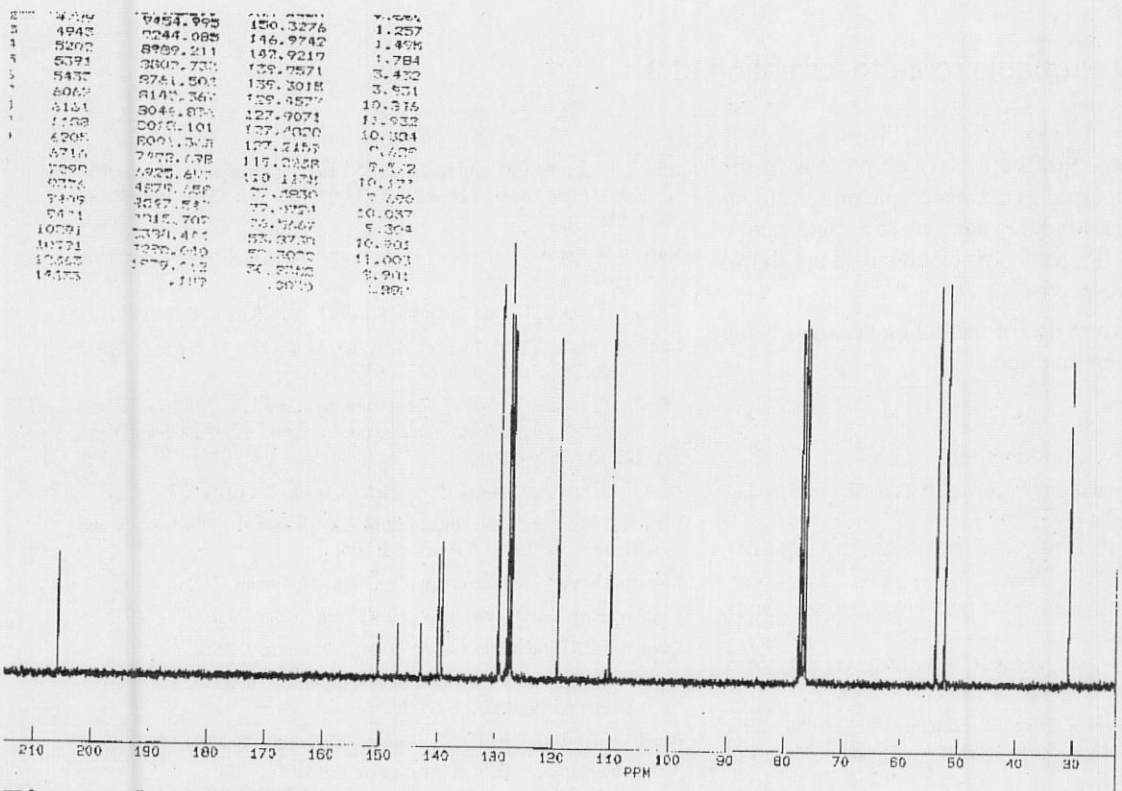

Figure 2. ^{13}C NMR Spectrum of **9a**

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CHEMICAL COMPOSITION AND BINDING POWER OF DRIED PULP WASTES PRODUCED FROM THE AFRICAN LOCUST BEAN (*PARKIA BIGLOBOSA*) IN LOW-COST FISH DIETS

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Abstract

The chemical compositions of pulp wastes produced from fermented African locust bean before and after fermentation were assessed in order to determine their possible utilization. The fermented bean pulp waste contained protein 11.75 %; ash, 15.86 %; crude fibre, 21.55 %; starch, 32.14 %; dry matter, 93.5% and moisture, 6.5 % while the unfermented pulp contained protein 10.13 %; ash content, 14.14%; crude fibre 22.63%; starch, 28.20%; dry matter, 92.5% and moisture, 7.5%. The unfermented locust bean pulp waste contained some essential amino acids including methionine, lysine, leucine and isoleucine and some non-essential amino acids include histidine, proline, glycine and tyrosine. Concentrations of tannin and oligosaccharides were generally very low. The pulp waste was used wholly and partially to replace corn starch (yellow maize) as a binder in the preparation of the diet of cultured fish (*Clarias gariepinus*). Six diets were formulated using the pulp waste in various proportions. The binding power and the crumbling rate were assessed. The crumbling rate declined with increased inclusion of the pulp. The unfermented locust pulp waste exhibited a stronger binding effect than corn starch after 12 weeks storage. Diets stored in jute bags showed minor quality deterioration due to insect infestation, while diets stored in polythene bags maintained good quality throughout the study period. The use of locust bean waste can thus serve the dual purpose of (a) turning the waste into a useful and value-added farm product and (b) effective substitution for corn starch as a binder in fish feeds.

Keywords: Chemical composition, binding power, locust bean, fish diets.

1. Introduction

The need for on-farm produced feed mixtures for aquaculture, using locally available technology and feed ingredients in many developing countries has been advocated (Coche, 1995). The use of cheaper feedstuffs has shown great potentials in terms of their nutrient supply as well as reduction in feeding costs (Falaye, 1992, 1995). The African locust bean (*Parkia biglobosa* Jacq) is a perennial tropical legume (Campbell-Platt, 1980). In West Africa, the seed of this leguminous tree crop is fermented to yield a traditional food condiment called "dawadawa" or "iru" (Odunfa, 1981). The freshly harvested African locust beans are contained in pods. The pods contain a yellow dry powdery pulp in which the dark brown seeds are embedded.

The first major operation in the production of dawadawa is the removal of the locust bean seeds from the pods. One of the by-products of this operation is the yellow pulp, which forms a thick pulp when water is added. This locust bean pulp is usually an eyesore and a menace, causing pollution in the water and even in the environment where harvesting and large scale processing of this savanna crop is a viable trade.

The possibility of using this waste (pulp) as fish feed concentrates and binder deserves some trial. Over the years, it has been discovered that the by-products and waste materials from mills, mill packing, fish processing industries, oil seed processing and other processing industries have considerable feed value (Lu and Kevern, 1975; Edwards, 1980; Wee and Ng, 1986; Falaye, 1992, 1998). This study is one of the major evaluation works in the investigation of the nutritional qualities and binding potential of the waste.

As of date, little information is available on the nutritional composition of the yellowish pulp of *P. biglobosa.* Our preliminary analyses of the pulp shows that it has among others, a higher protein value compared to corn starch, which is commonly used locally as fish diet binder. In fish nutrition, binders are food and non-food materials, which are incorporated in food constituents to help bind the nutrients together thereby reducing the leaching of soluble essential nutrients.

The commonest binder used in fish feed formulation is starch which is in form of dextrin, carboxyl-methyl-cellulose (CMC), sodium bentonite, Guam gum, agar and gelatin, vegetable oils such as soybean oil which contain crude lecithin as binder (Wallace, 1985).

In Nigeria, there is now an awareness of the need for supplementary binders for fish feed formulation among the small-scale fish farmers (Akegbejo-Samsons, 1986). It is

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envisaged that finding local sources of binders will help to reduce the dependence on imported binders in the feed industries of Nigeria.

This paper reports the assessment of the nutrient quality of the pulp of the locust bean and its potential use as a binder in the fish feed industry.

2. Methods

Preparation of the experimental pulp

The pods of *P. biglobosa* were collected from the trees within the University of Agriculture, Abeokuta premises, and stored in jute bags for several weeks. The seeds were removed from the pods and soaked in water for about 12 h so as to separate the mass from the seeds. Natural fermentation occurs during this period, thus making the removal of the seeds to be easy. The pulp which were obtained before the 12 h fermentation period was regarded as the 'unfermented pulp', while those obtained after the 12 h fermentation were regarded as the 'fermented pulp'. In the normal fermentation production of 'dawadawa' from locust beans, these pulps are discarded as wastes as no local use has been found for them.

The pulp wastes that were obtained were dried for four days using sunlight energy. The resultant dried yellow pulp was then ground into powder using a blending machine.

Preparation of low cost fish diets

Six dry diets were prepared using common fish feed ingredients (Table 1). The ingredients were purchased locally. The diets were isonitrogenous with 40% crude protein value. The prepared diets had the pulp replaced wholly or partially with corn starch (from yellow maize), i.e. from 0-100% pulp inclusion levels at 20% incremental rates with corn starch as follow: Diet 1: 100% cornstarch (control diet); diet 2: 20% pulp & 80% cornstarch; diet 3: 40% pulp & 60% cornstarch; diet 4: 60% pulp & 40% cornstarch; diet 5: 80% pulp & 20% cornstarch; diet 6: 100% pulp (no corn starch).

Proximate analysis of the diets

The proximate analysis of the diets was determined on dry weight basis using the AOAC (1990) methods. The analyses were carried out in triplicates.

Tannin contents of the pulp powder were assessed using the method described by Makkar and Goodchild (1996). The amino acid contents were assessed using colorimetric analysis (Rosen, 1957). The chromatographic estimation of the amino acids were carried out using the thin-layer chromatography plate guide strip in locating the position of amino-acid on plates.

Evaluation of binding characteristics of the pulp/Binding power assessment

The cohesive/binding power of the pulp was assessed by subjecting the diets containing the pulp powder to series of local shearing and shaking processes such as running the diets through hard plastic containers and polythene containers. The rate of crumbling were observed and assessed as follows: (a) A crumblier (a roller mill with rolls specially designed for breaking up pellets into smaller particles) was used. The diets were passed through the crumblier and the rate of crumbling assessed by comparing the initial sizes and conformation (before passing through) of the pellets with the final sizes after passing through the crumblier.

(b) A sifter (an oscillating separator with a number of screens) was further used to examine the particle sizes of the different diets.

Storage quality assessment

The prepared diets were stored in jute bags and polythene bags for a period of 12 weeks at ambient condition and temperature temperature (29°C $\pm$ 3°C). Appearance and odour were critically assessed in all the diets. The assessment of the diets during storage was carried out by a panel of five assessors made up of the students and staff of the Department of Home Sciences Studies of the University of Agriculture, Abeokuta. Samples of the prepared diets were compared with a control sample of freshly prepared fish feed from one of the industries. The physical appearance and odour were assessed using a three-point score method. The scoring system for the appearance and odour was as follows:

→3 = appearance and odour similar to control sample.

→2 = appearance and odour slightly different from control sample.

→1 = appearance and odour different from control sample and unappealing.

Statistical Analysis

Data obtained from all analyses were subjected to statistical analysis using analysis of variance (ANOVA) and correlation analysis system programme (SAS, 1988).

3. Results

Proximate composition of fermented and unfermented pulp

The proximate composition and dry matter (DM) contents of the fermented and unfermented pulp waste are presented in Table 1. The crude protein content of the unfermented pulp is slightly higher than that of the fermented pulp. The dry matter value of the fermented is however higher than that of the unfermented. Results from this work show that there is an increase in the fat, ash and crude protein contents in the fermented samples compared to the unfermented samples respectively (1.38, 15.86 and 11.75 %). The sugar content in the unfermented samples was higher than the fermented waste.

Decrease in crude fibre, carbohydrates and moisture content in the fermented samples was noticed, this is due to the softening of the pulp during the fermentation period.

The samples were strongly acidic with pH of 3.22 and 2.98 for fermented and unfermented pulp respectively. Both fermented and unfermented pulp were found to be very rich in

Table 1: Dry matter and proximate composition of the African locust bean pulp (waste) pulp.

COMPONENTS (%)	FERMENTED WASTE	UNFERMENTED WASTE
Moisture	6.50± 0.87	7.50± 0.87
Dry matter (DM)	93.50± 0.88	92.50± 0.67
Ash content	15.86±0.15	14.14± 0.03
Crude Fibre content	21.55± 0.04	22.63± 0.28
Crude protein content	11.75± 0.12	10.13± 0.06
Fat content	1.38± 0.03	1.30± 0.07
Sugars	8.94± 0.19	13.32± 0.58
Starch	32.14± 0.57	28.20± 0.09
NFE	28.70± 0.30	26.30± 0.02
Carbohydrates	44.06± 0.01	46.26± 0.70
pH	3.22±0.05	2.97± 0.05

Table 2: Amino acid profile of the African locust bean pulp (waste) pulp.

Name of amino acid	Fermented waste (mg/100g)	Unfermented waste (mg/100g)
Histidine	1.39± 0.02	1.94± 0.03
Methionine	0.55± 0.04	0.74± 0.03
Lysine	3.15± 0.04	3.89± 0.02
Leucine	4.26± 0.01	3.79± 0.01
Isoleucine	2.59±0.01	2.22± 0.02
Proline	6.67± 0.04	6.57± 0.01
Phenylalanine	3.70± 0.02	3.52± 0.02
Cysteine	1.11± 0.02	1.29± 0.01
Threonine	1.01± 0.02	1.20± 0.02
Glycine	2.32± 0.01	2.04±0.02
Tyrosine	2.41±0.02	1.85± 0.01

Table 3: Tannin and Oligosaccharides contents of the African locust bean pulp (waste) pulp.

Contents/ Form	Fermented waste	Unfermented waste
Tannin (mg/100g)	0.020± 0.001	0.520± 0.002
Oligosaccharides (%)	0.002± 0.001	20.22± 0.03

carbohydrates, this could be a viable substrate for agar or other microorganisms culture. There was no significant difference ($P<0.05$) in all values between the fermented and unfermented pulp, therefore either could be used as a binder in fish feed. When compared to that of yellow maize/corn starch, which had a lower crude fibre value (3.0) and a lower protein content value (9.5) respectively, it can therefore be concluded the fairly high crude fibre and protein contents of this pulp will make it a suitable source of feed for fish (Akegbejo-Samsons, 1999).

There was a very close similarity in proximate composition of the fermented and unfermented, except for the sugar. This may likely be due to sampling methods and probably not necessarily due to the effects of fermentation.

Amino acid profile of fermented and unfermented pulp

Table 2 shows the amino acid profile of both fermented and unfermented pulp waste. There were 11 amino acids identified in the samples at various levels of concentration. Proline had the value of 6.67 for the unfermented and 6.57 for the fermented. This is followed by leucine and phenylalanine in a descending order.

The lowest amino acid value in the samples was methionine. It can be inferred that inclusion of the pulp to fish feed will improve the amino acid supplementation of such feeds, when compared with the results obtained from the work of Akegbejo-Samsons (1999).

Tannin and oligosaccharides content

Table 3 shows the tannin and oligosaccharides contents in the samples of both fermented and unfermented pulp waste. The tannin content is very insignificant ($P<0.05$) with a value of 0.02 mg/100g for the fermented and 0.52mg/100g for the unfermented pulp. In spite of this low value, fermentation was observed to lower the value the tannin in the samples. The low tannin content suggest the ease of digestibility of the formulated feeds containing the pulp powder. Aletor (1993) observed that high molecular weight condensed tannins have limited solubility and extractability. Low tannin level in the pulp of the African locust bean causes good growth response and nutrient utilization in some fish species as shown in the work carried out by Akegbejo-Samsons and Olagunju (2002) similar feed of this component was fed to *Clarias gariepinus.*

The results of this study show that fermented pulp of the African locust bean pulp is nutritionally rich, and can be added as thickening agent, binder or energy source to fish feed. Its usefulness in supplementation and fortification of maize flour based diets can be well considered. It is envisaged that the environmental 'mess' that is left after the processing and extraction of the seeds can be considerably reduced by over 80% by the utilization of the pulp.

Gross and proximate composition of the diets

The different ingredients used for the preparation of the fish diets, gross and proximate composition of the diets used to assess the binding ability of the pulp is presented in Table 4, while the proximate composition of the diets is presented in Table 5.

The crude protein of the diets ranged from 39.6 % in diet 3 to 40.1 % in diet 5. Moisture content was least in diet 5 with a value of 5.88 and highest in diet 6 (8.72). Crude fibre ranged from 5.23% in diet 1 to 4.73 in diet 6.

Sensory, crumbling and binding evaluation

These diets were tested for sensory, crumbling and binding qualities.

The appearance and odour of all the 6 diets after storage inside the freezer (-4°C) for over 3 weeks remained unchanged. However the appearance and odour of all these diets changed considerably when stored in jute bags at room temperature. This was due largely to insect infestation and bacteria attack. Diets stored inside jute bags at room temperature did not show any infestation. The initial sweet smell was retained while the pelleted sizes were maintained. Based on the judgment of the assessors, the diets had a graded point of 3 for storage in freezer, and 2 for storage in jute bags and polythene bags at room temperature. However, all the diets had similar appearances and odour to the control feed sample when stored in the freezer. When they were stored in jute bags and polythene bags at room temperature, there were slight differences in appearance and odour when compared with the control sample. This shows that if well stored under hygienic conditions, diets prepared from *P. biglobosa* pulp will not loose its odour and appearance over a long time.

Crumbling evaluation and Binding power

After preparation and storage of diets for over 12 weeks, it was observed that diets with high concentration of pulp crumbled less than those with less concentration of the pulp, when passed through the crumblier. In other words, diet 6 with 100% pulp maintained the binding quality, having minimal crumbles. The control diet (diet 1) with 100% corn crumbled gradually into powder over time. Diet 5 (80% pulp) was next to the control diet. This was followed by diets 4, 3 and 2.

This study shows that the crumbling rate of diets containing higher quantity of corn starch as a binder (diets 1,2 and 3) were faster in crumbling than those with lesser inclusion of *P. biglobosa* pulp. They (those with higher cornstarch inclusion) were found with greater quantities of fine particles after passing through the sifter. However diets with higher quantities of *P. biglobosa* pulp had a higher sticking and binding properties for the fish diets when compared to corn starch.

4. Conclusion

A critical look at the demand for livestock feedstuffs, such as cereals by man for his own consumption, shows a precarious situation for both the human populace and his animals in Nigeria. Yellow maize, from which cornstarch is obtained is seasonally available at exhorbitant prices in the northern part of country, while *P. biglobasa* pulp waste is

Table 4: Gross composition of experimental diets (g)

Ingredients	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Corn starch	25.79	14.10	10.57	7.05	3.52	-
Pulp	-	3.52	7.05	10.57	14.10	24.68
Fish meal	16.34	18.38	18.38	18.38	18.38	16.61
Soybean meal	49.00	55.15	55.15	55.15	55.15	49.83
Bone meal	2.50	2.50	2.50	2.50	2.50	2.50
Oyster meal	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin/Premix	0.60	0.60	0.60	0.60	0.60	0.60
Salt	0.25	0.25	0.25	0.25	0.25	0.25
Vegetable oil	5.00	5.00	5.00	5.00	5.00	5.00
Total	100.00	100.00	100.00	100.00	100.00	100.00
[1]CPV (%)	40	40	40	40	40	40

[1]CPV= Calculated Protein Value

Table 5: Proximate compositions of the experimental diets, pulp and yellow maize

Nutrients→ Diets↓	Moisture content	Ash content	Crude protein	Fat content	Crude fibre	NFE
Diet 1	5.68	14.96	39.8	6.32	5.23	28.01
Diet 2	6.74	13.42	39.9	6.04	5.14	28.16
Diet 3	8.68	11.96	39.6	5.52	4.98	29.26
Diet 4	7.45	10.74	39.7	5.36	4.88	31.87
Diet 5	5.88	10.74	40.1	4.98	4.76	34.26
Diet 6	8.72	9.94	39.7	4.88	4.73	32.03
Pulp	9.34	12.30	7.9	2.25	18.94	49.23
Yellow maize	6.90	2.80	9.5	4.10	3.00	73.70

readily available from the African locust bean. The animal production sector in Nigeria spends over N4 billion every year sourcing for yellow maize. A reduction in the quantity of yellow maize when the locust pulp is incorporated is envisaged. As a result an estimated amount of about N1 billion would be saved by the feed industries.

Apparently, the use of this pulp waste is a viable step towards the recycling of the pulp, which constitutes environmental menace and economic waste in areas where the locust bean trees are harvested and processed. From the result of this study, it is evident that the pulp can be effectively substituted for cornstarch to obtain a cheaper fish feed for the culture of some fish species. The use of this pulp will further reduce the competition for yellow maize in the country.

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RESOURCE QUANTIFICATION OF A KAOLIN DEPOSIT USING THE ELECTRICAL RESISTIVITY METHOD – CASE STUDY FROM IKERE EKITI, SOUTHWEST NIGERIA

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Abstract

The vertical electrical sounding (VES) involving Schlumberger electrode configuration has been used to investigate a kaolin deposit at Ikere in Ekiti State of Nigeria. The survey which covered an areal extent of 20, 000 square meters, involved a sum total of 15 VES stations.

Four geoelectric layers were delineated from the survey area. The first layer is the topsoil whose thickness varies from 0.7 m to 1.5 m and resistivity values range from 150 to 1150 ohm-m. The second layer is lateritic clay with thicknesses ranging from 4.0 to 17.5 m and resistivity values of between 217 and 1460 ohm-m

The third layer which is the kaolin has thicknesses ranging from 19 m to 99.5 m and resistivity values range from 105 to 485 ohm-m. The fourth layer is the basement bedrock. The resistivity values of the bedrock range from 4370 ohm-m to infinity.

The investigated area was divided into eight square blocks. The sum of the product of the average thicknesses of the kaolin deposit per block and the surface area of each block was multiplied by an average density, 2.45 gcm^{-3} of the kaolin to determine the reserve of the deposit which was estimated at 2,732,305 tonnes. The volume of the excavable overburden was estimated at 219,818.9 cubic meters. The kaolin deposit can be open mined.

Keywords: Kaolin deposit, resistivity, reserve quantification.

1. Introduction

Electrical resistivity methods involve measurement of apparent resistivity of subsurface materials as a function of depth or position. The resistivity measured is a complex function of porosity, permeability, ionic content of the pore fluids, and clay mineralization. Hence different rocks will exhibit marked differences in resistivities. In varieties of rocks, variation in electrical resistivity will be accompanied by a discernable variation in lithology and chemical composition. Useful information on the structural disposition of bedrock and nature of subsurface can be obtained from surface distribution in resistivity, thus making electrical resistivity applicable to a number of areas of interest.

The electrical resistivity method is commonly used in engineering site investigation. It is relevant in depth to bedrock determination, structural mapping, determination of nature of superficial deposits etc (e.g., Early and Dyer, 1964; Bisdorf, 1985; Olorunfemi and Mesida, 1987; Lucius and Bisdorf, 1995; Bisdorf, 1996). It has become one of the most useful tools in investigations for groundwater e.g., Odufisan (1991) and Olorunfemi and Fasuyi (1993). The method can provide useful and relatively low-cost information on the extent and thickness of fresh-water lenses. Traditionally, vertical electric sounding (VES) has been used for this purpose (Fretwell and Stewart, 1981; Ayers and Vacher, 1986; Kauahikaua, 1986). The electrical resistivity method has proved successful in the mapping of salt water interfaces in many different hydrogeologic settings (Swart and Stewart, 1981; Olorunfemi 1985; Fretwell and Stewart, 1981; Zohdy *et al.*, 1993). The resistivity method is also used for mineral investigations e.g., Adepelumi and Olorunfemi (2000), Olowolafe (1991) used vertical electrical sounding (VES) to survey a kaolin deposit at the Ululuku area of Delta State.

In this study, an electrical resistivity survey involving vertical electrical sounding (VES) technique was carried out for the determination of the reserve of a kaolin deposit at Ikere in Ekiti State (Figure 1). It also aimed at the estimation of the excavable volume of the overburden over the deposit.

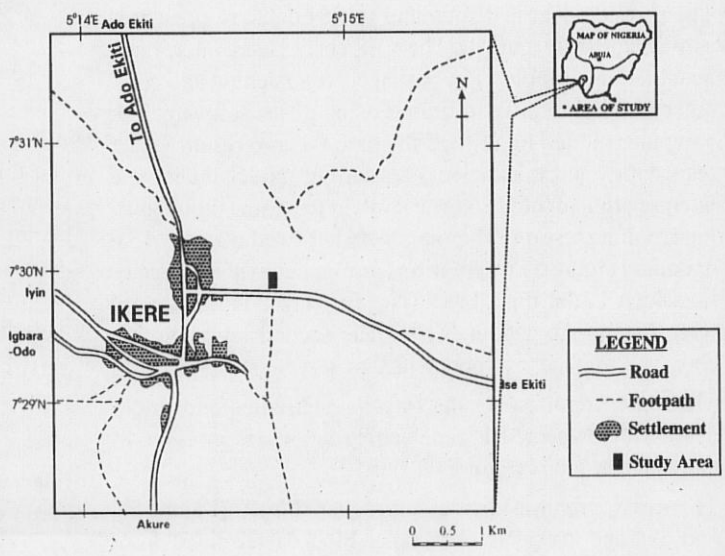

FIGURE 1. LOCATION MAP, SHOWING THE STUDY AREA
(EXTRACTED FROM FEDERAL SURVEY MAP, NIGERIA, 1967)

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Kaolin is a clay composed essentially of the mineral kaolinite. It is characterized by its white colour that may be changed according to the level of impurities present. Its melting point is 1785 °C and about 5 to 50 microns in particle size. It is formed as a result of the decomposition of the alumina - silicate minerals, especially the feldspars and hydrothermal alteration (Singh and Gilkes, 1991).

Kaolin is mostly used for chinaware, stoneware and refractories. It is also used in making various types of pottery, fillers in the manufacturing of paper, insecticides and pharmaceutical products. It also finds usage in paint making industry.

2. Geology of the area

Ikere-Ekiti is underlain by rocks of the basement complex of Nigeria. The Nigerian basement complex consist of six major groups or petrological units (Rahaman, 1988). The area investigated is underlain by rocks of the Older Granites suite and Charnockitic rocks. These two groups of rocks form the prominent topographic features within the area. The charnockitic rocks contain quartz, plagioclase, orthopyroxene, ± alkali feldspar ± fayalite ± clinopyroxene ± biotite ± hornblende (Rahaman, 1988). The Older Granite suite on the other hand consists of quartz, alkali feldspar, plagioclase, hornblende, biotite ± zircon, ±sphene, ± apatite (Rahaman, 1988). The parent rock of the investigated deposit, based on information from the geology of the area is coarse-grained charnockitic rock (Figure 2).

3. Methodology

In an attempt to determine the reserve of the kaolin deposit, as well as volume of excavable overburden, the vertical electrical sounding (VES) involving the Schlumberger array was used. The electrode spacing (AB/2) was varied from 1 – 100 m. A total of 15 sounding stations were occupied. The VES stations are shown in Figure 3.

A test pit was also dug in the premises of the survey area (Figure 3) to provide information on the subsurface sequence.

The vertical electrical sounding (VES) data are presented as VES curves (Figure 4). The VES curves were interpreted quantitatively (Table 1) by partial curve matching and computer assisted iterative technique using Resist software. The computer modelling utilised the partial curve results (layer resistivities and thicknesses) as starting models. Geologic interpretation of the VES results was aided by the lithological log from the test pit. The pit penetrated two layers at 15.0 m depth (Figure 5) where it was terminated within the kaolin which is the third layer. The first layer is the topsoil with thickness of about 1.2 m. The second layer lateritic clay with thickness of about 12.0 m.

The interpretation results (layer resistivities and thicknesses) are presented as geoelectric sections (Figures 6 – 8) and isopach maps (Figures 9 and 10).

The survey area was divided into square blocks (Figure 11) and average kaolin thickness per block determined from the isopach map. The volume of the kaolin deposit per block was calculated from square block surface area and the average thickness within each block. The summation of the volumes of the eight blocks gave the total volume of the kaolin deposit. The density of the kaolin was determined using simple displacement method. A standard density bottle was weighed and its weight noted. It was then filled with distilled water, covered and weighed. This weight was recorded as W_w. The water was discarded and 50g of air-dried sample was added to the bottle and filled with distilled water. The whole mixture was shaken together and filled with distilled water regularly to make sure that the bottle was filled to the brim. The weight was recorded as W_T. The procedure was repeated for each of the samples and density was computed from Equation 1.

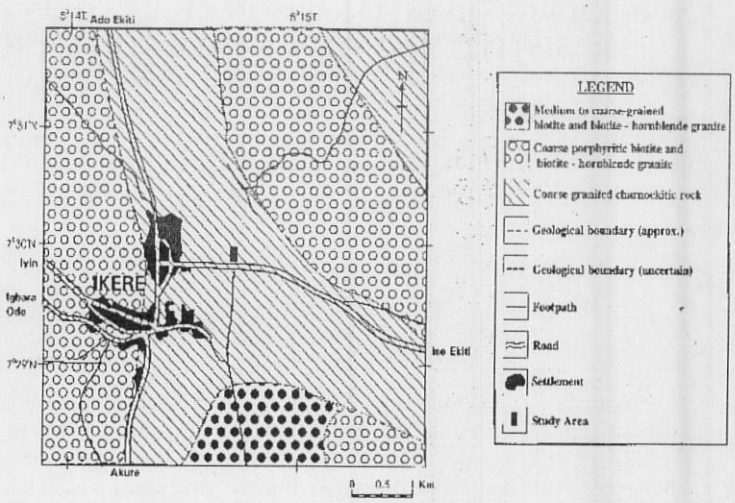

FIGURE 2. GEOLOGICAL MAP OF IKERE -EKITI AREA OF EKITI STATE (Modified, After Olarewaju 1987)

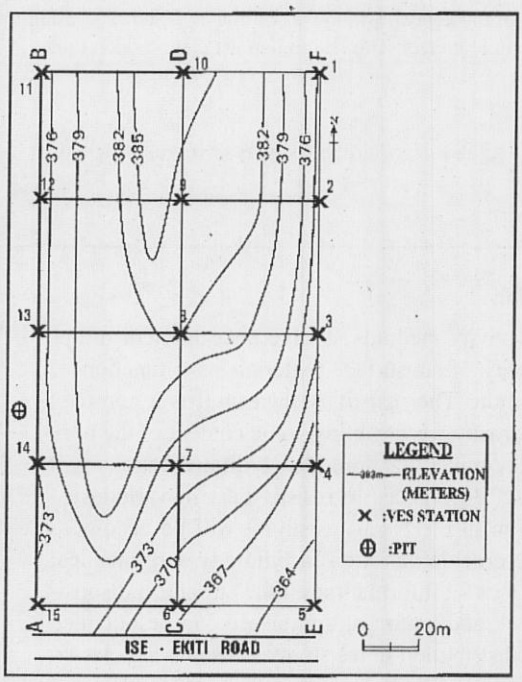

FIGURE 3. TOPOGRAPHICAL MAP OF THE AREA SHOWING THE VES STATIONS.

TABLE 1. VES INTERPRETED RESULTS.

VES STATION NUMBER	DEPTH (D_1 / D_2 / D_3 / . . . / D_n) (m)	LAYER RESISTIVITY (ρ_1 / ρ_2 / ρ_2 / . . . / ρ_n) (ohm-m)	CURVE TYPE
1	0.86 / 1.73 / 3.7 / 34	620 / 395 / 975 / 105 / ∞	HKH
2	1.18 / 4.47 / 10.3/ 40.2	358 / 1074 / 217 / 116 / ∞	KQH
3	0.75 / 10.5 / 30.0	520 / 636 / 130 / ∞	KH
4	1.08 / 4.43 / 13.5 / 60	380 / 242 / 480 / 105 / ∞	HKH
5	0.95 / 7.41 / 8.70/ 71.1/ 106	190 / 285/ 52 / 115 / 131/ ∞	KHKH
6	0.84 / 11.5 / 70.2	190 / 1350 / 165 / ∞	KH
7	1.1 / 14.2 / 70.46	220 / 440 / 105 / ∞	KH
8	1.43 / 5.29 / 29.8 / 73.92	325 / 163 / 300 / 172 / ∞	HKH
9	1.41 / 106 / 63.48	180 / 420 / 205 / 4370	KH
10	1.43 / 14.73 / 58.62	230 / 427 / 210 / 9750	KH
11	1.38 / 17.94 / 49.40	20 5/ 615 / 331 / 8740	KH
12	1.12 / 15.68 / 69.55	300 / 700 / 361 / ∞	KH
13	1.5 / 13.8 / 90.36	1150 / 1406 / 156 / 7020	KH
14	1.36 / 7.5 / 95.2	150 / 1350 / 485 / 9120	KH
15	0.72 / 7.2 / 78.5	318 / 389 / 206 / ∞	KH

TABLE 2. ESTIMATION OF VOLUME OF KAOLIN FROM ISOPACH MAP.

BLOCK	NUMBER OF SOUNDING POINT/SAMPLE CONTOUR LINE	THICKNESSES FROM CONTOUR LINE/VES STATION (m)	MEAN THICKNESS (m)	AREA OF BLOCK (m^2)	VOLUME OF KAOLIN (m^3)
SQ1	14	33,34,36,38,40,42, 44,46,48,50, 51.5,52,54.5.	40.64	2500	101,607.14
SQ2	16	54.5,51.5,67.5, 76,74,72,70,72, 74,76,78,80,82,83.	62.84	2500	157,100
SQ3	14	60,61,64,66,67.5, 68,70,72,74,76,78, 80,82,83.	71.61	2500	179,017.86
SQ4	14	60,62,64,66,68, 70,71.5,72,74,76, 78, 80,82,83.	71.89	2500	179,732.14
SQ5	14	31,31.5,32,34,36, 38,39,40,42,44,46, 48,50,51.5.	40.21	2500	100,535.71
SQ6	28	19,20,22,24,26,28, 30,31.5,32,34,36, 38,40,42,44,46,48, 50,51.5,52,54,56, 58,60,62,64,66,67.5.	42.91	2500	107,276.79
SQ7	28	19,20,22,24,26,28, 30,32,34,36, 38,40,42,44,46,48, 50,52,54,56, 58,60,62,64,66,67.5.	43.01	2500	107,548.08
SQ8	28	46,48,50,52,54,56, 58,60,62,64,66,68, 70,72,74,76,78,80, 82,84,86,88,90,92, 94,96,98,99.5.	72.96	2500	182,410.71

TOTAL VOLUME: 1,115,228.43m^3

TABLE 3. ESTIMATION OF VOLUME OF OVERBURDEN FROM ISOPACH MAP.

BLOCK	NUMBER OF SOUNDING POINT/SAMPLE CONTOUR LINE	THICKNESSES FROM CONTOUR LINE/VES STATION (m)	MEAN THICKNESS (m)	AREA OF BLOCK (m^2)	VOLUME OF KAOLIN (m^3)
SQ1	7	17.5,15,12,16,17, 14,13.	14.93	2500	37,321.43
SQ2	10	7,8,9,10,11,12,132,14,15,16.	11.5	2500	28,750.00
SQ3	11	7,7.5,8,9,10,11,12, 13,14,14.5,15.	10.97	2500	27,431.82
SQ4	11	7,7.5,8,9,10,11,11.5,12, 13,15.	10.68	2500	26,704.55
SQ5	12	4,5,6,7,8,9,10,11,12, 13,14,15.	9.5	2500	23,750.00
SQ6	6	7,8,9,10,11,12.	9.5	2500	23,750.00
SQ7	9	7,8,9,10,11,12,13,14, 14.5.	10.94	2500	27,361.11
SQ8	10	7.5,8,9,10,11,11.5, 12,13,14,14.5.	9.9	2500	24,750.00

TOTAL VOLUME: 219,818.91m^3

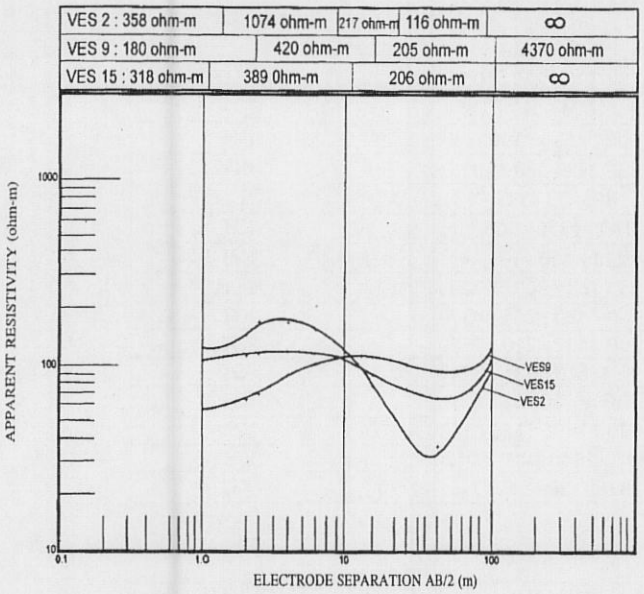

Figure 4. Typical depth sounding curves for the area of study.

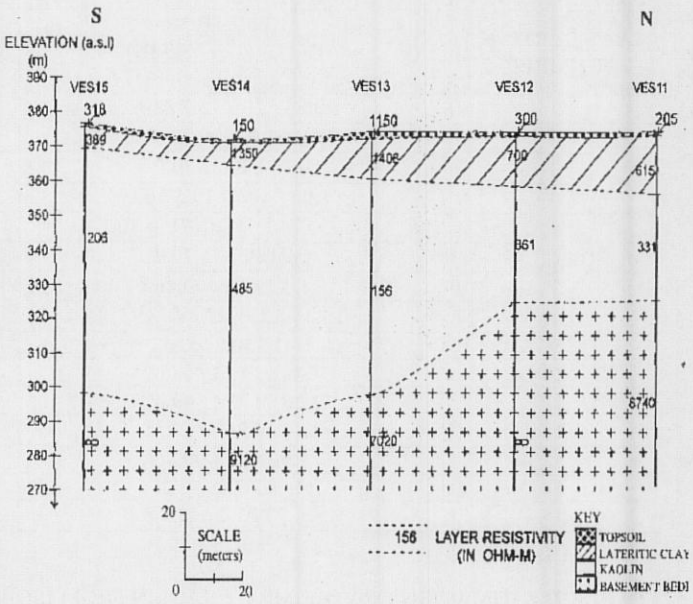

FIGURE 6. THE GEOELECTRIC SECTION ALONG TRAVERSE 1

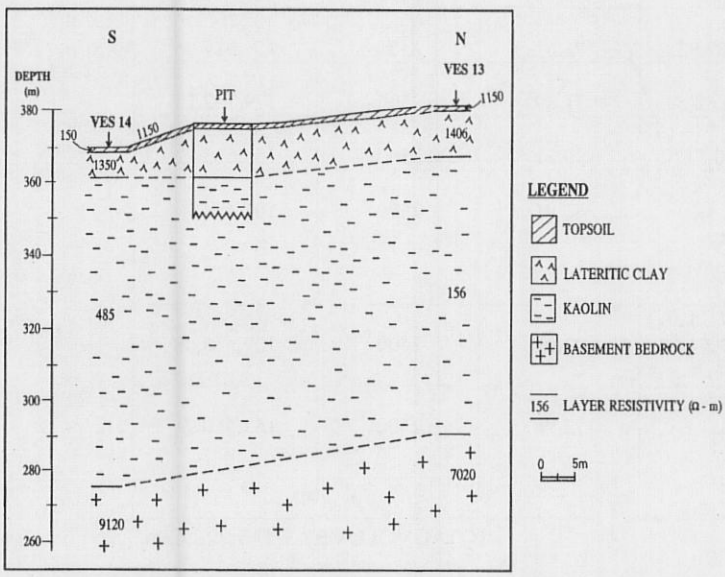

FIGURE 5: CORRELATION OF VES INTERPRETATION RESULT (VES 14 AND 13) WITH PIT LITHOLOGICAL LOG.

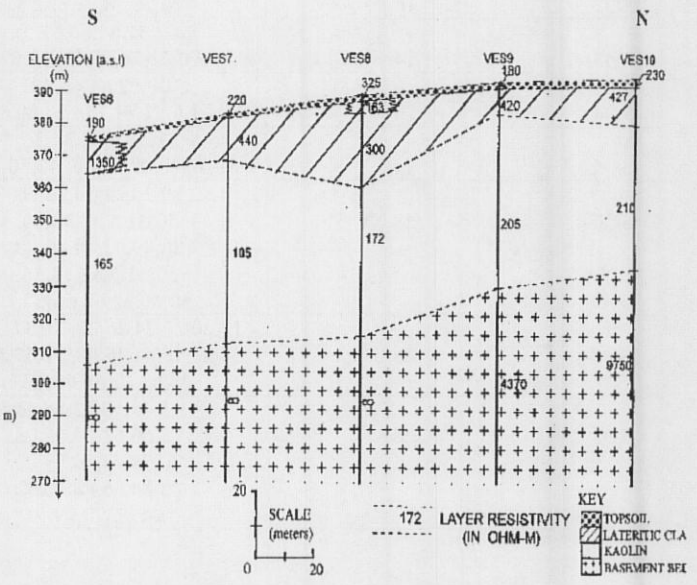

FIGURE 7. THE GEOELECTRIC SECTION ALONG TRAVERSE 3

$$d_s = \frac{W_s}{W_s - (W_T - W_w)} \qquad (1)$$

where d_s, W_s, W_w, W_T are density of sample, weight of air-dried sample, weight of water and density bottle and weight of bottle with water and air-dried sample respectively.

The average value of the density calculated was used in the determination of the reserve (tonnage) of the kaolin deposit. The volume of the excavable overburden was calculated in a like manner, as for the volume of kaolin deposit.

4. Results and Discussion

Three geoelectric sections were drawn along traverses 1,2,3 all trending in the north – south direction. The geoelectric section along traverse 1 (A – B) in Fig. 6, shows four distinct layers namely topsoil, lateritic clay, kaolin and basement. The topsoil has thicknesses varying from about 0.7m to 1.5m while its resistivity values vary from 150 to 1150 ohm-m. The second layer is lateritic clay with resistivity values ranging from 389 to 1350 ohm-m and thicknesses ranging from about 5m to 15m. The third layer is the kaolin deposit. The layer resistivity values vary from 156 – 485 ohm-m. While the thickness varies from 32m to 83m. The fourth layer is the basement bedrock with resistivity values of 7020 ohm-m and above.

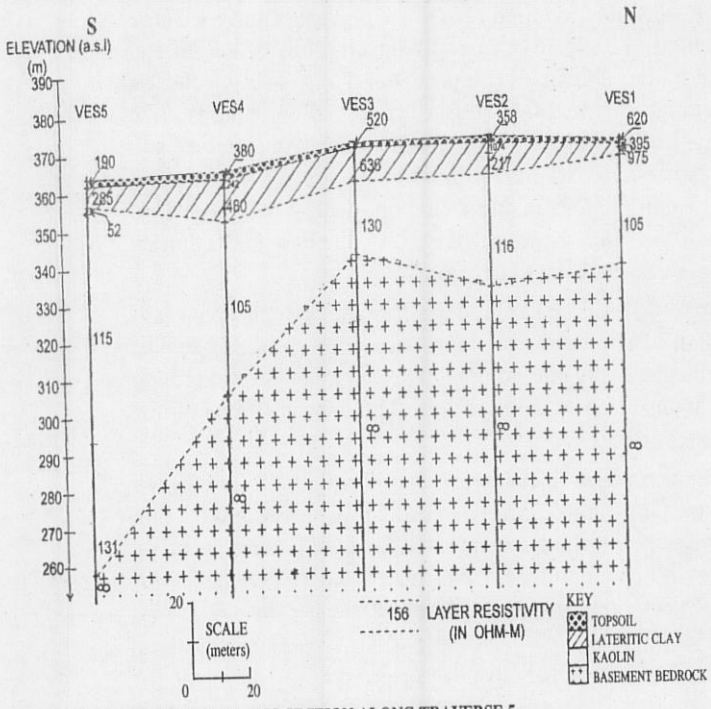

FIGURE 8. THE GEOELECTRIC SECTION ALONG TRAVERSE 5

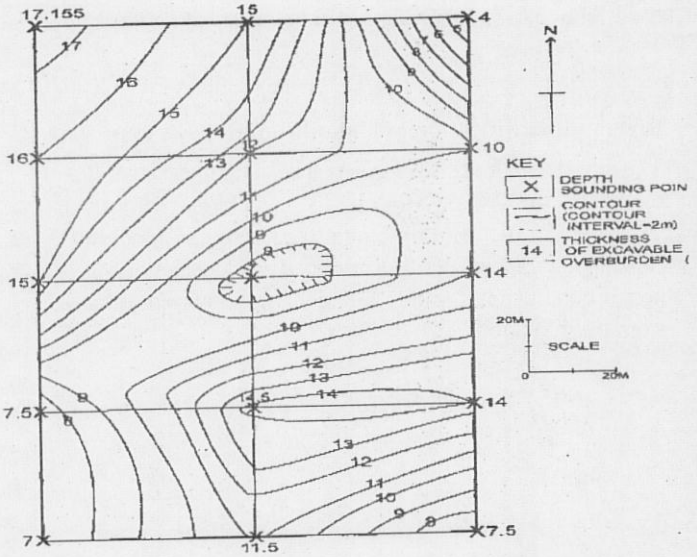

FIGURE 10. ISOPACH MAP OF OVERBURDEN

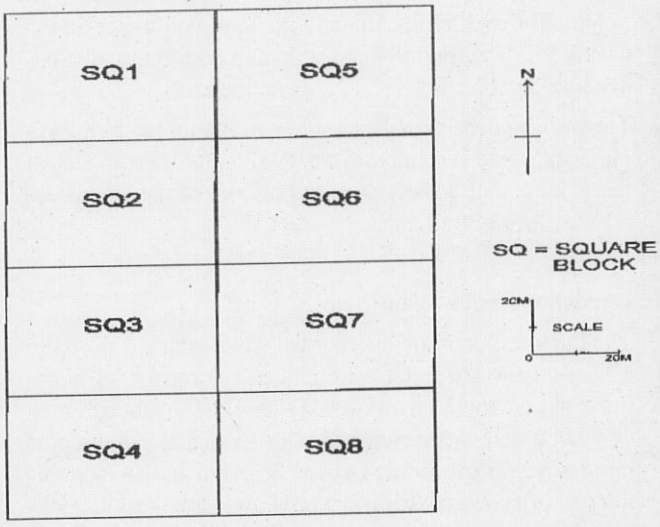

FIGURE 11. MAP THE AREA UNDER INVESTIGATIONS BROKEN INTO SQUARE BLOCKS.

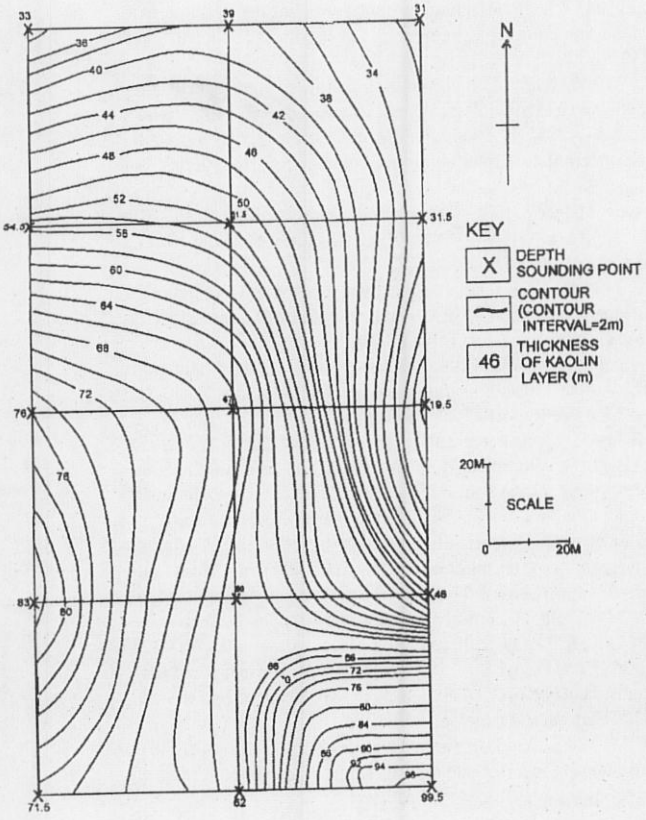

FIGURE 9. ISOPACH MAP OF KAOLIN

The geoelectric section along traverse 3 (C – D) in Fig. 7, also shows four geoelectric layers. The first layer is the topsoil with thicknesses varying from 0.8m to1.4m while its resistivity values vary from 190 to 325 ohm-m. The second layer is lateritic clay with resistivity values ranging from 163 to 1350 ohm-m and thicknesses ranging from 5m to 13m. The third layer is kaolin deposit with thicknesses varying from 43m to 62m and resistivity values ranging from 195 to 219 ohm-m. The fourth layer is the basement bedrock with resistivity values ranging from 4370 ohm-m to infinity.

The geoelectric section along traverse 5 (E – F) in Fig. 8, shows four geoelectric layers, just like the first two above. The first layer, which is the topsoil, varies in thickness from about 0.8m to 1.2m while its resistivity values range from 190 – 620 ohm-m. The second layer is lateritic clay having resistivity values, which range between 217 and 1074 ohm-m. The thickness of the lateritic clay varies between 3m and 13m. The third layer is kaolin with layer resistivity values varying from 105 to 131 ohm-m and thicknesses of between 19m and 99.5m. The fourth layer is the basement bedrock with an infinite resistivity.

Isopach map of kaolin deposit

The thicknesses of the kaolin obtained from the VES interpretation results were plotted against the VES stations and

contoured as shown in Fig. 9. A 2m contour interval was used. The map shows variation in thickness of kaolin from 19m to 99.5m.

Reserve quantification of kaolin from isopach map

The site under investigation has a rectangular shape. The rectangle was broken into eight square blocks (Fig. 11). The average thickness of kaolin in each square block was calculated from the VES determined thicknesses and values of contour lines within the block as shown in Table 2 from the Equation 2.

$$h_B = \frac{\sum_1^N h_N}{N} \qquad (2)$$

where, h_B, h_N, N are average thickness of deposit per block, value on the nth isopach contour within the block and the number of contours running across the block respectively.

Table 2 shows the estimated kaolin thicknesses, areas and volume for each block unit and the total volume of kaolin calculated. The total volume of kaolin within the area under investigation is 1,115,228 cubic meters.

The average density of the kaolin was determined from simple displacement method to be 2.45g/cm^3. The product of the volume and density gave the reserve of the kaolin deposit as 2,732,305 tonnes.

Isopach map of overburden

The thicknesses of the overburden were obtained from the VES data and the result were plotted against the VES stations and contoured as shown in Fig. 10. The isopach map of overburden was developed in order that the volume of the excavable overburden could be estimated. A contour interval of 1m was used. The map shows variations in thickness of the overburden from 4m to 17.5m.

Estimation of volume of overburden from isopach map of overburden

The volume of the excavable overburden was calculated in the same manner as the volume of kaolin was calculated. That is, the survey area was broken into block and superimposed on the isopach map of overburden. The average thickness per block was determined from the contour values and VES results. The summation of the product of the area of each block and the average thickness (Table 3) gave the volume of the excavable overburden as 219,818.91m^3.

Conclusion

This paper describes the electrical resistivity survey of the Ikere kaolin deposit. The survey involved a total of 15 Schlumberger vertical electrical resistivity soundings (VES) uniformly distributed within the survey area. The electrode separation (AB/2) varied from 1m to 100m. The vertical electrical sounding (VES) data were presented as VES curves interpreted quantitatively by partial curve matching and computer aided iterative technique using Resist software.

The VES interpretation results are presented as geoelectric sections (Figures 6 – 8) and isopach maps of kaolin and overburden thickness (Figures 9 and 11). The geoelectric sections identified four subsurface layers – topsoil, lateritic clay, kaolin and the basement. The layer thicknesses and resistivities for the upper three layers are 0.7 – 1.5m; 3 – 15m and 19 – 99.5m and 1150 ohm-m; 163 – 1406 ohm-m and 105 – 485 ohm-m respectively. The basement bedrock is in most places infinitely resistive.

The reserve of the kaolin deposit in the area under investigation is estimated to be approximately 2,732,305 tonnes while the excavable volume of overburden is estimated to be about 219,819 cubic meters. The deposit can be mined economically.

The overburden thicknesses range from 4 to 17.5m, hence surface mining technique could still be adopted. The geoelectric sections indicate that the kaolin layer probably extends beyond the area investigated. Therefore, the kaolin deposit could be investigated beyond the present study area.

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HIGHWAY PAVEMENT FAILURE INDUCED BY POOR GEOTECHNICAL PROPERTIES AT A SECTION ALONG THE F209 OKITIPUPA – IGBOKODA HIGHWAY, SOUTHWESTERN NIGERIA

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Abstract

Failure of highway pavement is a common feature on many major highways in Nigeria. Extensive field and laboratory investigations on kilometer 20 to kilometer 25 along the F209 Okitipupa– Igbokoda highway, Southwestern Nigeria, revealed that the road was deformed by the development of potholes resulting from poor physical properties of the pavement subsoil materials.

Laboratory soil mechanics tests carried out on the disturbed soil samples collected from the failed sections of the road showed that the natural soil moisture ranges from 7.10% to 9.7%, liquid limit from 22% to 43.50%, linear shrinkage from 2.30 to 5.20, and the specific gravity from 2.60 to 2.66.

The California bearing ratio (CBR) value was 55% showing a considerable reduction in strength as a result of surface water ingress into primary cracks that later developed into potholes. X-ray diffraction studies showed the presence of abundant kaolinite peaks and a subdued goethite peak without any trace of montmorrilonite. The presence of excess fines in the pavement construction materials (soils) contributed to the failure of the highway pavement at this locality. The low CBR value is also a noted cause of highway pavement failure.

Keywords: Kaolinite, potholes, pavement failure.

1. Introduction

As far back as the pre-independence times, highway pavement failure had been a common phenomenon on Nigeria roads (Pollit, 1950; Jegede, 1998). The rate, frequency and magnitude of pavement deformation in virtually all major highways in Nigeria have reached an alarming proportion. Various forms of road deformation features characterise most major highways in Nigeria, particularly Southwestern Nigeria. However, the most common of these road deformation features include, cracking, corrugation; potholes, pavement incision; routing and rutting.

Occasionally, flooding of highways resulting from "bathub" on the road surface and defective road shoulder due to lack of drainage facility constitute some of the major deformation features on Nigerian highways (Chukweze, 1988; Jegede, 1998).

There is no gainsaying the fact that transportation systems in general and the highway network in particular are indispensable physical infrastructure. Over 80% of Nigeria's peoples are agrarian and most of the food and cash crops produced need to be transported from the locality to another, either for sale or for consumption purposes. The railway network is poor especially in the Southwest and air travel is very expensive. Therefore, the highway system still constitutes the primary mode for movement of passengers and freight (Jegede, 1998, 1999, 2000).

Good roads will certainly create a tremendous multiplier effect on the Nigerian national economy. There is therefore the need for the highways to be in good motor-able condition at all times. The development of good roads in Nigeria is a pre-requisite to the overall economic growth and technological advancement of the nation in all ramifications (Jegede, 1999).

Okitipupa is a recognized commercial centre in Ondo State of Nigeria. Ondo State is so richly blessed with vast commercial deposits of various minerals and very large forest resources. There is a very large plantation of palm trees at Okitipupa where the State Government had long established a palm oil, palm kernel etc company. Ondo state, in which Okitipupa is situated is poised for an industrial boom given the limitless presence of virtually all kinds of raw materials to support most of industries. In the section of the F209 highway, precisely in between kilometer 20 to 25 along Okitipupa – Igbokoda stretch of the road, are found potholes of various geometry and dimension including different types of cracks. All these pavement deformation features constitute dangers for motorists trafficking the road. Several motor accidents claiming many precious lives and valuable properties have occurred along this section of the road. There is therefore the need for thorough geotechnical investigation into the main causes of the failure of the road pavement in this section of the F209 Okitipupa –Igbokoda highway.

2. Materials and Methods

The investigation involved both field and experimental work. The field work aspect involved extensive road travel and trekking in order to observe and record highway pavement failure features, such a potholes, corrugation, pavement routing, rutting and incision. The geological settings of the immediate environment of the road including drainage conditions were also investigated (see Fig. 1).

Bulk disturbed representative soil samples were collected from under the failed sections of the road including the base level material; because the base is the most vitally important layer of highway prism or configuration.

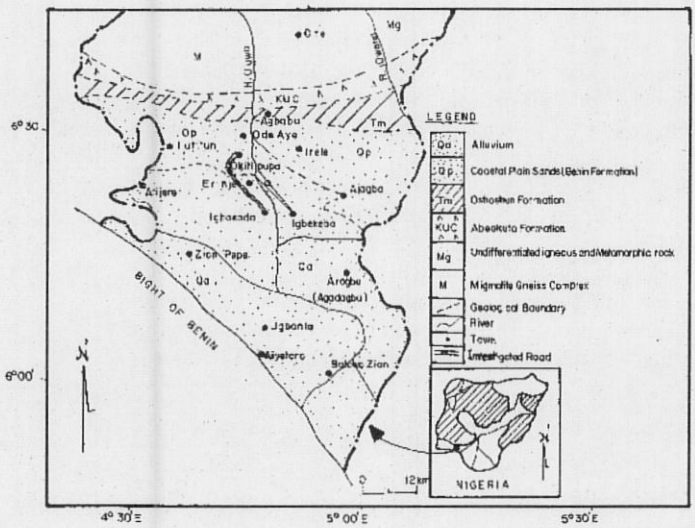

Fig.1: Geological map of the study area (after GSN 1974)

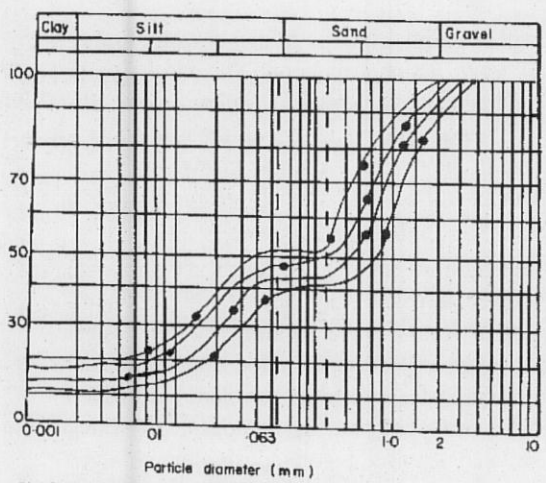

Fig. 2: Gap graded curves for Okitipupa subsoils

The geotechnical tests carried out on the representative soil samples collected include natural moisture content, Atterberg (consistency) limits, linear shrinkages, specific gravity, compaction, the California bearing ratio, and X–ray diffraction (XRD) analysis.

The tests were performed in accordance with the procedures specified by the American Society for Testing and Materials and the British standards Institution (ASTM 1289, 1979; BS 1377, 1975).

The test results are presented in Table 1. The laboratory tests were carried out at both the Universita Degli Studi di Milano, Italy and the Federal University of Technology, Akure, Nigeria.

3. Discussion

The geotechnical properties of the subsoil materials that were used in constructing the road are presented in Table 1. The soil textural analysis indicated that it classified as gap graded silty clay. The percentage fine is well above 25% (Fig. 2). There is clear deficiency of coarse fraction especially gravelly fraction in the soil profile. The definite increase in the soil natural moisture content may be best explained by decreasing evaporation trend from surface to subsurface along a soil profile in the crust. High plasticity and high in situ moisture content imply that a soil will take longer periods to dry after the heavy rains associated with the wet season (Jegede, 1995, 1997, 1998, 2000).

The consistency (Atterberg) limit values plot in the region of CL – CI and ML – MI groups (Fig. 3). This indicates that the soil contains both clay and silt fractions. It also implies that they may both be regarded to consist of clay of low to intermediate plasticity and silt of low to intermediate plasticity (Casagrande, 1974; Jegede, 1994, 2000). The liquid limit values of the soil may be considered high, ranging from 22% to 43.50%. This further supports the lack of high plasticity in the soil. Therefore, the soil with regard to the extremely high liquid limit values may be considered too weak in strength. The linear shrinkage values because of their relatively low values indicated a non-shrinking, non-heaving soil (Brink *et al.*, 1982).

Table 1: Geotechnical properties of a section along the F209 Okitipupa-Igbokoda highway.

Sampling Index Number	Depth (m)	Natural Moisture (%)	Liquid Limit (%)	Plastic Limit (%)	Plasticity Index	Linear Shrinkage	Specific Gravity	Soil Description
OK 1	0.6	7.10	44	34.10	9.40	5.0	2.60	Reddish brown silty clay
OK 2	1.0	8.90	37	28.73	8.27	4.0	2.61	
OK 3	1.5	9.00	41	24.20	11.80	3.0	2.61	
OK 4	2.0	9.30	40	28.57	11.443	4.1	2.62	
OK 5	2.5	9.70	33	30.90	2.10	5.2	2.66	
OK 6	3.0	9.60	22	10.38	11.62	2.3	2.65	

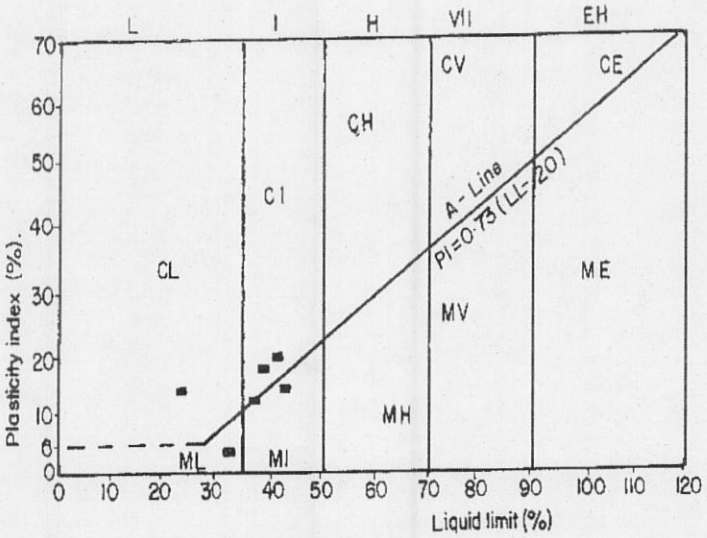

Fig.3: Casagrande's plasticity chart for studied soil samples (Okitipupa).

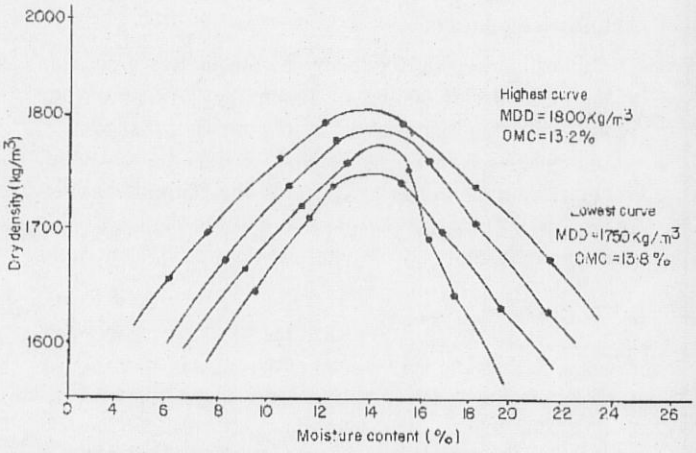

Fig.5: Moisture / density relationship for Okitipupa subsoils

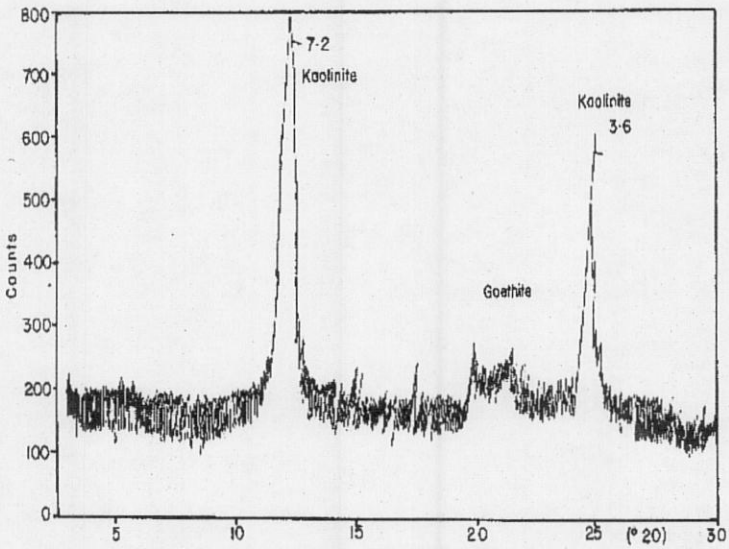

Fig.4: Diffractogram for the clay (2 micron) fraction from Okitipupa locality.

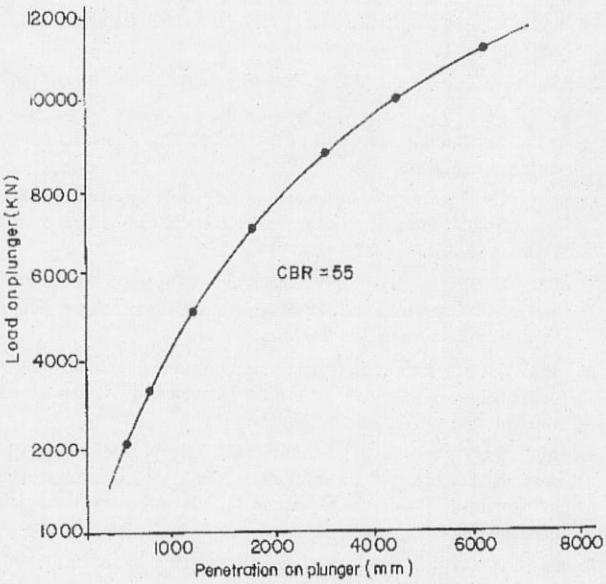

Fig.6: CBR Curve for Okitipupa studied soil sample

X-ray diffractogram showed very sharp and prominent Kaolinite peaks (more than 80% and a subdued goethite peak (less than 10%) without any trace of montmorillonite (Fig. 4). The presence of abundant kaolinite in the soil confirmed the linear shrinkage test results (Table 1). Both information confirm the presence of a non-heaving, non-shrinking clay soil material (Jegede, 1998). The specific gravity values range from. 2.60 to 2.66.

The moisture density relationship, i.e. the compaction curves (Fig. 5) showed a maximum dry density raging from 1750 kg/m^3 for the lowest curve and 1800 kg/m^3 for the highest curve at an optimum moisture content of 13.8% and 13.2% respectively. The CBR value of 55% (Fig. 6) is indicative of a strong reduction in the strength of the soil (pavement) materials. The extremely low value of 55% may be best explained as being due to the possible incursion of surface water through tiny openings which are mainly cracks and joints of differing geometry. The downward percolation or infiltration of this moisture into the base layer of the road prism further aggravates weakening and destruction of the road pavement (Jegede, 2000). It should be noted that the collapsible coastal plain sands, that is the Benin formation, may also have contributed to the failure of the road at this location.

The reason being that, this lithology is compressible and is liable to settlement under extreme load by the vehicular traffic on the road.

4. Conclusion

Highway pavement failure at a section along the F209 along Okitipupa – Igbokoda highway had been mainly induced by very poor soil properties as revealed by the excess fines (above 25%), high liquid limit values and very low California bearing ratio value of just 55%. The very low CBR value was mainly induced by surface water ingress into lower layers of the road prism including the base elevation, which resulted into failure of the highway pavement at this locality. The strength of the soil may be improved by soil stabilization techniques while cracks and joints may be sealed during road maintenance programme. The collapsible coastal plain sands at this location also contributed to pavement failure.

Acknowledgements

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ASSESSMENT OF TRACE METALS COMPOSITION OF VEGETABLE *AMARANTHUS SPINOSUS* (LINN SPECIES) GROWN ALONG URBAN MOTORWAYS USING TXRF TECHNIQUE

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Abstract

Vegetables of *Amaranthus Spinosus Linn* species, commonly grown along urban motorways, were sampled in three different locations along motorways in Ile Ife, Nigeria. The washed and unwashed leaves, stems and roots portions of the vegetable samples as well as the soil samples from each site were separated for independent analysis. All samples were digested using analytical grade acids and subsequently analysed using Total Reflection X-ray Fluorescence (TXRF) technique. Elements identified mainly in the samples are K, Ca, Ti, Mn, Fe, Cu, Zn, Br, Rb, Sr and Pb. Enrichment factors and soil-to-plant transfer ratios (TR_{s-p}) of the metals for each vegetable sample were determined. The enrichment factors reveal that anthropogenic contributions increased the levels of some elements: Cr, Mn, Br, Sr and Pb, in the vegetable samples.

Keywords: TXRF, *Amaranthus Spinosus Linn*, soil-to-plant transfer ratio, toxicity, enrichment factor

1. Introduction

Macronutrients and micronutrients required for plant growth are derived from the soil. These macronutrients include Ca, K, Mg and Fe. The micronutrients which are also known as the minor or trace elements include Mn, Zn, and Cu (Delaune *et al.*, 1986). Nutritional elements widely required by human body structure and functions are derived essentially from the plants consumed as food. *Amaranthus Spinosus Linn* vegetable is widely consumed in the western part of Nigeria because of its nutrients composition, cheapness and availability. These nutritional elements are Ca, K, Fe, Cu, Mn, and Zn. Calcium and K occur in the greatest quantity in all parts of the plant. Magnesium and Ca play important roles in the glycolytic activities in cells (Chu-Fang, 1996). Magnesium occurs in teeth and bones and acts as a cofactor to certain enzymes. Iron is essential to haemoglobin formation and is part of cytochrome molecule (Hay, 1984). Of the essential elements, Cu, Mn and Zn are required in minute traces only (Hay, 1984). Copper deficiency leads to anaemia since Fe cannot be used without it. Manganese is an activator of enzymes and Zn is associated with proper growth of hair (Chu-Fang, 1996). It is also very important to mention that some elements such as As, Br and Pb are known to be toxic to human bio-system if they exceed the threshold limit (Baker, 1983). Although it has been discovered that high proportion of ingested toxic elements are excreted, the populace still face serious health risk from vegetable poisoning as large portions of the consumed vegetables are grown on contaminated plots along motorway within the city (Loppi *et al.*, 1998).

Naturally, only very small concentrations of these elements, particularly, Pb are present in soils and plants, but as a result of anthropogenic activities the concentrations of these elements in plant increase (Chu-Fang, 1996). Swaine (1955) estimated the average content of lead in the Earth's crust to be 16 ppm and gave a range of total lead in agricultural soils of 2 to 200 ppm. Connor (1961) found a range of 16 to 710 ppm lead in some surface soils from New Jersey, Pennsylvania, and Maryland.

Obviously when the soil is impacted, the plant will also be impacted but the level of impaction of the plant from the soil depends on the soluble portion of the pollutants. It has been observed that the accumulation of heavy metals in plants depends upon many factors. These are the availability of the elements in soluble form to the plants, the characteristics of the plants- species, age, state of health, type of reproduction, etc; and other such parameters as temperature, available moisture, substratum characteristics, etc. (Conti and Cecchetti, 2001). Plant uptake of pollutant from soil will be better related to soluble than to total pollutant in soils. Concentrations of the pollutants in plants also depend on the part of the plant being analyzed.

Studies made of transplanted *Evernia prunastri* highlighted the fact that the capacity for Pb accumulation after transplanting. It was found that the relationship between the concentration after the transplanting of *Evernia prunastri* and the initial concentration value is 10.2 in France, 3.7 for Germany and 4.4 for the city of Rome, Italy (Bartoli *et al.*, 1994; Moriarty, 1999). In Italy, different biomonitoring studies carried out using lichens have shown that Pb is still very widespread in spite of the introduction of lead-free petrol. This indicates that high levels of this metal are still released (and/or resuspended) by vehicle traffic (Cardarelli *et al.*, 1993; Conti, 1996; Deruelle, 1996). Vehicular traffic seems to be the main source of atmospheric Cr, Cu, and Pb in the central Italian sites (Monaci *et al.*, 1997). Some elements – Cd, Cu, Cr, Ni, Pb and Zn detected in the analysis of roadside

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soils, vegetation and crops were found to have elevated concentrations attributable to traffic pollution (Ndiokwere, 1984). It has been reported that a considerable fraction of Pb particles are deposited on the soil and crops within a few hundred feet of the highways and that contact between Pb and the crops occurs both above and below ground. It was also reported that barley plants grown on soils treated with up to 800 ppm lead indicated less than 3 ppm in leaves but almost 800 ppm in the roots after analysis. (Schuck and Locke, 1970). They also reported as high as 890 ppm of lead in the roots of lemon cuttings grown in solution culture, whereas less than 3 ppm was found in the leaves. This means that automotive lead particulates exists mostly as a simple topical coating on vegetation and that the highest Pb concentrations are associated with those portions of the plant which have the greatest surface to volume ratio, e.g., leaves.

In this work, the urban grown *Amaranthus Spinosus Linn* vegetable is considered because of its availability and frequency of consumption among the food-stuff make-up of most people in the area. The trace elements in washed and unwashed *Amaranthus Spinosus Linn* vegetables, soil and water (used for wetting the vegetables) samples are presented. Elemental concentrations of the vegetable, soil and water samples were compared to know the source of increased level of some elements in the vegetable samples.

2. Materials and Methods

2.1 Site Location

This work was carried out in Ile-Ife, southwestern Nigeria. The vegetable plots on swampy lands are along motorways at about 10 and 100 meters from the motorways. Three sampling sites (which are totally free from tree cover) were chosen with samples collected randomly at the sites. Sample 1 means the samples from Site 1, etc.

2.2 Sampling

(a) Vegetables

The vegetable samples were randomly uprooted within an area of about 10m x 10m at each of the selected sites. Each sample was regarded as a composite sample. In the laboratory, the vegetables were grouped into two with one part washed and the other unwashed. The samples in each of the two groups were separated into leaves, stems and roots. The washing was carried out using double distilled water and all samples were dried at 60°C for 48 hours. The dried vegetable materials were homogenized in a 5% nitric acid-conditioned mortar and pestle.

Digestion of the vegetable samples was carried out according to Ogner *et al.* (1991). Sub-samples of 200mg were weighed into a 125ml Teflon bomb and 4ml of a 3:1 mixture of ultra-pure HNO_3 and H_2O_2 added. The Teflon bombs were then heated in a microwave oven at 300W, 450W and 600W, for 7 min. at each level. The solution was then analyzed after cooling.

(b) Soils

Ten samples of soils of approximately 50g were taken at each site. All vegetation, coarse rock fragments and roots were removed manually. The samples were dried and passed through a 2-mm sieve to remove stones prior to digestion. Each soil sample was homogenized and grounded with mortar and pestle. It was then digested as described above.

(c) Water

Irrigation water samples were collected from each of the sampling sites. The appropriate sample container was prepared in advance for actual sample collection for the analytes of interest. Water samples were collected directly into the containers from the stream in accordance with Quality Assurance Project Plan (QAPP) and Field Safety Plan (FSP) (USEPA, 1992). Three sub-samples of 1.0 ml were prepared, after thorough shaking, from each of the water samples and immediately analysed for the heavy metals.

2.3 Sample Analysis

A 5µl aliquot of each of the samples, with Gallium as internal standard, was pipetted on to a quartz sample carrier and dried using an infrared lamp. The dried samples were analyzed using a total reflection X-ray fluorescence spectrometer. The samples were irradiated with X-rays from a Mo-secondary target source operated at 40 kV and 20 mA. Fluorescence X-rays from the irradiated sample was collected and sorted using an X-ray spectrometer consisting of a Si(Li) detector, GENIE2K inspector hardware and GENIE2K software running on a PC. Each sample was analysed thrice and the spectra obtained were quantified using the QXAS software package. The data sets presented are the averages of the three measurements in respect of the spectra analyses and the averages for the soil and vegetable samples from a particular site.

2.4 Statistical Analysis

The soil-to-plant transfer ratio (TR_{s-p}) and the enrichment factor (EF) can be used as means of inferring the relationship between the soil and the vegetables. The TR_{s-p}, defined as the ratio of the concentrations of element x in vegetable and soil samples, is given by:

$$TR_{s-p} = \frac{(C_x)_{veg}}{(C_x)_{soil}} \qquad (1)$$

where C_x is the concentration of element x. The EF is the quotient of the ratio of the concentration of element x to the concentration of reference element f in the vegetable sample to the same ratio in the reference soil. It is expressed as:

$$EF = \left(C_x / C_f\right)_{veg} \Big/ \left(C_x / C_f\right)_{ref.soil} \qquad (2)$$

where C_x and C_f are the concentrations of element x and reference element f in vegetable and reference soil samples.

Table 1: Average elemental concentration *(μg/g)* of unwashed and washed vegetable samples

Sampling Site 1 (10 m from the motorway)						
	ROOT		STEM		LEAF	
Elements	Unwashed	Washed	Unwashed	Washed	Unwashed	Washed
K	45764.3	36601.6	45554.8	41031.2	32280.7	31224.5
Ca	10219.7	7483.0	10214.7	9857.4	16819.9	15362.9
Ti	160.7	92.5	84.3	37.0	141.1	73.0
Cr	35.0	14.0	74.8	37.4	118.8	17.8
Mn	93.9	36.7	30.5	29.6	91.6	81.1
Fe	2010.5	515.9	1085.5	366.3	1361.6	804.9
Cu	27.2	19.6	14.3	14.0	25.1	24.4
Zn	49.5	41.7	41.7	40.5	78.5	54.6
Br	10.4	6.7	25.3	24.7	20.9	17.0
Rb	98.0	77.6	35.9	33.3	57.9	41.8
Sr	124.6	94.8	115.6	105.5	135.7	128.0
Pb	95.7	31.6	270.9	112.5	138.7	113.5

Sampling Site 2 (100 m from the motorway)						
	ROOT		STEM		LEAF	
Elements	Unwashed	Washed	Unwashed	Washed	Unwashed	Washed
K	20325.9	19016.9	46223.3	40005.9	38020.3	24194.8
Ca	7077.6	6502.2	10474.7	9853.3	15447.1	14586.4
Ti	92.9	47.2	35.9	16.9	55.6	41.2
Cr	71.5	60.7	72.5	55.6	104.1	29.2
Mn	36.4	28.1	21.4	15.8	54.3	42.8
Fe	1440.6	801.2	457.7	306.2	744.1	513.7
Cu	13.7	13.2	11.2	5.5	25.9	21.6
Zn	45.3	41.2	40.3	35.3	87.6	77.9
Br	4.5	3.2	7.2	7.1	12.1	6.3
Rb	31.0	28.8	72.7	62.2	71.1	38.7
Sr	67.0	66.2	106.9	99.2	87.8	70.4
Pb	138.8	126.2	78.6	58.1	50.7	48.3

Sampling Site 3 (10 m from the motorway)						
	ROOT		STEM		LEAF	
Elements	Unwashed	Washed	Unwashed	Washed	Unwashed	Washed
K	39417.4	38228.3	38188.4	35765.1	26850.3	21021.3
Ca	9436.6	8830.6	10972.7	9601.6	21811.7	21717.6
Ti	195.4	87.6	44.3	25.7	120.6	37.0
Cr	43.9	40.5	35.4	28.4	28.0	14.0
Mn	43.7	38.9	14.8	11.4	46.6	36.2
Fe	2493.1	1298.9	434.6	374.2	1068.3	592.0
Cu	17.6	15.7	13.9	6.6	25.5	23.5
Zn	71.8	68.8	64.4	59.3	114.9	94.1
Br	5.2	4.3	4.4	3.4	5.0	4.3
Rb	85.5	81.5	68.2	57.2	84.0	72.1
Sr	84.3	82.9	91.2	86.8	122.0	116.5
Pb	49.6	45.7	39.2	33.1	54.5	51.5

Table 2: Elemental enrichment factor of the vegetable samples

	Cr	Mn	Br	Sr	Pb
Sampling Site 1					
Leaf	13.7	6.6	3517.8	26.6	622.2
Stem	56.6	4.7	10107.8	43.2	2928.7
Root	8.5	2.3	1089.6	15.5	136.6
Sampling Site 2					
Leaf	39.6	6.1	2297.1	25.9	468.7
Stem	184.4	5.5	6322.7	88.8	1374.7
Root	72.1	3.5	1014.4	21.2	1070.6
Sampling Site 3					
Leaf	21.2	5.8	1765.2	47.6	556.5
Stem	61.9	2.6	2008.2	51.1	514.7
Root	25.9	2.6	748.1	14.3	208.7

Table 3: Elemental enrichment factor of soil samples

	Cr	Mn	Sr	Pb
Site 1	-	2.1	13.4	46.2
Site 2	2.8	1.4	12.9	37.9
Site 3	2.8	1.2	14.2	182.5

Table 4: Average elemental concentration (*µg/g*) of soil samples

	Sample-1	Sample-2	Sample-3
K	3517.0	4654.4	5774.9
Ca	8414.4	6446.9	11557.2
Ti	5600.1	3411.9	4106.5
Cr	0.0	409.0	329.0
Mn	2122.4	1706.3	1132.2
Fe	46436.9	56271.4	44614.4
Ni	37.3	38.3	0.0
Cu	50.1	30.5	29.3
Zn	281.6	213.9	390.2
Rb	44.3	48.3	83.5
Sr	139.9	184.7	161.9
Ba	2808.6	1296.1	2542.6
Pb	564.2	560.9	2137.3

Table 5: Average elemental concentration (µg/g) of water samples

	Sample-1	Sample-2	Sample-3
K	128.5	600.5	329.9
Ca	666.6	1171.1	541.7
Ti	12.8	19.9	8.2
Mn	48.4	18.4	16.3
Fe	89.8	109.6	101.2
Ni	6.5	6.4	0.0
Cu	8.2	4.9	1.8
Zn	14.7	10.5	12.8
In	300.4	525.9	209.4
Ba	36.9	62.0	24.0

Table 6: Soil-to-plant transfer ratio for washed parts of *Amaranthus* vegetable

Sample-1	Ti	Cr	Mn	Sr	Pb
Leaf	0.01	-	0.04	0.92	0.20
Stem	0.01	-	0.01	0.75	0.48
Root	0.02	-	0.02	0.68	0.06
Sample-2					
Leaf	0.01	0.07	0.03	0.38	0.09
Stem	0.01	0.14	0.01	0.54	0.10
Root	0.01	0.15	0.02	0.36	0.22
Sample-3					
Leaf	0.01	0.04	0.03	0.72	0.02
Stem	0.01	0.09	0.01	0.54	0.02
Root	0.02	0.12	0.03	0.51	0.02

It is agreed in principle that if the value of TR_{s-p} is greater than 0.9, the vegetables possessed high uptake ability for the element from sources other than soil or indicate that the soil is not the only source of contribution of the element (Jenne, 1968). It is to be noted, however, that large proportions of the pollutants are washed to the soil from the atmosphere and get leached to various levels of the soil profile.

3. Results and Discussion

In Table 1, the average elemental concentrations of twelve major, minor and trace elements in *Amaranthus Spinosus Linn* (green variant) for both washed and unwashed samples from each of the sample sites are reported. For lithophilic elements like Ti, Mn and Fe, elemental concentrations were higher for the unwashed samples than the washed samples. The difference in elemental concentrations between the washed and the unwashed samples are however minimal for Cu, Zn, Br, Rb Sr and Pb. This calls for serious concern in view of the immediate toxic nature of these heavy metals at high concentrations, apart from the long time accumulation effects. It was observed that Br, Sr and Pb were highly enriched and Cr and Mn moderately enriched, taking EF > 5 as indication of possible anthropogenic influence (Tables 2 and 3). The vegetable plots are strongly impacted from wind and automobiles related re-entrained particulates that settle soon after re-entrainment as a result of their closeness to highways since. Plots far from highways or totally outside urban centers would be less impacted especially from automotive related sources. It is interesting to note from Tables 4 and 5 that Ba was detected in soil samples and In and Ba in water samples but the two elements were not detected in the vegetable samples. It could therefore be stated that the uptake level for these elements is low. The soil-to-plant transfer ratio, TR_{s-p}, (Table 6) showed that the elements Ti, Cr, Mn and Sr were majorly taken up from the soil. The observed increased levels of Cu, Zn, Br and Pb detected in the leaves as compared to the stem and root parts of the vegetable samples could be attributed to strong contributions from surface contaminations from anthropogenic sources especially road traffic. This is because of the closeness of the sampling sites to the motorways and the established fact of the said elements being source finger-print elements for road traffic (Monaci *et al.*, 1997).

4. Conclusion

This study has shown that contamination of vegetables grown within urban centers could be significant. Though research has shown that high proportions of ingested toxic elements are excreted, the populace still face serious health risks from food poisoning in respect of heavy metals like Pb, Cu, Zn and Cr. This is because of the almost daily consumption of this food item leading to accumulation of such toxic metals over time. The cultivation of such food items in urban centers should be discouraged in view of its contamination from anthropogenic sources. It has been observed that thorough washing removes a lot of the lithophilic elemental contaminants so that clean cooking habits at homes should be emphasized.

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HIGH FIELD ^{13}C NMR SPECTROSCOPIC ANALYSIS OF THE TRIACYLGLYCEROLS OF *ADENOPUS BREVIFLORUS* SEEDS OIL

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Abstract

High resolution Carbon-13 NMR (gated decoupled) spectra of the carbonyl, saturated and olefinic carbons in *Adenopus breviflorus* seeds oil have been used for direct determination of the acyl composition and acyl positional distribution on the glycerol backbone. The spectra revealed the presence of saturated, oleic and linoleic fatty acids. Semi-quantitative analysis using the integrals of the allylic carbons signals gave the percentage composition of the oil as saturated 25.00%, oleic 14.00% and linoleic 60.90%. These percentage composition were confirmed by gas chromatography. The spectra further revealed that while the saturated fatty acids are distributed between the 1,3 (α) and 2 (β) glyceridic positions, oleic acid is attached only at the α glyceridic positions while linoleic acid is attached mostly at the (β) glyceridic position.

1. Introduction

Most seed oils are composed of triacylglycerols which contain an array of fatty acids, saturated as well as unsaturated and distributed among the three positions of the glycerol backbone. In defining the acyl positional distribution between the α-(i.e. the 1- and 3- positions of the glycerol) and β-(i.e. the 2-position of glycerol), carbon-13 NMR has been found most useful (Wollenberg, 1990). There have also been some efforts in the past (Ng, 1984; Gunstone, 1993; Lie Ken Jie and Pasha, 1996) where ^{13}C NMR was used to identify , confirm or evaluate the fatty acids composition of different seed oils. These reports indicated that except for lack of differentiation of the saturated fatty acids, the ^{13}C NMR technique provided the same information as the time-consuming conventional gas chromatographic technique for establishing fatty acid composition of oils and the tedious enzymatic hydrolysis for identifying the positional distribution of the oils' acyl groups.

Adenopus breviflorus (Cucurbitacea) grows in the wild in Savanah forest of Southern Nigeria. It has about 55-60% oil (Esuoso and Bayer, 1998). Oderinde (1990) and Oshodi (1996) reported the fatty acids composition of the *Adenopus breviflorus* seeds oil. We have characterized the oil and indicated some possible uses of the seeds oil (Akintayo, 2002a). In an earlier investigation, we have tried to identify *Adenopus breviflorus* seeds oil from other seed oils using the peak area ratio $(-CH_2-)/(=CCH_2C=)$ derived from their ^1H NMR spectroscopy (Akintayo, 2002b) as a qualitative index. In continuation of our efforts on the systematic studies of the lesser known and under-utilised tropical seeds oils, the present effort aims at the ^{13}C NMR spectroscopic analysis of *Adenopus breviflorus* seed oil to (i) confirm the presence of the reported fatty acids, (ii) identify and semi-quantiate the fatty acids and most importantly (iii) determine the fatty acids distribution on the glycerol backbone. The quantitative integrity of the NMR derived fatty acid composition is verified by Gas Chromatographic analysis of the oil.

2. Experimental

Adenopus breviflorus (ADB) seeds were purchased from some markets in Ibadan, Akure and Ado-Ekiti in the South-Western part of Nigeria. The seeds were screened, washed and dried in the oven (103°C) and the oils extracted with hexane for 20hr by Sohxlet method. The extracts were desolventised under reduced pressure on a rotavapour.

The extracted oil was purified. 2g of extracted oil was percolated through a silica gel (15g) column with a mixture of petroleum ether (b.p. 40-60°C) and diethyl ether (95:5, v/v, 150ml). The eluate was evaporated under reduced pressure to 5ml portion and this portion further concentrated by a gentle blow of nitrogen gas to yield a mixture of triacylglycerols (1.72g).

The ^{13}C NMR of the sample dissolved in deuteriated Chloroform were recorded on the BRUKER AMX-400 (BRUKER Instruments, Inc. Karlsruhe, Germany) Fourier transform spectrometer operating at 100.6MHz. The gated decoupling pulse sequence was used with the following parameters. Number of scans 512, acquisition time 1.3665sec, pulse width 10.3µsec, delay time 1.0sec. Free induction decay (FID) was transformed and zero filled to 300K to give a digital resolution of 0.366Hz/point.

Fatty acid methyl ester (FAME) of the oil was prepared as follows: approximately 2mg crude seeds oil was transferred into a 5-10ml glass vial and 1ml of diazomethane-ether solution added. The mixture was shaken thoroughly and allowed to stand for 1 min. Then 16µl of 3.33M CH_3ONa/CH_3OH solution was added, mixture shaken and allowed to stand for 10 min after which 10µl acetic acid was added. The equation of the transmethylation is shown above.

The clear supernatant was used for gas chromatographic analysis. 0.2µl of the FAMES was injected into Hewlett-Packard 5890 GC (Hewlett-Packard Co, Palo Albo CA). The column was HPUltra Performance coated with crosslinked 5% Phenol +95% Polysiloxane, 30x0.25nm, 0.2µ coating thickness. Temperature programming was as follows: Initial

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Table 1: ^{13}C NMR chemical shifts of *Adenopus breviflorus* seed oil

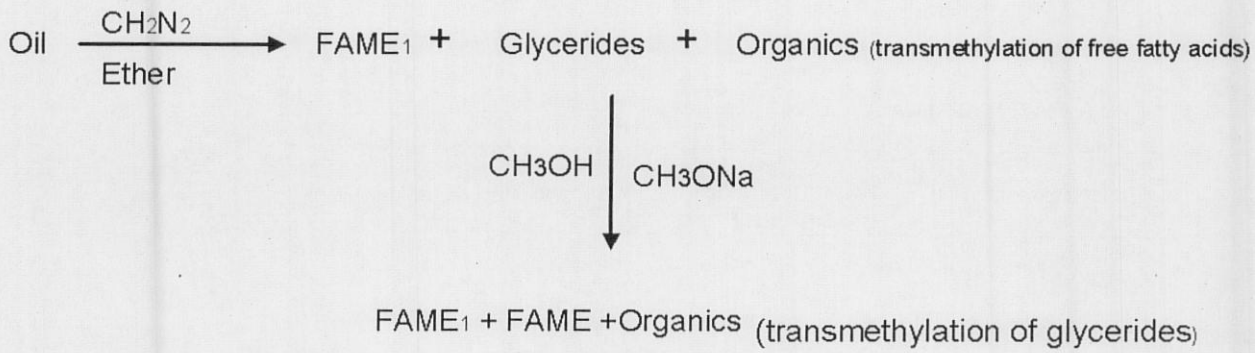
^{13}C NMR Shift of ADB	^{13}C NMR shift of standard esters*	Assignments
173.3188	173.312	C-1, Sat
173.2752	173.171	C-1, L(α)
172.8606	172.771	C-1, L(ß)
130.2467	130.182	C-13, L
130.0357	130.029	C-10, O
130.0067	129.980	C-9, L
129.7375	129.720	C-9, O
128.1083	128.114	C-10, L(ß)
128.0938	128.095	C-10, L(α)
127.9265	127.932	C-12, L(α)
127.9120	127.920	C-12, L(ß)
34.2180	34.242	C-2, Sat(ß)
34.1307	34.190	C-2 L(ß)
34.0798	34.041	C-2, O(α)
34.0507	34.074	C-2, Sat(α)
24.9082	24.896	C-3, L(α)
24.8718	24.860	C-3, L(ß)
27.2575	27.254	C-11, O
27.2356	27.226	C-14, L
27.2065	27.202	C-8, O
25.6573	25.655	C-11, L
31.9632	31.962	ω3, Sat
31.9414	31.956	ω3, O
31.5632	31.557	ω3, L
22.7335	22.733	ω2, Sat
22.7189	22.726	ω2, O
22.6098	22.610	ω2, L

* Established data as reported by Lie Ken Jie,*et. al.* (1992, 1993, 1995).

Table 2: Fatty acid composition of *Adenopus breviflorus* seed oil

Fatty acids	*a* (%)	*b* (%)	*c* (%)	^{13}C NMR
Palmitic	10.10	10.10	10.84	*
Stearic	2.50	9.90	14.06	*
Oleic	24.56	19.40	13.84	14.10
Linoleic	62.86	60.70	61.26	60.90
∑Saturated	12.60	19.90	24.90	25.00
∑Unsaturated	87.42	80.10	75.10	75.00

(*a*) Percentage Fatty acid composition as reported by Oderinde (1990)
(*b*) Percentage Fatty acid composition as reported by Oshodi (1996).
(*c*) Percentage Fatty acid composition as obtained in the present effort byGas Chromatographic method
(*) Percentage Fatty acid composition reported together as total saturated.



temperature, 160°C for 2 min, temperature increased at 2.5°C/min up to 300°C and maintained at this final temperature for 5min. Injector and dectector temperature were 280°C and 340°C respectively.

3. Results and Discussion

In this discussion we abbreviate saturated acyl groups as Sat., oleate[18:1(9*Z*)] as O and linoleate [18:2(9*Z*,12*Z*)] as *L* (where the first number in bracket denotes the number of carbon atoms in fatty acid chain, the second number denotes the number of double bonds, the other numbers denote the position of double bonds, and *Z* stands for the *Z* configuration of the corresponding double bond). The structures of oleate and linoleate and the respective carbon numbers used throughout this discussion are as given below.

Table 1 presents the important signals in the ^{13}C NMR spectra of ADB oil (Fig. 1) along with those of established data (Lie Ken Jie *et.al.*, 1992, 1993, 1995) and their assignments.

The high resolution ^{13}C NMR spectrum of the carbonyl carbons of the triglycerides of ADB is presented in Figure 2 and it shows three signals at 173.3188ppm, 173.2752ppm and 172.8606ppm. Ng (1983) has reported that the carbonyl carbons of saturated chains appear as the highest frequency peak in the NMR spectrum (at approximate δ of 173.3). The highest chemical shift in the spectrum of ADB oil 173.3188ppm can therefore be assigned to carbonyl carbon of Sat in α position. Ng (1983) has also shown that C-1 of O and L attached to either of the 1, 3 glyceridic carbons (i.e. at α position) occur at a slightly lower field to that of Sat. occupying the same position (O differs by 0.029±0.002ppm while L differs by 0.041±0.002ppm).

Rather than relying solely on chemical shift values, we have also made use of the difference values to ascertain the type of the ester and their positions on the glycerol backbone throughout this discussion. The higher value of the pair of signals, 173.2752ppm differs from the 173.3188ppm signal by *ca* 0.043ppm. Referring to Ng (1983) and Lie Ken Jie *et.al.* (1992, 1993, 1995), the pair of signals 173.2752ppm/ 172.8606ppm could therefore be assigned to *L* in α and β positions. Signals observed in the carbonyl region of this oil indicate the presence of Sat and *L*. Earlier reports by Ng (1983) and Shiao and Shiao (1989) have shown that resonances of saturated fatty acids were not resolved in the carbonyl region.

Oleate - $^{18}CH_3{}^{17}CH_2{}^{16}CH_2{}^{15}CH_2{}^{14}CH_2{}^{13}CH_2{}^{12}CH_2{}^{11}CH_2{}^{10}CH={}^{9}CH{}^{8}CH_2{}^{7}CH_2{}^{6}CH_2{}^{5}CH_2{}^{4}CH_2{}^{3}CH_2{}^{2}CH_2{}^{1}COO^-$

Linoleate - $^{18}CH_3{}^{17}CH_2{}^{16}CH_2{}^{15}CH_2{}^{14}CH_2{}^{13}CH={}^{12}CH{}^{11}CH_2{}^{10}CH={}^{9}CH{}^{8}CH_2{}^{7}CH_2{}^{6}CH_2{}^{5}CH_2{}^{4}CH_2{}^{3}CH_2{}^{2}CH_2{}^{1}COO^-$,

where the superscripts stand for carbon numbers.

The ^{13}C NMR signal profiles in the upfield region (20-36ppm) of the ADB oil (Figure 3) were also found to be very characteristic and could be used for identification of the acyl groups and their positional distribution on glycerol backbone. There are two sub-regions in the spectra that are useful for carbon shift region (*ca* 34ppm) and (ii) the C-3 (*ca* 24ppm), allylic (25-27ppm), C-17(*ca* 22ppm) and C-16 (*ca* 31ppm) carbon shift region.

C-2 carbon shift region (ca 34ppm)

Four signals 34.2180ppm, 34.1307ppm, 34.0798ppm and 34.0507ppm appear in this region. Two of the signals 34.2180ppm/34.0507ppm could be paired (shift difference of 0.167ppm). These shifts are assigned to the C-2 carbon atoms of Sat in the β and α positions. The 34.1307ppm is assigned to L in β glyceridic position and the 34.0798ppm assigned to O in α glyceridic position. These assignments were based on established data, Lie Ken Jie (1992, 1993, 1995).

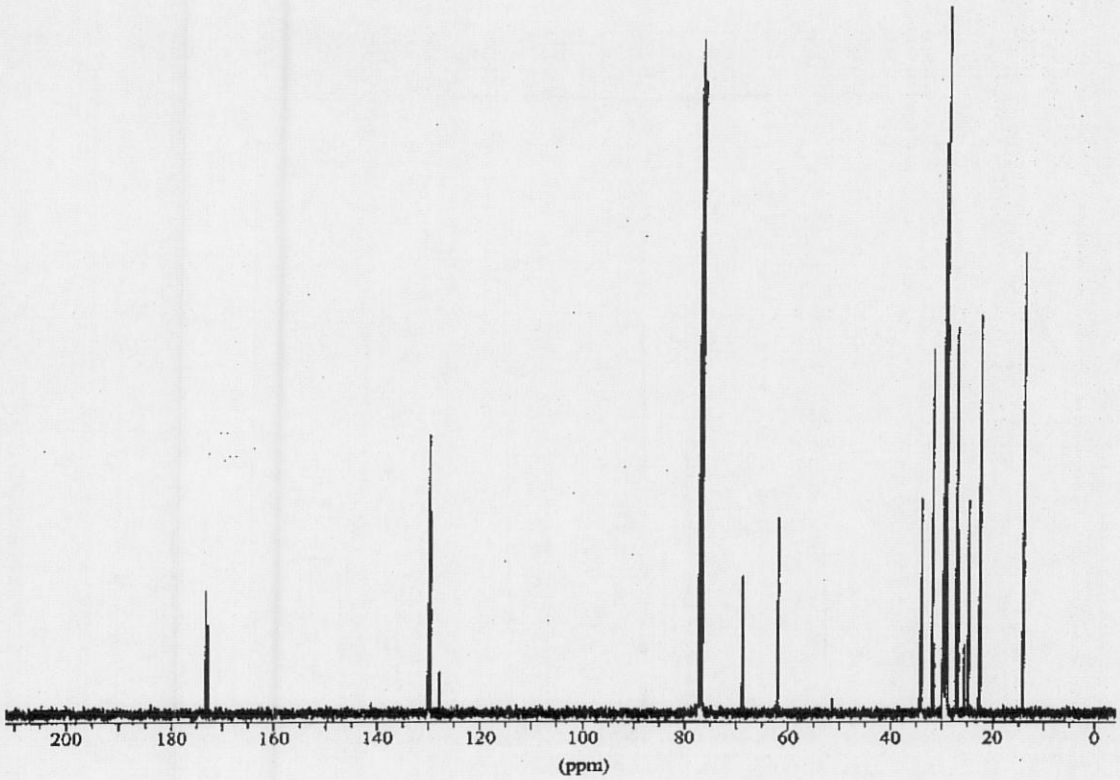


Figure 1: Proton decoupled high resolution ^{13}C NMR (100.6MHz) spectrum of the Triacylglycerols in *Adenopus breviflorus* seeds oil

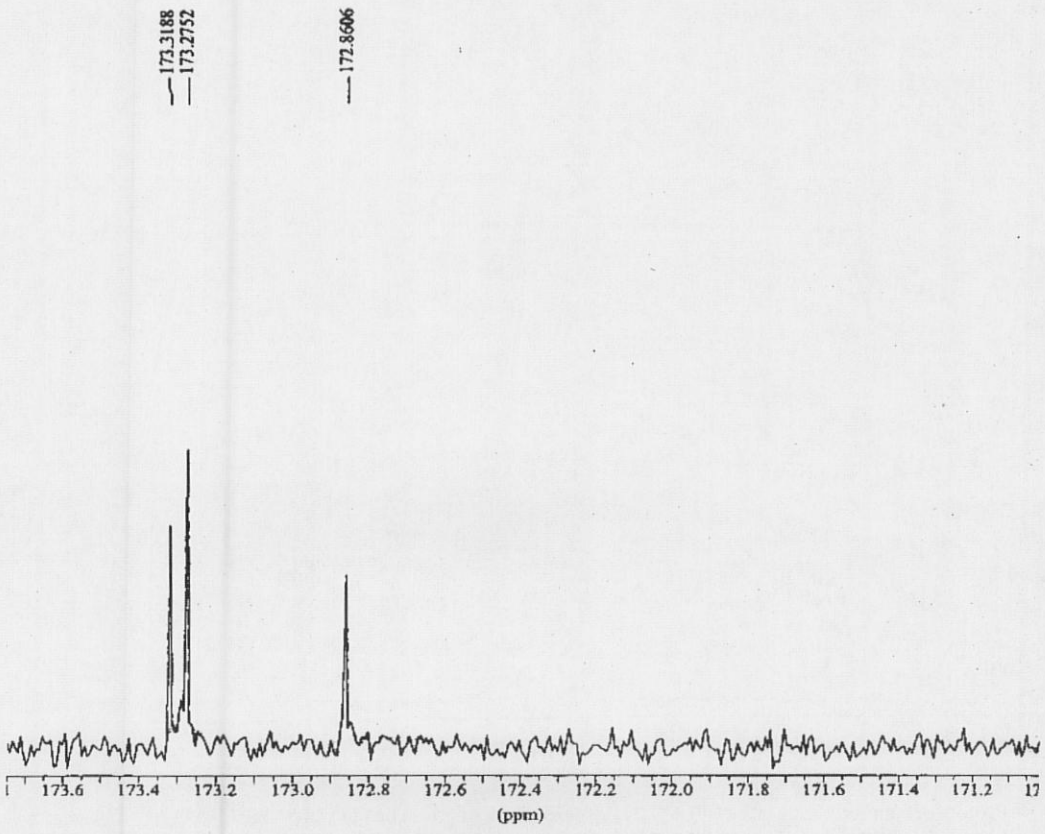


Figure 2: Proton-decoupled high resolution ^{13}C NMR (100.6MHz) of the carbonyl carbons of the triacylglycerols in *Adenopus breviflorus* seeds oil

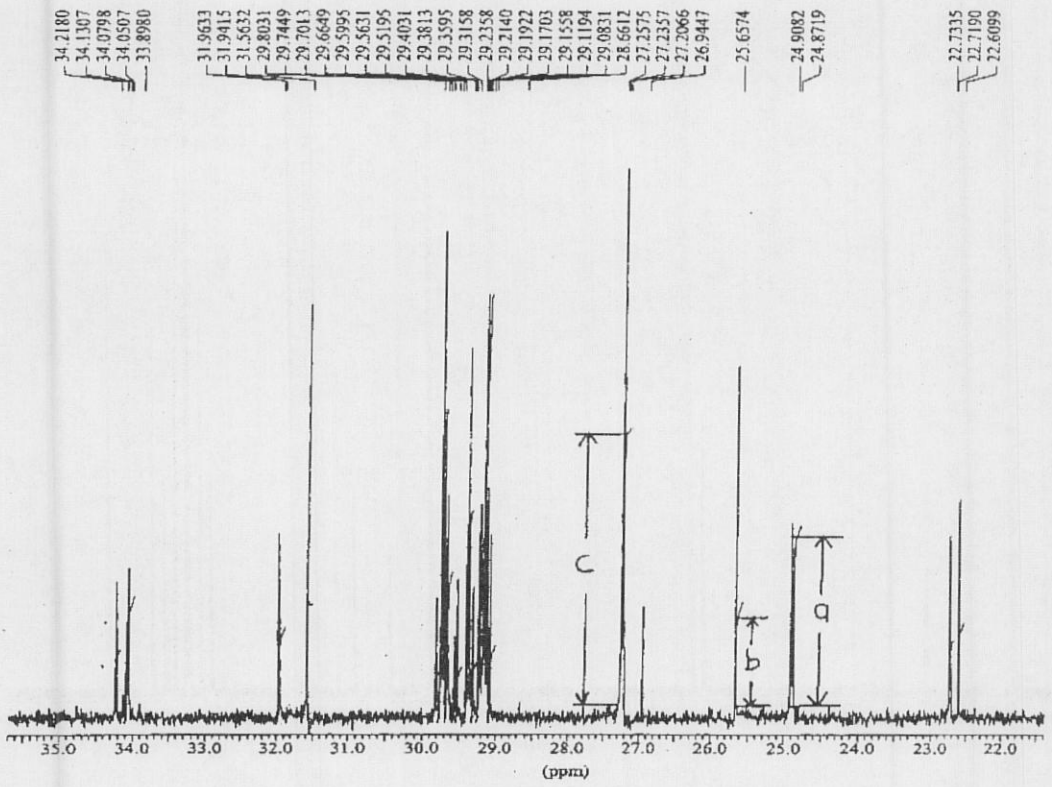


Figure 3: Proton-decoupled ^{13}C NMR (100.6MHz) of the saturated carbons of the fatty acid chains in *Adenopus breviflorus* seeds oil. The integral value *'a'* is for the peak at *ca* 24ppm, *'b'* is for the peak at *ca* 25ppm and *'c'* is for the peak at *ca* 27ppm.

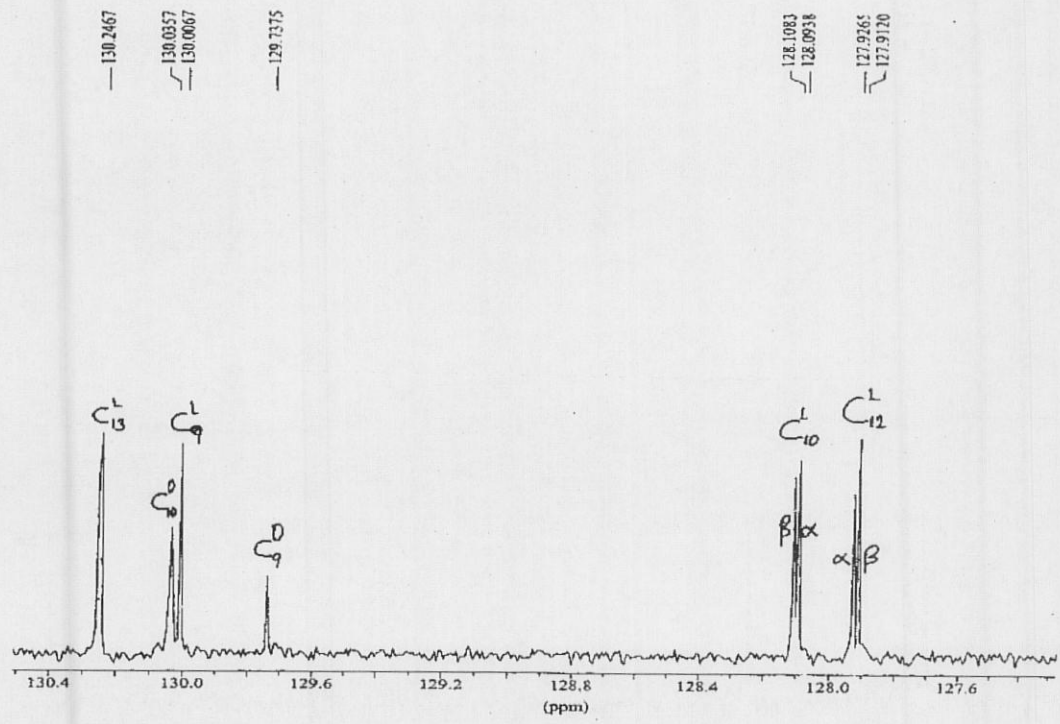


Figure 4: Proton-decoupled ^{13}C NMR (100.6MHz) of the olefinic carbons of the triacylglycerols of *Adenopus breviflorus* seeds oil. In the assignment of the peaks, the superscripts of symbol *C* are defined as follows, *O* for oleic and *L* for linoleic. The subscripts of symbol *C* represents the specified carbon in the fatty acid chain.

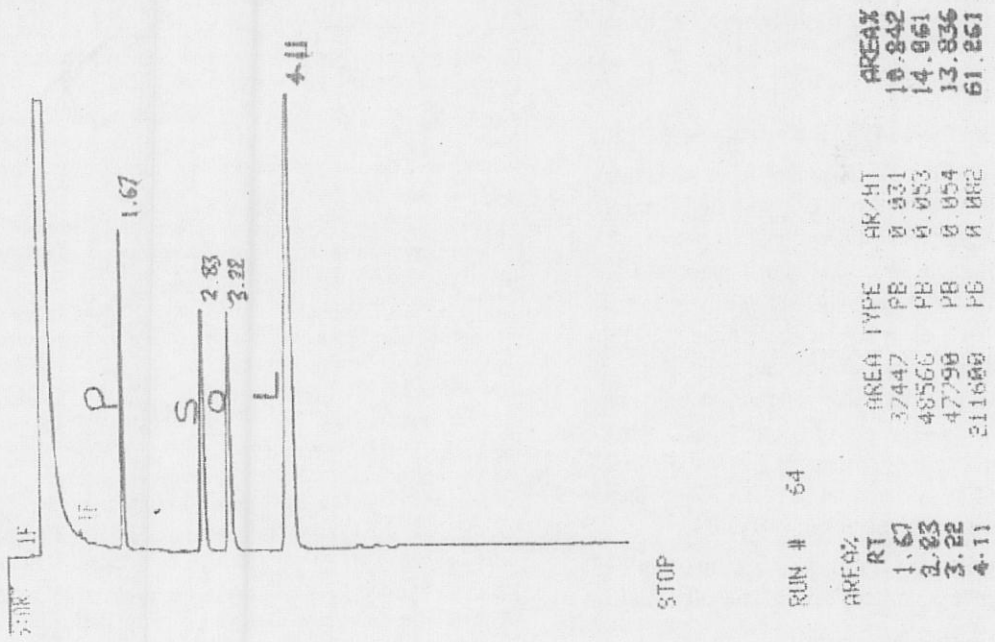


Figure 5: *GC* Chromatogram of *Adenopus breviflorus* seeds oil. The numbers are retention times. The symbols are: *P* for Palmitic acid, *S* for stearic acid *O* for oleic acid and *L* for linoleic acid.

C-3, allylic, C-17 and C-16 carbon shift region.

The two signals in the C-3 region (*ca* 24ppm) 24.9082ppm and 24.8718ppm can be paired having a chemical shift difference ($\Delta\delta$) of 0.036ppm. Referring to reported data, this pair of signals are assigned to C-3 of L distributed in the α and β glyceridic positions. No signal is found in the region *ca* 32ppm, hence the presence of trans ethylenic systems in the seeds oil can be ruled out.

Ten signals appear in the region (20-27ppm). The signal at 27.2575ppm is due to C-11 carbon atom of O, the 27.2356ppm signal is due to C-14 carbon atom of L, the 27.2065ppm is due to C-8 carbon atom of O and L and the 25.6573ppm signal is due to C-11 of L. The relative intensities of the allylic methylene protons are distinct and the signals profile and intensity could serve as fingerprint for identification of the oil. Lie Ken Jie and Lam (1995) have observed a deshielding order for the shifts of C-16 carbon nuclei as follows, Sat (31.976ppm) > O (31.954ppm) > L (31.567ppm). This trend was also observed by the same authors for C-17 carbon nuclei. The spectra of ADB also shows this deshielding effect, so the signals at 31.9632ppm, 31.9414ppm and 31.5632ppm are assigned to the shift of C-16 carbon nuclei of Sat, O and L respectively present in the ADB oil. In the same manner, the 22.7335ppm, 22.7189ppm and 22.6098ppm are assigned to the shift of C-17 carbons of Sat, O and L respectively.

Another very characteristic region in the ^{13}C NMR spectra of oils that defines the acyl composition and positional distribution on glycerol backbone is the olefinic carbon shift region. ^{13}C NMR spectrum of ADB oil in this region is shown in Figure 4.

Ng (1983) had observed that the chemical shift between a pair of peaks become smaller for the olefinic carbon nearer to the methyl end of the fatty acid chain, i.e. in the O chain, magnitude of the peak separation is in the order C-9 > C-10 > C-12 > C-13. He also observed that in the O chain, the peak β glyceridic position appears at a lower field than that attached at the α- position and that the reverse order holds for C-10. These high/low field alterations in peak position were also observed among the olefinic carbons of L chain. In general, in the O chain, $\Delta\delta$ between C-10 and C-9 α-positions is 0.30ppm and that between their β- positions is 0.34ppm. In the L chain $\Delta\delta$ between C-13 and C-9 α-positions is 0.20ppm and $\Delta\delta$ between their β-positions is 0.24ppm while $\Delta\delta$ between C-10 and C-12 α-positions is 0.17ppm and their β positions is 0.19ppm. Based on these difference values and other established data, the peaks in the olefinic regions are assigned as shown in Figure 3. The spectrum clearly shows the presence of O and L and absence of any triene ester. The intensity of the peaks shows that L is more abundant than O in ADB oil. The sharpness of the C-9 and C-10 of O clearly indicate that they are single peaks. However the chemical shift difference ($\Delta\delta$ = 0.30ppm) points to the fact that O is attached only at the α glyceridic position. The chemical shift difference between the C-13 and C-9 of L ($\Delta\delta$ = 0.24ppm) and the intensities of the pair of peaks observed for the C-10

and C-12 shows that L is mostly attached at the β glyceridic position. These results corroborate our observations from other regions of the spectra especially the C-3 carbon region which had indicated the distribution of L in the α and β glyceridic positions and the C-2 carbon shift region which had indicated presence of O in α position and L in mainly β position.

Semi-quantitative analysis of the fatty acid composition

The results discussed above revealed that ADB oil is composed mainly of Sat, O and L. For oils with non complex composition like this, the peaks at *ca* 24ppm represents the total number of saturated, monoene and diene chain.The peaks at *ca* 25ppm belongs to C-11 that is allylic to both double bonds of a *cis-cis* diene (linoleic) such that they represent the total number of diene chains, and the peaks at *ca* 27 ppm belong to the two carbons allylic to *cis* double bond i.e. C-8, C-11 of O and C-8, C-14 of L, such that they represent twice the total number of monoene (O) and diene (L) chain (Ng and Ng, 1984). The areas of these peaks therefore permit quantitative analysis of Sat., O and L.

Integrals of these peaks are identified as *a*, *b* and *c* in Figure 3 and the percentage composition of the oil is calculated as:

$$\text{Percentage of } Sat. = [\ (a - 0.5c)/a\ ] \times 100$$

$$\text{Percentage of } O = [\ (0.5c - b)/a\ ] \times 100$$

$$\text{Percentage of } L = [\ b/a\ ] \times 100$$

For the ADB, $a = 0.46$, $b = 0.28$ and $c = 0.69$. The percentage of the acyl composition derived from the NMR spectra is presented in Table 2 along side those obtained by gas chromatography by Oshodi (1997) and Oderinde (1990) and also obtained by GC methods in the present effort. The chromatogram obtained in the present effort is presented in Figure 5. The NMR results confirm the GC results that L is the most abundant fatty acid in ADB oil. Our GC results compare very well with our NMR extrapolated results. However results of other workers differ especially in their O and S contents. These variations may be due to geographical and environmental factors. Going by the agreement between our two results obtained by two independent methods, we can reasonably state that in our sample of ADB oil, percentage saturated fatty acids is *ca* 25% and unsaturated fatty acids is *ca* 75% comprising of oleic(*ca* 14%) and linoleic(*ca* 61%) acids.

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AN ASSESSMENT OF HEALTH IMPLICATIONS OF NATURAL RADIOACTIVITY IN THE TAR SAND DEPOSIT OF ONDO STATE, SOUTHWESTERN NIGERIA

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Abstract

Gamma ray spectrometric and Energy-dispersive X-ray fluorescence analyses were carried out on samples of bituminous sand deposits of Ondo State. The objectives were to determine the presence and level of radioactivity; assess the impact of radioactivity on the environment and provide a geochemical baseline data for its exploitation.

The average specific activity concentrations obtained for Bi-214, T1-208 and Ra-226 in the overburden were 165.64 ± 2.91, 150.25 ± 2.91 and 60.97 ± 2.27 $BqKg^{-1}$ respectively. Only Ra-226 with a range of (18.12 ± 3.53 to 36.13 ± 3.15) $BqKg^{-1}$ and Pb-214 with a range of (17.17 ± 0.46 to 31.01 ± 0.84) $BqKg^{-1}$ were detected in the bituminous sands. The calculated average dose rate in the overburden and the bituminous sands were 68.49nGyhr $^{-1}$ and 8.66nGyhr $^{-1}$ respectively. The mean equivalent doses in the overburden and the bituminous sands were about 0.59mSvyr^{-1} and 0.07m

In general, the study confirmed the presence of radionuclides in the overburden and the bituminous sands but their activity levels are within the background values. The calculated radiation values fall below the recommended exposure limit to the populace and thus are not expected to constitute any health hazard.

Keywords: Health implications, radioactivity, tar/bituminous sand, dose-limits.

1. Introduction

Renewed interest in the exploration and exploitation of the vast bituminous sand deposit of Nigeria and recent reported cases of possible high levels of radioactive materials in some bituminous nodules in Chez Republic (Bohdan *et al.*, 1999) emphasizes the need for new studies on the presence and level of radioactivity within the bituminous sand of Ondo state.

Tar sand deposits with enormous reserves are known to occur within a belt that cuts across Lagos, Ogun, Ondo and Edo States in South-western Nigeria. Outcrops however, cover an area of 120 km by 6 km in an East-West belt (Fig. 1). Tar sands are composed of sands, heavy oil (bitumen) and mineral rich clay in various proportions (Adegoke *et al.*, 1980). The heavy oil in the tar sand is a viscous complex mixture of hydrocarbons and other heterocyclic substances.

Radioactive elements occur in trace amounts in all rocks and minerals and are readily detectable by the gamma radiation emitted during their decay. Natural radioactivity is associated with natural sources such as uranium deposits, oil and natural gas fields (Boyle, 1982). The primary source of radiation received by humans is from the store of natural radioactivity in soils, those due to medical examination/ therapy and artificial radionuclides produced by thermonuclear testing. All over the world, radioactivity measurements have revealed a lot of useful information about radiation levels in natural and polluted environments. External radiation exposures from naturally occurring radionuclides have been reported to contribute about half of the average annual dose to humans from all radiation sources (National Research Council, 1988). The health effect of radon is a subject of much debate; recent reports from the U.S indicate that a portion of the population may be exposed to potentially harmful level of radiation from radon progeny. Radon produces two short-lived isotopes of polonium, ^{218}Po and ^{214}Po, which are non-gaseous and tend to attach to particulates and aerosols in the air and can be inhaled. In such condition, it lodges in the lungs and can cause damage to the lungs through the emission of alpha radiation as it decays. Gates and Gundersen (1992) showed that the decay of short-lived daughters of some radionuclides especially ^{222}Rn causes induced cancer and was responsible for an estimated 15,000 to 20,000 deaths in the U.S.

Up till now, no work has been reported on the health implications of the natural radioactivity distribution in the tar-sand deposits of Ondo State and the environmental implications for its exploration and exploitation. The main objective of this study is to determine the absorbed doses and dose equivalents due to selected radionuclides in both the soil and bituminous sand samples, thus to assess their health impact.

2. Geology and Stratigraphy

The study area falls within the Nigerian sector of the Dahomey basin. Klemme (1975) showed that it is a coastal, sedi-

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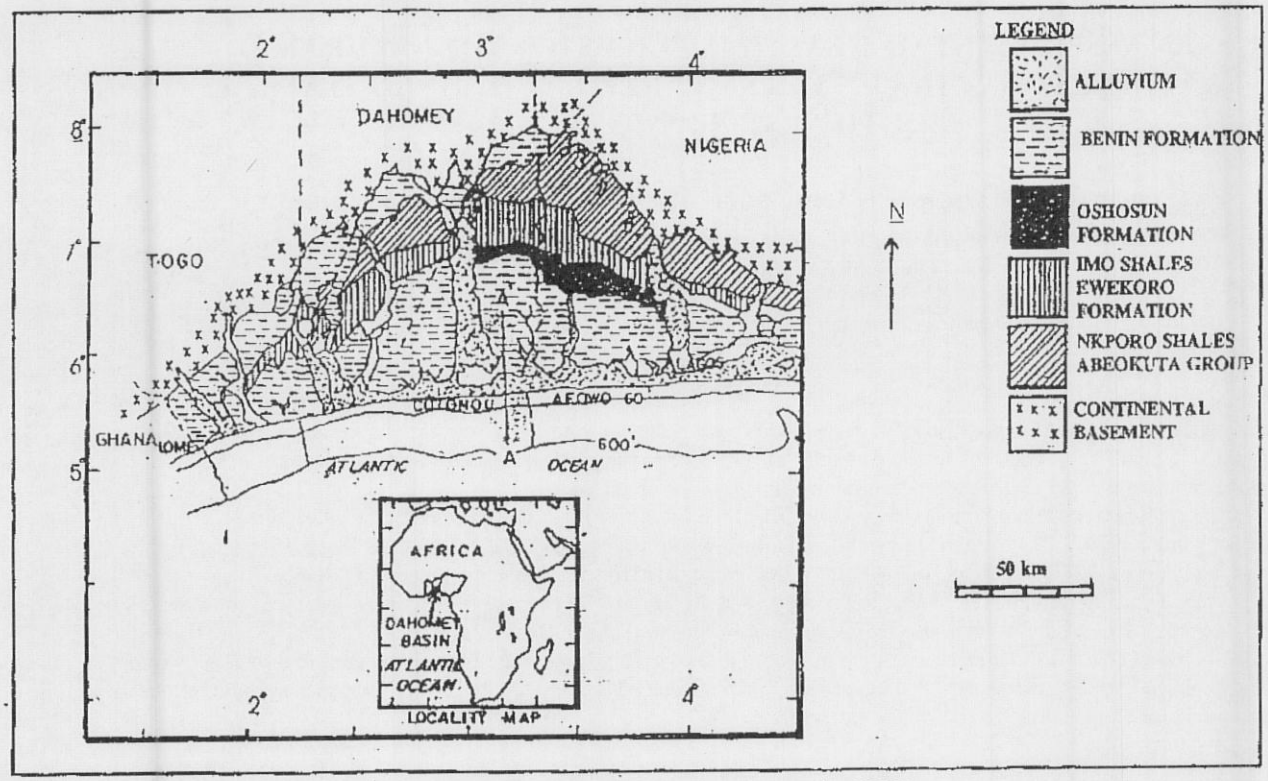

Figure 1: Regional Geological map of Dahomey Basin (after Adegoke *et al.*, 1980)

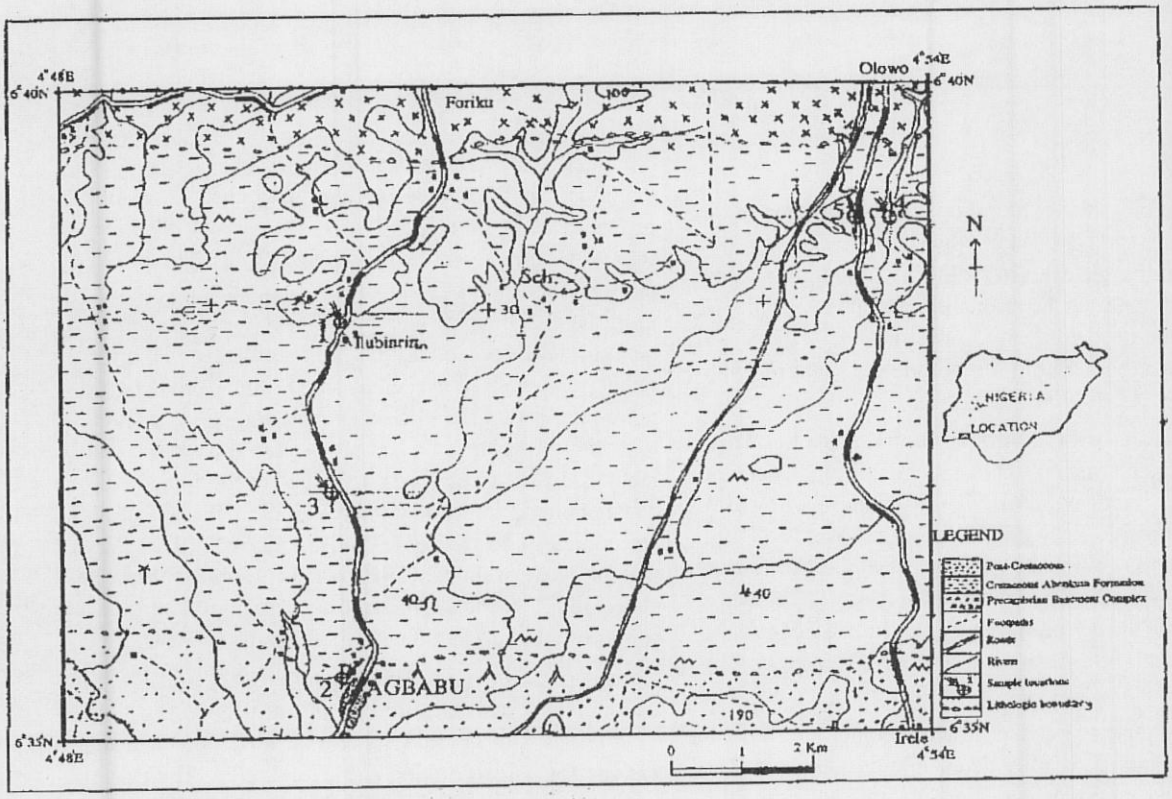

Figure 2: Sample location map of study area (after Oyawale, 2003)

mentary, marginal (Type 5) pull apart basin. The basin extends from Ghana-Ivory coast boundary across Togo, and republic of Benin to Western Nigeria. Durham and Picket (1966) subdivided the basins sedimentary fill into three lithological units. The geology of the Nigerian sector of the Dahomey basin was later reviewed by Omatsola and Adegoke (1981). They showed that the bitumen were found impregnating sedimentary deposits previously referred to as the Abeokuta Formation in the literature. They recognized three Formations belonging to the Abeokuta group. These are Ise, Afowo and Araromi Formations. The main habitat, for the bituminous sands are the Afowo Formation,

which is made up of a thick sequence of sands, interbedded with organic shale and siltstones. The tar bearing sands occur as two distinct stratigraphic bands (X and Y) separated by a uniformly thick oil shale (Enu, 1985). The thickness of the oil shale ranges between 6 and 15m, and the average thickness of the tar sand horizons is about 12m.

3. Materials and Methods

The study area falls within latitudes 6°35' and 6°39' N and longitudes 4°48' and 4°54' E. Ten surface and pit samples were collected from five locations (Ilubinrin, Agbabu, Mile 2, Camp Looda 1 and Camp Looda 2 (Fig. 2). The samples include both the bituminous sands and the overlying materials generally referred to as the overburden.

For the purposes of gamma-ray spectrometric analysis, except for samples 2A and 3A that are viscous bitumen, the samples were homogenized and dried in free air until constant weight was achieved. The samples were sealed (air tight) for a minimum of 28 days to attain secular equilibrium, which is done to prevent the gaseous daughters of the two natural decay series headed by U-238 and Th-232. Energy and efficiency calibrations were done using a well-calibrated mixed source soil standard obtained from the Federal Radiation Protection Services (FRPS), University of Ibadan, Nigeria and traceable to the standard calibration laboratory of the IAEA. These calibrations aided the identification and quantification of the radionuclides present in each sample. The analysis was done using a Gamma ray spectrometer fitted with a calibrated Canberra vertical coaxial high purity Germanium Detector System located at the laboratory of the Centre for Energy, Research and Development (CERD), Obafemi Awolowo University, Ile-Ife. The spectra for each sample was measured by counting for 36,000 secs using an HPGe detector that was shielded by a 5cm thick lead castle which was constructed to maintain low background radiation level. Also, an empty container was counted to serve as background count. The soil standard that contains "certified" radioactivity concentrations due to ^{238}U, ^{232}Th and ^{40}K (by weight) was also counted for a minimum of 36,000s in accordance with standard practice (IAEA, 1989). The spectra produced were analyzed using a computer program Sampo-90, which matched γ-energies at various energy levels to a library of possible radioisotopes.

For the purposes of EDXRF study, about 10g of the air dried samples were disaggregated by pestle and mortar and later pulverized in a disc mill. Except for the sticky tar-sand, each sample powder was homogenized and quartered to obtain a representative portion. Pellets of 19mm diameter were prepared from 0.3g powder mixed with cellulose in a 1:2 weight ratio and pressed at 5 tons. Measurements were made at the Centre for Energy, Research and Training (CERT), Ahmadu Bello University, Zaria, using an annular 25mCi ^{109}Cd and ^{55}Fe excitation sources. The ^{109}Cd emits Ag-K X-rays (22.1KeV) and ^{55}Fe emits Mn-K X-rays (5.89KeV) so that all elements with low characteristics excitation energies were detectable in the samples.

The corresponding absorbed dose rates were calculated using the relationship derived by Beck *et al.*, (1972), which is given as:

$$D = 0.042\, A_{c(K)} + 0.429\, A_{c(U)} + 0.666\, A_{C(Th)} \quad (nGyhr^{-1})$$

where,

D = the absorbed dose rate in air

$A_{C(K)}$ = Activity concentration of potassium ($BqKg^{-1}$)

$A_{C(U)}$ = Activity concentration of uranium ($BqKg^{-1}$)

$A_{C(TH)}$ = Activity concentration of thorium ($BqKg^{-1}$)

The Dose equivalent in ($mSvyr^{-1}$) was obtained by converting the absorbed dose in $nGyhr^{-1}$ to $mSvyr^{-1}$ thus:

$$X\ (nGyhr^{-1}) = (X * 24hrs * 365days / 1000000)\ mSvyr^{-1}$$

4. Results and Discussions

Table 1 shows the results of the various radioisotopes identified and their specific activity concentration in the soil/bituminous samples. The radionuclides identified and quantified from the gamma ray spectra are decay daughter products of naturally occurring radioactive elements ^{238}U and ^{232}Th. The other naturally occurring but non-series radioactive isotope, ^{40}K whose photo peak was identified could not be quantified. In spite of its ubiquitous occurrence in most environments, ^{40}K were observed to occur below the limit of detection of our gamma spectrometer. Feldspars and mica have been reported to occur in very small amount; also clays are not present in high percentage in the tar sands <5% (Adegoke *et al.*, 1980). All these are potential sites for K, the absence of which could result in low value of ^{40}K in the measured samples.

The average specific activity concentrations of ^{214}Bi, ^{208}Tl and ^{226}Ra in the overburden are 165.64 ± 9.56 $BqKg^{-1}$, 150.25 ± 10.36 $BqKg^{-1}$ and 60.97 ± 4.92 $BqKg^{-1}$. In the bituminous sands/viscous tar Pb-214 and Ra-226 showed average values of 24.09 ± 0.65 $BqKg^{-1}$ and 25.25 ± 3.16 $BqKg^{-1}$ respectively. The other radionuclides were below the detectable limits of the instrument. The activity concentrations of the radionuclides vary partly with depth and partly with lithology and the values are within the same range when compared with values obtained for similar radionuclides on non-mineralized soils from Kanawa uranium mineralization area in N.E. Nigeria (Funtua, 1994), with U, Th, ^{226}Ra and ^{210}Pb having 30-71ppm, <250 $BqKg^{-1}$, <300$BqKg^{-1}$ and <300$BqKg^{-1}$ respectively.

The low level of radioactive elements in the bituminous sand correlates with the results of natural gamma-ray logs earlier reported by (Adegoke *et al.*, 1980). They observed a low magnitude of gamma deflection opposite clean sands, a high magnitude of gamma deflection opposite clay/shale horizons and an invariably low gamma-ray response in the zone of bitumen-saturated sands.

Tables 2 and 3 show the summary of elemental concentration in the overburden and the bituminous sands respectively. In the overburden, the values of Th varied from 11 ± 4 ppm to 20 ± 6 ppm with an average of 16.6 ± 4.22 ppm while U varied from 12 ± 3 ppm to 15 ± 3 ppm with an average of 13.2 ± 1.2 ppm. In the bituminous sands, the values of Th varied from 13 ± 5 ppm to 32 ± 6 ppm while U

Table 1: Concentration of radionuclides in ($BqKg^{-1}$)

Sample	^{238}U			^{232}Th	
	^{214}Bi	^{214}Pb	^{226}Ra	^{208}Tl	^{228}Ac
Lateritic Soil (1A)	263.83±4.25	107.60±2.38	93.98±1.20	152.92 ±6.62	73.17 ±8.8
Clayey Soil (1B)	97.87 ± 1.89	59.62 ± 1.53	69.17± 4.02	61.86 ± 2.28	62.24 ± 8.5
Sandy clay Soil (1C)	41.75 ± 0.85	17.01 ± 0.59	71.61± 1.99	353.78±15.19	67.81 ± 8.9
Laterite Soil (4A)	275.22±4.67	60.71 ± 1.56	47.67± 2.83	63.02 ± 2.65	99.25±11.4
Loose sand (5B)	149.58±2.89	31.91 ± 0.97	22.42± 1.33	119.50±5.78	15.43 ± 2.61
Bituminous sand (1D)	-	17.77 ± 0.46	36.13± 3.15	-	-
Viscous tar (2A)	-	-	18.12± 3.53	-	-
Viscous tar (3A)	-	31.01 ± 0.84	27.83± 3.53	-	-
Bituminous sand (4B)	-	-	18.92± 2.46	-	-
Bituminous sand (5A)	-	-	-	-	-

Table 2: Elements Concentration in the Overburden (Values in ppm except where indicated).

Elements	Samples					
	1A Lateritic soil	1B Reddish brown clayey soil	1C Sandy clay	4A Lateritic top soil	5B Loose medium grained sand	Limit of detection (LOD)
K (wt)%	0.31 ± 0.08	0.55 ±0.23	0.52 ± 0.22	0.72 ± 0.24	0.80 ± 0.24	0.30
Ca (wt)%	0.20 ± 0.06	0.39 ± 0.05	0.38 ± 0.05	0.54 ± 0.08	0.49 ± 0.06	0.144
Ti	320 ± 20	470 ± 40	500 ± 40	660 ± 50	360 ± 40	0.08
Mn	200 ± 10	60 ± 10	70 ± 10	1300 ± 100	20 LOD	0.039
Fe (wt)%	24.63 ± 0.28	12.18 ± 0.13	15.88 ± 0.16	19.32 ± 0.19	0.62 ± 0.03	0.27
Zn	57 ± 10	52 ± 10	4.3 ± 8	52± 10	26 ± 8	10
Rb	11 ± 3	15 ± 4	19 ± 4	12 ± 3	10 ± 2	0.18
Sr	126 ± 4	248 ± 6	161 ± 5	74 ± 5	135 ± 4	2.9
Y	9 ± 2	19 ± 3	12 ± 3	12 ± 3	33 ± 3	5.2
Pb	68 ± 14	53 ± 18	43 ± 10	63 ± 10	30 ± 8	0.18
Ta	<LOD	<LOD	<LOD	<LOD	<LOD	0.13
W	<LOD	<LOD	<LOD	<LOD	<LOD	0.18
Th	18 ± 6	20 ± 6	16 ± 5	18 ± 6	11 ± 4	0.24
U	12 + 3	12 ± 3	13 ± 4	15 ± 3	14 + 4	0.41
Zr	346 ± 5	446 ± 6	365 ± 6	255 ± 5	223 ± 4	0.85
Nb	15 ± 2	19 ± 2	10 ± 2	18 ± 3	6 ± 2	0.10

< LOD = Value lower than limit of detection

Table 3: Elements Concentration in Bituminous sands (Values in ppm except where indicated)

Elements	Samples				
	1D Bituminous sands	2A Viscous tar	3A Viscous tar	4B Slightly impregnated tar sand	5A Highly impregnated bituminous sand
K (wt)%	0.56 ± 0.22	0.11<LOD	0.06 LOD	0.75±0.25 0.44	0.08 LOD
Ca (wt)%	0.54 ± 0.12	0.10<LOD	0.22 ± 0.04	±0.07	0.06 LOD
Ti	400 ± 40	3 LOD	30 ± 4	230 ± 40	20 ± 1
Mn	30 ± 10	10 LOD	20 LOD	20 LOD	30 LOD
Fe (wt)%	3.35 ± 0.06	0.04 ± 0.01	0.52 ± 0.06	0.76 ± 0.04	0.03 ± 0.003
Zn	30 ± 12	51 ± 10	7 ± 2	30± 12	3 LOD
Rb	8 ± 3	24 ± 5	2 LOD	8 ± 2	1 LOD
Sr	17 ± 3	19 ± 3	25 ± 1	14 ± 3	4 ± 0.40
Y	10+ 3	18 + 4	4 + 1	6 ± 3	1 LOD
Pb	33 ± 7	69 ± 18	6 LOD	31 ± 6	4 LOD
Ta	<LOD	<LOD	<LOD	<LOD	< LOD
W	<LOD	<LOD	<LOD	<LOD	< LOD
Th	14 ± 6	32 ± 8	< LOD	13 ± 5	< LOD
U	9 ± 3	33 ± 6	4 ± 1	10 ± 3	< LOD
Zr	136 ± 3	18 ± 2	76 ± 1	75 ± 3	15 ± 1
Nb	6 ± 2	14 ± 2	1 LOD	4 ± 1	1 LOD

< LOD = less than limit of detection

Table 4: Calculated Absorbed Dose Rate of the Lateritic Overburden.

Sample no	Sample Description	Ac(U) Ra-226 ($Bqkg^{-1}$)	A_C (Th) Ac-228 ($Bqkg^{-1}$)	Absorbed dose rate ($nGyhr^{-1}$)	Dose equivalents ($mSvyr^{-1}$)
1A	Lateritic Top-soil	93.98± 1.20	73.17± 8.84	89.05	0.78
1B	Reddish brown clayey soil	69.17 ± 4.02	62.24 ± 8.53	71.12	0.62
1C	Sandy clay	71.61± 1.99	67.81± 8.90	75.88	0.66
4A	Lateritic Top- soil	47.67 ± 2.83	99.25 ± 11.46	86.55	0.76
5B	Loose medium grained sand.	22.42 ± 1.33	15.43± 2.61	19.89	0.17

Table 5: Calculated Absorbed Dose Rate of bituminous sand ($nGyhr^{-1}$)

Sample no	Sample Description	Ac (U) Ra-226 ($Bqkg^{-1}$)	A_C (Th) Ac-228 ($Bqkg^{-1}$)	Absorbed dose rate ($nGyhr^{-1}$)	Dose equivalents ($mSvyr^{-1}$).
1D	Bituminous sand	36.13± 3.15	-	15.49	0.135
2D	Viscous tar	18.12 ± 3.52	-	7.77	0.068
3A	Viscous tar	27.13± 3.35	-	11.94	0.105
4B	Slightly impregnated bituminous sand	18.92± 2.46	-	8.12	0.07
5A	Highly impregnated bituminous sand	-	-	-	-

Table 6: Dose limits and their biological effects

Radiation dose rate	Duration of exposure	Likely effects/implications
10,000 mSv	Short-term dose	Immediate illness and subsequent death within a few weeks.
1,000 mSv	Short-term dose	Nausea and decreased white blood cell, but not death. Above this, severity of illness increase with dose.
50 mSv/yr	Over 5years	Conservatively, the lowest dose rate where there is any evidence of cancer being caused. Above this, the probability of cancer occurrence increases with dose.
20 mSv/yr	Over 5years	Limit for nuclear industry employees and uranium miners, who are closely monitored.
2 mSv/yr (approx)		Normal background radiation from natural sources, including an average of 0.7mSv/yr from radon in air.
0.3-0.6 mSv/yr		Artificial sources of radiation, mostly medical equipment.

Source: (ICRP, 2002) Uranium information center.

varied from 4 ± 1 ppm to 33 ± 6 ppm. This confirms the presence of these elements in both the overburden and the bituminous sands.

The results of the absorbed dose rate vary between 19.89 and 89.05 $nGyhr^{-1}$ with a mean of 68.49 $nGyhr^{-1}$ in the overburden while the average value of absorbed dose rate in the bituminous sand is 8.66 $nGyhr^{-1}$. (Tables 4 and 5). The absorbed dose rate itself does not give an indication of possible biological effects until it is converted to the dose equivalent, which is measured in Sieverts (Sv). The mean dose equivalents are 0.59 $mSvyr^{-1}$ in the overburden and 0.076 $mSvyr^{-1}$ in the bituminous sands. On the risk of cancer-induction due to exposure of the population, observational evidence shows that radiation induced cancer in humans comes largely from exposures due to large doses over a short period of time. However, for setting of environmental standards and for gauging the consequences of exposures routinely received by the general public, the most important doses are relatively small doses received over long period of time. Comparing the results of this study with the dose limit set by the International Commission on Radiological Protection (ICRP) in Table 6, the calculated dose equivalent falls below harmful level which is 1 mSv/yr to the general public and 20 mSv/yr for radiation workers in most environment.

5. Conclusions

The results of gamma spectrometer and energy dispersive XRF analyses showed that measurable traces of radioisotopes that belong to ^{238}U and ^{232}Th are present in both the overburden and the bituminous sands. The result shows that the dose equivalents at a distance of 1 m from the ground are 0.59$mSvyr^{-1}$ in the overburden while it is 0.076$mSvyr^{-1}$ in the bituminous sands. This is still below harmful dose to man. However, the mode of exploitation and extraction could contribute to an increase in the rate of release of the harmful radon gas and could therefore be an important health factor in the future. Open cast mining might expose and concentrate Radon in the overburden during excavation, which may lead to significant health hazards. For low levels of radiation exposure, the biological effects are so small they may not be detectable. Radiation protection standards assume however, that the effect is directly proportional to the dose, even at low levels. A baseline study of this nature, coupled with data from other sampling media such as well water, stream water and vegetation could help the government to enact policies on safety and monitoring when full exploitation begins.

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ANALYSIS OF CONVERGENCE FOR CONTROL PROBLEMS GOVERNED BY EVOLUTION EQUATIONS INEQUALITY AND EQUALITY CONSTRAINTS WITH MULTIPLIERS IMBEDDED

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Abstract

The convergence of a scheme to minimize a class of a system of continuous optimal control problems characterized by a system of evolution equations and a system of linear inequality and equality constraints with multiplier imbedding is considered. The result is applied to some problems and the scheme is found to exhibit geometric convergence.

Keywords: Evolution equations, multiplier imbedding, constraints, geometric convergence.

1. Introduction

Recently, Olorunsola (2002) constructed a control operator specifically for a wider class of systems of continuous optimal control problems characterized by a system of evolution equations and a system of linear inequality and equality constraints with multiplier imbedding. He however failed to address the issue of the nature of convergence of this scheme as it applies to the combination of the standard penalty method and the multiplier imbedding algorithm (Glad, 1979). This paper deals with the issue of the convergence of this scheme and a good updating approach as it applies to this scheme.

For the purpose of clarity and completeness, it is therefore relevant at this stage to look at the class of problems dealt with in the above scheme whose convergence is being addressed.

Problem 1.1

$$\text{Minimize } \int_0^T x^T(t)Px(t) + u^T(t)Qu(t)dt \qquad (1.1)$$

$$\text{Subject to } \dot{x}(t) = Rx(t) + Gx(t-r) + Wu(t) \qquad (1.2)$$

$$Cx(t) + Du(t) \geq 0 \qquad (1.3)$$

and

$$Ex(t) + Fu(t) = 0 \qquad (1.4)$$

where $x(o) = x_o$, $t \in [0,T]$, $x(t) = h(t)$ for $t \in [-r,0]$.

$x(t), x(t-r) \in R^n$, $u(t) \in R^m$ are respectively the state variable, delay and the control variables. P and Q are n and m-symmetric positive definite square matrices respectively. The matrices R, G, C and E are any n-square matrices while W, D and F are n x m matrices. In case m < n, products of the form Qu(t), Wu(t) .... etc., control vectors u(t) can be made conformable by adjoining n-m zeroes.

Using the combination of the standard penalty function method (Glad, 1979) and the method of the multiplier imbedding Extended conjugate gradient (Olorunsola, 1991), a control operator A for the class of Problem 1.1 was obtained in Olorunsola (2002) as in Theorem 1.1 below.

THEOREM 1.1 The exact control operator that satisfies the given optimization problem 1.1 is given by

$$(AZ)(t) = \begin{bmatrix} a_{11} & a_{12} & \ldots\ldots\ldots\ldots a_{16} \\ a_{21} & a_{22} & \ldots\ldots\ldots\ldots : \\ : & & : \\ : & & : \\ a_{61} & \ldots\ldots\ldots\ldots & a_{66} \end{bmatrix} \tag{1.5}$$

where $(a_{11}x)(t) = \mu_1\left[x_0(t) - (R+G)\dot{x}_0\right]Sinh(t) + \mu_1\int_0\left\{\dot{x}(s) - (R+G)x(s)Cosh(t-s)ds -\right.$

$$\int_0\left\{[P + \mu_1 R^T(R+G)x(s) - \mu_1 R^T\{x(s) + \mu_2 M^T Mx(s)Sinh(t-s)\}ds -\right.$$

$$\mu_1\int_0^{-r}\left\{G^T(R+G)x(s+r)Sinh(t-r-s)\right\}ds + \left\{\left[P + \mu_1 R^T(R+G)\right]x_0 + \mu_2 M^T Mx_0 - \mu_1 R^T x_0(t) +\right.$$

$$\mu_1 G^T(R+G)x(r) - \mu_1 G^T\dot{x}(s+r)Cosht + \frac{Sinht}{SinhT}\{[P + QR^T(R+G)]x(T) - \mu_1 R^T\dot{x}(T) + \mu_2 M^T Mx(T) +$$

$$\mu_1 G^T x(T-r) - \mu_1 G^T x(T-r) - \left[(P + \mu_1 R^T(R+G)x_0 - \mu_1 G^T x_0)\right] + \mu_2 M^T Mx_0 + \mu_1 G^T(R+G)x(r)\}CoshT$$

$$\mu_1\left[\dot{x}(t) - (R+G)x_0\right]SinhT + \int_0^T\left\{P + \mu_1 R^T(R+G)x(s) - \mu_1 R^T\dot{x}(s) + \mu_2 M^T Mx(s)\right\}SinhT(T-S)ds$$

$$+ \mu_1\int_0^{-r}\left\{G^T(R+G)\dot{x}(s+r)\right\}Sinh(T-r-s)ds - \mu_1\int_0^T\left\{\dot{x}(s) - (R+G)x(s)\right\}Cosh(T-s)ds \tag{1.6}$$

$$(a_{21}x)(t) = \nu_1 W^T(R+G)x(t) - \mu_1 W^T\dot{x}(t) + \mu_2 N^T Mx(t) \tag{1.7}$$

$$(a_{31}x)(t) = \nu_1 G^T[(R+G)x(t+r) - \dot{x}(t)] + \mu_2 N^T Mx(t) \tag{1.8}$$

$$(a_{41}x)(t) = \dot{x}(t) - (R+G)x(t) \tag{1.9}$$

$$(a_{51}x)(t) = \mu_2 Mx(t) \tag{1.10}$$

$$(a_{61}x)(t) = 0 \tag{1.11}$$

$$(a_{12}u)(t) = \mu_1 u_0 \sinh t - \mu_1\int_0^t Wu(s)Cosh(t-s)ds - \int_0^t\left\{\mu_1 R^T Wu(s) + \mu_2 M^T Nu(s)Sinh(t-s)\right\}ds$$

$$-\mu_1\int_0^{-r} G^T Wu(s+r)Sinh(t-r-s)ds + \left[\mu_1 R^T Wu_0 + \mu_2 M^T Nu_0 + \mu_1 G^T u(r)\right]Cosht +$$

$$\frac{Sinht}{SinhT}\left\{\mu_1 G^T u(T) + \mu_2 M^T Nu(T) + \mu_1 G^T Wu(T-r)\right\} - \left[\mu_1 R^T Wu_0 + \mu_2 M^T Nu_0 + \mu_1 G^T Wu(T)\right]CoshT$$

$$-\mu_1 Wu_0 SinhT + \int_0^T \left\{\mu_1 R^T Wu(s) + \mu_2 M^T Nu(s)\right\} Sinh(T-s)ds + \mu_1 \int_0^{-r} G^T Wu(s+r) Sinh(t-r-s)ds +$$

$$\mu_1 \int_0^T Wu(s) Cosh(T-s)ds \tag{1.12}$$

$$(a_{22}u)(t) = \left\{Q + \mu_1 W^T W + \mu_2 N^T N\right\} u(t) \tag{1.13}$$

$$(a_{32}u)(t) = \mu_1 G^T Wu(t-r) \tag{1.14}$$

$$(a_{42}u)(t) = -Wu(t) \tag{1.15}$$

$$(a_{52}u)(t) = \mu_2 Nu(t) \tag{1.16}$$

$$(a_{62}u)(t) = \tfrac{1}{\mu_2} a_{52}u(t) \tag{1.17}$$

$$(a_{13}h)(t) = \mu_1 Gh_0 Sinht - \mu_1 \int_0^t Gh(s) Cosh(t-s)ds - \mu_1 \int_0^t R^T Gh(s) Sinh(t-s)ds + \mu_1 R^T Gh_0 Cosht$$

$$\frac{Sinht}{SinhT}\left(\mu_1\left[R^T Gh(r) - R^T Gh_0 Coshr - Gh_0 Sinht\right] + \mu_1 \int_0^r R^T Gh(s) Sinh(r-s)ds\right.$$

$$\left. + \mu_1 \int_0^r Gh(s) Cosh(r-s)ds\right) \tag{1.18}$$

$$(a_{23}h)(t) = \mu_1 W^T Gh(t) \tag{1.19}$$

$$(a_{33}h)(t) = \mu_1 G^T Gh(t) \tag{1.20}$$

$$(a_{43}h)(t) = -Gh(t) \tag{1.21}$$

$$(a_{14}y)(t) = -\mu_2 \int_0^t y(s) Sinh(t-s)ds - \mu_2 y_0 Cosht - \frac{Sinht}{SinhT}\left\{\mu_2 y(T) + y_0 Cosht + \mu_2 \int_0^T y(s) Sinh(T-s)ds\right\}$$

(1.22)

$$(a_{24}y)(t) = -\mu_2 Ny(t) \tag{1.23}$$

$$(a_{54}y)(t) = -\mu_2 y(t) \tag{1.24}$$

$$(a_{62}y)(t) = \tfrac{1}{\mu_2} a_{52}y(t) \tag{1.25}$$

$$(a_{15}\lambda)(t) = -\lambda_0 Sinht + \int_0^t \lambda(s) Cosh(t-s)ds + \int_0^t \lambda^T(s) RSinh(t-s)ds - \lambda_0^T RCosht + \frac{Sinht}{SinhT}\left(-\lambda(T) + \lambda_0^T RCosht\right.$$

$$\left. + \lambda_0 SinhT - \int_0^T \lambda^T(s) RSinh(T-S)ds - \int_0^T \lambda(s) Cosh(T-s)ds\right) \tag{1.26}$$

$$(a_{25}\lambda)(t) = \lambda^T(t) W \tag{1.27}$$

$$(a_{35}\lambda)(t) = -\lambda^T(t) G \tag{1.28}$$

$$(a_{16}\rho)(t) = -\int_0 \rho(s)Sinh(t-s)ds + \rho_0 Cosht + \frac{Sinht}{SinhT}\left\{\rho(T) - \rho_0 CoshT + \int_0 \rho(s)Sinh(T-S)ds\right\} \tag{1.29}$$

$$(a_{26}\rho)(t) = \rho^T(t)N \tag{1.30}$$

$$(a_{34}y)(t) = (a_{44}y)(t) = 0 \tag{1.31}$$

$$(a_{44}\lambda)(t) = (a_{55}\lambda)(t) = (a_{63}\lambda)(t) = (a_{65}\lambda)(t) = 0 \tag{1.32}$$

$$(a_{36}\rho)(t) = (a_{46}\rho)(t) = (a_{56}\rho)(t) = (a_{66}\rho)(t) = 0 \tag{1.33}$$

$$(a_{53}h)(t) = 0 \tag{1.34}$$

Remark 1.1

Although the Scheme reliably converges at low iterations and that the constraints are well satisfied for varying penalty constants μ_1 and μ_2, the nature of its convergence was not addressed. Hence we discuss in this paper the nature of its convergence.

We now state and prove the main results.

2. Convergence of the Scheme

The aim is to prove that the optimal control problem 1.1 exhibits geometric convergence.

Definition 2.1 Let $\{\xi_n\}$ be a sequence of vectors in a Hilbert Space H with limit $\xi^* \in H$

Such that $\{\|\xi_{n+1} - \xi^*\|\}/\|\xi_n - \xi^*\|$ tends to a limit $\gamma < 1$ as n $\to\infty$, then $\{\xi_n\}$ is said to converge geometrically to ξ^* with a convergence ratio γ, as reported by (Ibiejugba, Otunta and Olorunsola, 1992)

We shall recall here for the purpose of clarity and explicitness the various steps of the conjugate gradient algorithm that generates the convergent sequence $\{Z_n(t)\}$ of solutions of Problems 1.1 according to Dipillo, Grippo and Lampariello (1974). The algorithm employs the explicit knowledge of the control operator A developed in Theorem 1.1.

STEP 1 Choose initial values for the conjugate descent algorithm

$$Z_0^t(t) = \{x_0(t), u_0(t), h_0(t), y_0(t), \lambda_0(t), \rho_0(t)\} \qquad Z_0(t) \in H.$$

Compute $P_0 = -g_o$

The remaining members of the sequence are then found as follows:

STEP 2 Update x_o, u_o, h_o, y_o, λ_o, ρ_o, such that

$$\left.\begin{aligned} x_{n+1}(t) &= x_n(t) + \alpha_n P_{x,n} \\ u_{n+1}(t) &= u_n(t) + \alpha_n P_{u,n} \\ h_{n+1}(t) &= h_n(t) + \alpha_n P_{h,n} \\ y_{n+1}(t) &= y_n(t) + \alpha_n P_{y,n} \\ \lambda_{n+1}(t) &= \lambda_n(t) + \alpha_n P_{\lambda,n} \\ \rho_{n+1}(t) &= \rho_n(t) + \alpha_n P_{\rho,n} \end{aligned}\right\} \tag{2.1}$$

where α_n and $P_{\cdot n}$ are the step length and the descent directions respectively.

STEP 3 Update gradients and descent directions using the updating rules:

$$\left.\begin{aligned} g_{x,n+1} &= g_{x,n} + \alpha_n AP_{x,n} \\ g_{u,n+1} &= g_{u,n} + \alpha_n AP_{u,n} \\ g_{h,n+1} &= g_{h,n} + \alpha_n AP_{h,n} \\ g_{y,n+1} &= g_{y,n} + \alpha_n AP_{y,n} \\ g_{\lambda,n+1} &= g_{\lambda,n} + \alpha_n AP_{\lambda,n} \\ g_{\rho,n+1} &= g_{\rho,n} + \alpha_n AP_{\rho,n} \end{aligned}\right\} \tag{2.2}$$

where $g_{\cdot,n}$ is the gradient at the n^{th} iteration and A is the control operator in Theorem 1.1

$$\alpha_n = \left\langle g_{\bullet,n} \quad g_{\bullet,n} \right\rangle_H \Big/ \left\langle P_{\bullet,n} \quad AP_{\bullet,n} \right\rangle_H$$

$$g_{\bullet,n}^T = (g_{x,n}, g_{u,n}, g_{h,n}, g_{y,n}, g_{\lambda,n}, g_{\rho,n}) \text{ and } p_{\bullet,n}^T = (p_{x,n}, p_{u,n}, p_{h,n}, p_{y,n}, p_{\lambda,n}, p_{\rho,n})$$

$$(AP_n)(t) = \begin{pmatrix} (a_{11}p_{x,n})(t) + (a_{12}p_{u,n})(t) + (a_{13}p_{h,n})(t) + (a_{14}p_{y,n})(t) + (a_{15}p_{\lambda,n})(t) + (a_{16}p_{\rho,n})(t) \\ (a_{21}p_{x,n})(t) + (a_{22}p_{u,n})(t) + (a_{23}p_{h,n})(t) + (a_{24}p_{y,n})(t) + (a_{25}p_{\lambda,n})(t) + (a_{26}p_{\rho,n})(t) \\ (a_{31}p_{x,n})(t) + (a_{32}p_{u,n})(t) + (a_{33}p_{h,n})(t) + (a_{34}p_{y,n})(t) + (a_{35}p_{\lambda,n})(t) + (a_{36}p_{\rho,n})(t) \\ \vdots \\ \vdots \\ (a_{61}p_{x,n})(t) + (a_{62}p_{u,n})(t) + (a_{63}p_{h,n})(t) + (a_{64}p_{y,n})(t) + (a_{65}p_{\lambda,n})(t) + (a_{66}p_{\rho,n})(t) \end{pmatrix}$$

$$= \begin{pmatrix} AP_1(t) \\ AP_2(t) \\ \vdots \\ \vdots \\ AP_6(t) \end{pmatrix} \tag{2.3}$$

Setting

$$\left.\begin{aligned} J_{x,n} &= \nabla_{x,n} J(x_n(t), u_n(t), h_n(t), y_n(t), \lambda_n(t), \rho_n(t), \mu_1, \mu_2) \\ J_{u,n} &= \nabla_{u,n} J(x_n(t), u_n(t), h_n(t), y_n(t), \lambda_n(t), \rho_n(t), \mu_1, \mu_2) \\ J_{h,n} &= \nabla_{h,n} J(x_n(t), u_n(t), h_n(t), y_n(t), \lambda_n(t), \rho_n(t), \mu_1, \mu_2) \\ J_{y,n} &= \nabla_{y,n} J(x_n(t), u_n(t), h_n(t), y_n(t), \lambda_n(t), \rho_n(t), \mu_1, \mu_2) \\ J_{\lambda,n} &= \nabla_{\lambda,n} J(x_n(t), u_n(t), h_n(t), y_n(t), \lambda_n(t), \rho_n(t), \mu_1, \mu_2) \\ J_{\rho,n} &= \nabla_{\rho,n} J(x_n(t), u_n(t), h_n(t), y_n(t), \lambda_n(t), \rho_n(t), \mu_1, \mu_2) \end{aligned}\right\} \tag{2.4}$$

where

$$J(x_n(t), u_n(t), h_n(t), y_n(t), \lambda_n(t), \rho_n(t), \mu_1, \mu_2) = \int_0^T x^T(t)Px(t) + u^T(t)Qu(t)dt +$$

$$\mu_1 \int_0^T \left\{ \left[x(t) - Rx(t) - Gx(t-r) - Wu(t) \right]^T \left[x(t) - Rx(t) - Gx(t-r) - Wu(t) \right] \right\} dt +$$

$$\mu_2 \int_0^T \{[Mx(t)+Nu(t)-y(t)]^T [Mx(t)+Nu(t)-y(t)]\}dt + \int_0^T \lambda^T(t)[x(t)-Rx(t)-Gx(t-r)-Wu(t)]+$$

$$\int_0^T \rho^T(t)[Mx(t)+Nu(t)-y(t)]dt \qquad (2.5)$$

and $\nabla_{\bullet,n} J$ is the gradient of any compliment at the nth step. We obtain the following relations

$$p_{x,n} = -\int_0 \nabla_{x,n} J(x_n(t), u_n(t), h_n(t), y_n(t), \lambda_n(t), \rho_n(t), \mu_1, \mu_2)ds$$

or more generally $p_{\bullet,n} = -\int_0 \nabla_{\bullet,n} J(x_n(t), u_n(t), h_n(t), y_n(t), \lambda_n(t), \rho_n(t), \mu_1, \mu_2)ds$ for $P_{x,n}$, $P_{u,n}$, $P_{h,n}$, $P_{y,n}$, $P_{\lambda,n}$, and $P_{\rho,n}$.

Therefore

$$(AP_1)(t) = \mu_1[J_{x,0}(t) - (R+G)p_{x,0}]Sinht + \mu_1 \int_0 \{p_{x,n}(s) - (R+G)J_{x,n}(s)Cosh(t-s)ds$$

$$- \int_0 \{[P + \mu_1 R^T (R+G)J_{x,n}(s) - \mu_1 R^T \{p_{x,n}(s) + \mu_2 M^T M J_{x,n}(s)Sinh(t-s)ds]$$

$$- \mu_1 \int_0^{-r} \{G^T (R+G)J_{x,n}(s+r)\sinh(t-r-s)\}ds + ([p + \mu R^T (R+G)J_{x,0} + \mu_2 M^T M J_{x,0}(t)$$

$$- \mu_1 R^T J_{x,0}(0) + \mu_1 G^T (R+G)J_{x,n}(r) - \mu_1 G^T P_{x,n}(s+r)Cosht + \frac{Sinht}{SinhT}\{[P + QR^T (R+G)]J_{x,n}(T)$$

$$- \mu_1 R^T P_{x,n}(T) + \mu_2 M^T M J_{x,n}(T) + \mu_1 G^T J_{x,n}(T-r) - \mu_1 G^T J_{x,n}(T-r) -$$

$$[(P + \mu_1 R^T (R+G)J_{x,0}(0) - \mu_1 G^T J_{x,0}(0) + \mu_2 M^T M J_{x,0}(0) + \mu_1 G^T (R+G)J_{x,n}(r)]CoshT$$

$$+ \mu_1[p_{x,n}(t) - (R+G)J_{x,0}(0)]SinhT + \int_0^T \{p + \mu_1 R^T (R+G)J_{x,n}(s) - \mu_1 R^T p_{x,n}(s) +$$

$$\mu_2 M^T M J_{x,n}(s)\}Sinh(T-S)ds + \mu_1 \int_0^{-r} \{G^T (R+G)P_{x,n}(s+r)\}Sinh(T-r-s)ds -$$

$$+ \mu_1 \int_0^T \{P_{x,n}(t) - (R+G)J_{x,n}(s)\}Cosh(T-S)ds + \mu_1 j_{U,0}(0)\sinh t - \mu_1 \int_0 WJ_{u,n}(s)Cosh(t-s)ds$$

$$- \int_0 \{\mu_1 R^T WJ_{u,n}(s) + \mu_2 M^T NJ_{u,n}(s)Sinh(t-s)\}ds - \mu_1 \int_0^{-r} G^T WJ_{u,n}(s+r)Sinh(t-r-s)ds$$

$$+ [\mu_1 R^T WJ_{u,0}(0) + \mu_2 M^T NJ_{u,0}(0) + \mu_1 G^T J_{u,n}(r)]\cosh t + \frac{Sinht}{\sinh T}(\mu_1 G^T J_{u,n}(T) + \mu_2 M^T NJ_{u,n}(t)$$

$$+ \mu_1 G^T WJ_{u,n}(T-r) - [\mu_1 R^T WJ_{u,0}(0) + \mu_2 M^T NJ_{u,0}(0) + \mu_1 G^T WJ_{u,n}(T)]\cosh T - \mu_1 WJ_{u,0}(0)\sinh T$$

$$+ \int_0^T \{\mu_1 R^T WJ_{u,n}(s) + \mu_2 M^T WJ_{u,n}(s)Sinh(T-s)\}ds - \mu_1 \int_0^{T-r} G^T WJ_{u,n}(s+r)Sinh(t-r-s)ds$$

$$+ \mu_1 \int_0^T WJ_{u,n}(s)\cosh(T-s)ds + \mu_1 GJ_{h,0}(0)Sinht - \mu_1 \int_0 GJ_{h,n}(s)Cosh(t-s)ds - \mu_1 \int_0 R^T GJ_{h,n}(s)$$

$$Sinh(t-s)ds + \mu_1 R^T GJ_{h,0}(0)\cosh t + \frac{Sinht}{SinhT}\left\{\mu_1\left[R^T GJ_{h,n}(r) - R^T GJ_{h,0}(0)\cosh r - GT_{h,0}(0)\sinh t\right]\right.$$

$$\left. + \mu_1 \int_0 R^T GJ_{h,n}(s)\sinh(r-s)ds + \mu_1 \int_0 GJ_{h,n}(s)Cosh(r-s)ds\right\} - \mu_2 \int_0 J_{y,n}(s)Sinh(t-s)ds$$

$$- \mu_2 J_{y,0}(0)\cosh t - \frac{Sinht}{SinhT}\left\{\mu_2 J_{y,n}(T) + J_{y,0}(0)Cosht + \mu_2 \int_0^T J_{y,n}(0)Sinh(T-s)ds\right\} - J_{\lambda,0}(0)Sinht$$

$$+ \int_0 J_{\lambda,0}(0)\sinh t + \int_0 J_{\lambda,n}(s)Cosh(t-s)ds + \int_0 J^T_{\lambda,n}(s)R\sinh(t-s)ds - J^T_{\lambda,0}(0)RCosht +$$

$$\frac{Sinht}{SinhT}\left\{- J_{\lambda,n}(T) + J^T_{\lambda,0}RCosht + J_{\lambda,0}(0)\sinh T - \int_0^T J^T_{\lambda,n}(s)R\sinh(T-s)ds - \int_0^T J_{\lambda,n}(s)Cosh(T-s)ds\right\}$$

$$- \int_0 J_{p.0}(s)Sinh(t-s)ds + J_{p,0}(0)Cosh + \frac{Sinht}{SinhT}\left\{J_{p.n}(T) - J_{p,0}(0) + \int_0 J_{p,n}(s)Sinh(T-s)ds\right\}$$

(2.5)

$$(AP_2)(t) = \mu_1 W^T(R+G)J_{x,n}(t) - \mu_1 W^T P_{x,n}(t) + \mu_2 N^T MJ_{x,n}(t) + [Q + \mu_1 \| W \| + \mu_2 \| N \|]J_{u,n}(t)$$
$$+ \mu_1 W^T GJ_{h,n}(t) - \mu_2 NJ_{y,n}(t) + J_{\lambda,n}(t)W + J_{\rho,n}(t)W$$

(2.6)

$$(AP_3)(t) = \mu_1 W^T(R+G)J_{x,n}(t) - \mu_1 W^T P_{x,n}(t) + \mu_2 N^T MJ_{x,n}(t) + \mu_1 G^T WJ_{u,n}(t-r) +$$

$$\mu_1 J_{h,n}(t)\| G \| - J_{\lambda,n}(t)G \qquad (2.7)$$

$$(AP_4)(t) = P_{x,n}(t) - (R+G)J_{x,n}(t) - WJ_{u,n}(t) - G^T J_{h,n}(t) \qquad (2.8)$$

$$(AP_5)(t) = \mu_2 MJ_{x,n}(t) + \mu_2 NJ_{u,n}(t) - \mu_2 J_{y,n}(t) \qquad (2.9)$$

$$(AP_6)(t) = NJ_{x,n}(t) - \mu_2 J_{y,n}(t) \qquad (2.10)$$

The main result is stated below in Theorem 2.1.

REMARKS 2.1

The following remarks are in order as we state and prove theorem 2.1

(i). Z* is the optimal value required in Problem 1.1

(ii). The expression $\left\|Z_{n+1} - Z^*\right\|\left\|Z_n - Z^*\right\|^{-1}$ is the convergence ratio of the terms of the sequence {Zn(t)} in Hilbert Space H.

(iii). According to Hasdorff (1976), the general quadratic functional in the Hilbert space H to be minimized is given by $\varphi(z) = F_0 + \langle a, z\rangle_H + \frac{1}{2}\langle Z, AZ\rangle_H$ where A is the symmetric positive definite n-square matrix. If $F_0 = \langle a, z\rangle_H = 0$. We just have $\varphi(z) = \langle Z, AZ\rangle_H$. A is the control operator of Theorem 1.1.

(iv). Note that $\{P_{z,n}\}$ are conjugate with respect to the linear operator A

i.e. $\langle P_{z,n}, AP_K\rangle_H = 0$ n < k

THEOREM 2.1

The sequence $\{Z_n(t)\}$ of elements generated in solving Problem 1.1 using the explicit knowledge of the control operator A constructed in Theorem 1.1 converges geometrically, to Z*(t) with ratio γ given by

$$\gamma = 1 - \beta \text{ where } \beta = \frac{1}{\| Z_0 \|} \max . \| AZ_n \|^3 \left(< AP_{z,n}, P_{Z,n} >_H \right)^{-1}$$

Proof: Let $\varphi(z) = \langle z - z^*, AZ \rangle_H$ and $Z^T(t) = (x(t), u(t), h(t), y(t), \lambda(t), \rho(t))$ optimality condition demands that $AZ^*(t) = 0$.

In general for Z, Z_n, $\in$ H

$$\varphi(z_n) = \langle Z_n - Z^*, A(Z_n - Z^*) \rangle = < Z_n, AZ_n > - < Z^*, AZ_n > \tag{2.11}$$

and

$$\varphi(z_{n+1}) = \langle Z_n + \alpha_n p_{z,n} - Z^*, A(Z_n + \alpha_n p_{z,n} - Z^*) \rangle$$

$$= < Z_n, AZ_n > + \alpha_n < Z_n, AP_{z,n} > + \alpha_n < P_{z,n}, AZ_n > + \alpha_n^z < P_{z,n}, AP_{z,n} >$$

$$- < Z^*, AZ_n > - \alpha_n < Z^*, AP_{z,n} > \tag{2.12}$$

From 2.11 and 2.12

$$\varphi(Z_n) - \varphi(Z_{n+1}) = \frac{\| AZ_n \|^2}{< P_{z,n}, AP_{z,n} >} x \frac{\varphi(Z_n)}{< AZ_n, Z_n >} \tag{2.13}$$

Since A is a Self-adjoint operator, (Rickart, 1960; Olorunsola, 2002) $AZ^* = 0$

$$\text{Again } < AZ_n, Z_n > = < AZ_n, Z_0 > + \sum_{k=0}^{N-1} \alpha_k < AZ_n, P_{z,k} >, \quad but \quad \sum_{k=0}^{N-1} \alpha_k < AZ_n, P_{z,k} > = 0 \quad \forall n \neq k$$

Hence $<AZ_n, Z_n> = <AZ_n, Z_0>$ (2.14)

A is a bounded operator implies that there exist numbers $\bar{m} > 0 \; and \; \bar{M} \in R$ such that for every $z \in H$

$$\bar{m} \| Z - Z^* \|^2 \leq \| A(Z - Z^*) \|^2 \leq \bar{M} \| Z - Z^* \|^2 \tag{2.15}$$

Hence $$\langle AZ_n, Z_n \rangle \leq \| AZ_n \| \| Z_0 \| \tag{2.16}$$

Substituting (2.16) in (2.13) we obtain

$$\varphi(Z_n) - \varphi(Z_{n+1}) = \frac{\| AZ_n \|^2 . \varphi(Z_n)}{< P_{z,n}, AP_{z,n} > \| AZ_n \| \| Z_0 \|}$$

Hence $$\varphi(Z_{n+1}) \leq \left(1 - \frac{\| AZ_n \|^3}{< P_{z,n}, AP_{z,n} > \| Z_0 \|} \right) . \varphi(Z_n) \tag{2.17}$$

But by (2.15), $\varphi(Z_n) \geq \bar{m} \| Z_n - Z^* \|^2$ so that (2.17) becomes

$$(\| Z_{n+1} - Z \|^2)(\| Z_n - Z^* \|^2)^{-1} \leq \frac{\varphi(Z_{n+1})}{\varphi(Z_n)} \leq 1 - \frac{\| AZ_n \|^3}{< P_{z,n}, AP_{z,n} >} \| Z_0 \|^{-1}$$

Hence the result.

Table 1(a): Solution Profile for numerical Example 3.1. We compute the objective functional $J(x(t),u(t))$ and the constraint Satisfaction $CSAT(x,u) = \|\varphi(x,u)\|^2$

Penalty Constant μ_1	Penalty Constant μ_2	Iteration number K	Objective Functional $J(x(t),u(t))$	Constraint Satisfaction $\|\varphi(x(t),u(t))\|^2$
10^{-2}	10^{-2}	0	5.0000	25.7825
		1	2.86444	5.98355×10^{-1}
		2	1.942341	5.822259×10^{-1}
		3	1.390102	5.91823×10^{-1}
		4	1.021468	5.54895×10^{-1}
2×10^{-2}	2×10^{-2}	0	5.00000	25.7825
		1	2.988816	5.786152×10^{-1}
		2	2.225503	5.435591×10^{-1}
		3	1.831498	5.224789×10^{-1}
		4	1.58644	5.103435×10^{-1}
		5	1.440511	5.16819×10^{-1}
3×10^{-2}	3×10^{-2}	0	5.00000	25.7825
		1	2.930837	5.06737×10^{-1}
1×10^{-2}	3×10^{-2}	0	5.0000	25.7825
		1	2.90313	5.993069×10^{-1}
		2	2.102618	5.737325×10^{-1}
		3	1.730622	5.572019×10^{-1}
		4	1.615126	5.415913×10^{-1}
2×10^{-2}	3×10^{-2}	0	5.00000	25.7825
		1	2.986242	5.754655×10^{-1}
		2	2.306321	5.301353×10^{-1}
		3	2.171998	4.891628×10^{-1}
1×10^{-2}	4×10^{-2}	0	5.00000	25.7825
		1	2.942877	6.000822×10^{-1}
		2	2.150869	5.677288×10^{-1}
		3	1.904404	5.355533×10^{-1}
2×10^{-2}	4×10^{-2}	0	5.00000	25.7825
		1	2.925442	5.969161×10^{-1}
		2	2.141035	5.629162×10^{-1}
		3	1.899237	5.290906×10^{-1}

Table 1(b): Convergence ratio for numerical Example 3.1

Penalty	Constants	Iteration	Convergence Ratio
μ_1	μ_2	N	$\gamma^2 = 1 - \beta$
0.01	0.01	1	0.204197
		2	0.1827247
		3	0.1761002
		4	0.1769987
		5	0.1877945
0.02	0.02	1	0.2042600
		2	0.1827929
		3	0.1759149
		4	0.1773238
		5	0.1907254
0.03	0.03	1	0.2044095
		2	0.1827606
		3	0.1755998
		4	0.1775935
		5	0.193959
0.04	0.04	1	0.2046224
		2	0.1826889
		3	0.1751335
		4	0.17778556
		5	0.193959
0.05	0.05	1	0.2048749
		2	0.1824520
		3	0.17443623
		4	0.1778679
		5	0.200913

3. Numerical Results

Example 3.1 Minimize the following quadratic functional governed by a system of linear of evolution equations and a system of linear inequality and equality constraints.

$$\text{minimize} \quad \int_0^t x^T(t)Px(t) + u^T(t)Qu(t)dt$$

$$\text{Subject to} \quad \dot{x}(t) = Rx(t) + Gx(t-r) + Wu(t)$$

$$Cx(t) + Du(t) \geq 0$$

$$\text{and} \quad Ex(t) + Fu(t) = 0$$

where

$$P = \begin{pmatrix} 1 & 1 \\ 1 & 2 \end{pmatrix}, Q = \begin{pmatrix} 1 & 0 \\ 0 & 1 \end{pmatrix}, R = \begin{pmatrix} 1 & 0 \\ 1 & 1 \end{pmatrix}, G = \begin{pmatrix} 1 & 0 \\ -1 & 1 \end{pmatrix}, W = \begin{pmatrix} 1 & 0 \\ 1 & 1 \end{pmatrix},$$

$$C = \begin{pmatrix} 1 & 0 \\ -1 & 1 \end{pmatrix}, D = \begin{pmatrix} 1 & 0 \\ 1 & 1 \end{pmatrix}, E = \begin{pmatrix} 1 & 0 \\ -1 & 0 \end{pmatrix}, F = \begin{pmatrix} -1 & -1 \\ 0 & 1 \end{pmatrix}$$

The solution to this problem is presented in Table 1(a) below. Table 1(b) shows that numerical example 3.1 converges geometrically according as in Theorem 2.1. Table 2(a) shows the solution profile for numerical Example 3.2.

Table 2(a): Solution Profile for numerical Example 3.2

Penalty Constant μ_1	Penalty Constant μ_2	Iteration number K	Objective Functional J(x(t),u(t))	Constraint Satisfaction $\|\|\varphi(x(t),u(t))\|\|^2$
0.01	0.01	0	6.00000	22.07000
		1	3.700829	5.14899×10^{-1}
		2	2.620612	5.173527×10^{-1}
		3	1.945335	6.043216×10^{-1}
		4	1.86899	9.016032×10^{-1}
0.02	0.01	0	6.00000	22.06948
		1	3.862853	5.039536×10^{-1}
		2	2.934001	4.368277×10^{-1}
		3	2.323645	4.995465×10^{-1}
		4	1.90697	6.417061×10^{-1}
0.03	0.01	0	6.00000	22.05934
		1	4.192574	4.67285×10^{-1}
		2	3.9332	4.209466×10^{-1}
0.05	0.01	0	6.0000	22.05934
		1	3.927693	4.930718×10^{-1}
		2	3.160065	4.655135×10^{-1}
		3	2.716471	4.547522×10^{-1}
		4	2.375047	4.661923×10^{-1}
0.06	0.01	0	6.00000	22.04826
		1	3.82174	5.26245×10^{-1}
		2	2.497295	5.16670×10^{-1}
		3	1.886065	5.025523×10^{-1}

Table 2(b): Convergence ratio for Example 3.2

Penalty Constants		Iteration	Convergence Ratio
μ_1	μ_2	N	$\gamma^2 = 1 - \beta$
0.01	0.01	1	0.350039
		2	0.308697
		3	0.3088348
		4	0.309716
		5	0.3177035
0.02	0.02	1	0.3467576
		2	0.3427000
		3	0.3053388
		4	0.305913
		5	0.315491
0.03	0.03	1	0.3438357
		2	0.302687
		3	0.302739
		4	0.3023933
		5	0.313749

Example 3.2

$$\text{minimize} \quad \int \{(x_1 + 0.5x_2)^2 + 0.75x_2^2 + 2(u_1 + 0.5u_2)^2 + 0.5u_2^2\}dt$$

$$\text{Subject to} \quad x_1 + x_1(t-0.5) = x_1 - x_2 + x_2(t-0.5) + 2u_1 + 2u_2$$

$$x_2 + x_2(t-0.5) = x_1 + x_2 - x_1(t-0.5) - u_2$$

$$x_1 + 2x_2 - u_1 \geq 0$$

$$x_2 - 0.5x_1 - 0.5u_2 \geq 0$$

$$x_1 - x_2 + 0.5u_1 = 0$$

$$x_2 + 0.5x_1 - u_1 = 0$$

Again we compute the objective functional J(x,u) i.e. OBJ and the constraint satisfaction CSAT(x,u) = $\|CSAT(x,u)\|^2$

4. Comments and Conclusions

In Table 1(a) the solution to example 3.1 converges for varying penalty parameters in at most five iterations. It is observed that as μ_1 and μ_2 increase converges takes place at the 3rd iteration. The same pattern is observed in Table 2(a) for solution to example 3.2. Convergence takes place in at most five iterations even when μ_1 increases and μ_2 is kept constant.

Table 1(b) and 2(b) confirm that the scheme developed earlier, described and extended in this paper converges geometrically. The general trend is that as the iteration increases the convergence ratio falls rapidly from iteration N =1 to N = 3. For example, In Table 1(b) for $\mu_1 = \mu_2 = 0.01$, N = 1, $\gamma^2 = 0.2049197$ and for N = 3, $\gamma^2 = 0.17610$. γ^2 falls initially and increases as N increases is the general pattern in the convergence profile. In all cases $\gamma^2 < 1$, showing that the scheme exhibits geometric convergence.

For numerical Example 3.1, $\gamma^2 \approx 0.18 < 1$ and for Example 3.2, $\gamma^2 \approx 0.31 < 1$.

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GEOELECTRIC/ELECTROMAGNETIC VLF SURVEY FOR GROUNDWATER DEVELOPMENT IN A BASEMENT TERRAIN – A CASE STUDY

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Abstract

A geophysical investigation for groundwater development involving the electrical resistivity and electromagnetic VLF methods was carried out in the premises of the Conference Centre, Obafemi Awolowo University, Ile-Ife, southwestern Nigeria.

The investigation involved three reconnaissance VLF-EM profilings and six vertical electrical soundings (VES). The VLF normal and filtered real component anomalies identify five major geological interfaces suspected to be faults/fractured zones. One of the interfaces coincided with a river channel which is suspected to be structurally controlled.

The geoelectric section prepared from VES interpretation results delineate four subsurface layers which include the topsoil, weathered layer, partly weathered/ fractured basement and the fresh basement. The weathered layer and the partly weathered/ fractured basement constitute the aquifer units. The weathered layer is relatively thin (1.8m - 4.7m) while the partly weathered/fractured basement is significantly thick (15.7m - 67.3m) and extensive with tendency for significant groundwater discharge capacity.

The lithological log from a test borehole at one of the VES stations corroborated geophysically predicted subsurface sequence and its structural disposition. Fractured basement columns were identified at depth ranges of 2.5 - 7m and 12 - 45m within a column of fresh basement rock. The borehole whose aquifer is primarily fractured basement discharges about 2.0 L/s.

Keywords: electromagnetic, electrical resistivity, lithological log, groundwater development.

1. Introduction

Faults, lithological contacts/boundaries, network of joints, fractures/fissures and shear zones are structural features with hydrogeological significance in crystalline basement complex rocks. These geological features deform the basement rocks creating inhomogenieties which in turn enhance groundwater storage and groundwater flow.

Geophysical methods play an increasingly important role in the search for these suitable and productive groundwater reservoirs. Electrical resistivity method has been used routinely in exploration for groundwater. However, several other geophysical methods have been applied successfully either singly or in combination, for prospecting for groundwater resources in varying geologic situations. Olorunniwo and Olorunfemi (1987) used combination of magnetic, electromagnetic (VLF) and electrical resistivity methods to map buried bedrock relief for groundwater exploration in the Precambrian terrain of Ikare, southwestern Nigeria. The electromagnetic (VLF) method has found useful applications in groundwater investigation in basement terrain, most especially as a reconnaissance tool (de Jong *et al.*, 1981; de Rooy *et al.*, 1986; Hazell *et al.* 1988; Amadi and Nurudeen 1990; Olayinka 1990; Olorunfemi *et al.*, 1995). Olorunfemi *et al.* (2001) applied the spontaneous potential (SP) and electrical resistivity to understand the nature and groundwater development feasibility of a suspected spring in Ajegunle-Igoba, Akure.

In this paper, we present the results of the application of electromagnetic (EM) and electrical resistivity methods in the delineation of favourable hydrogeophysical regime for productive aquifers in the premises of the Conference Centre, Obafemi Awolowo University, Ile-Ife, southwestern Nigeria (Fig. 1).

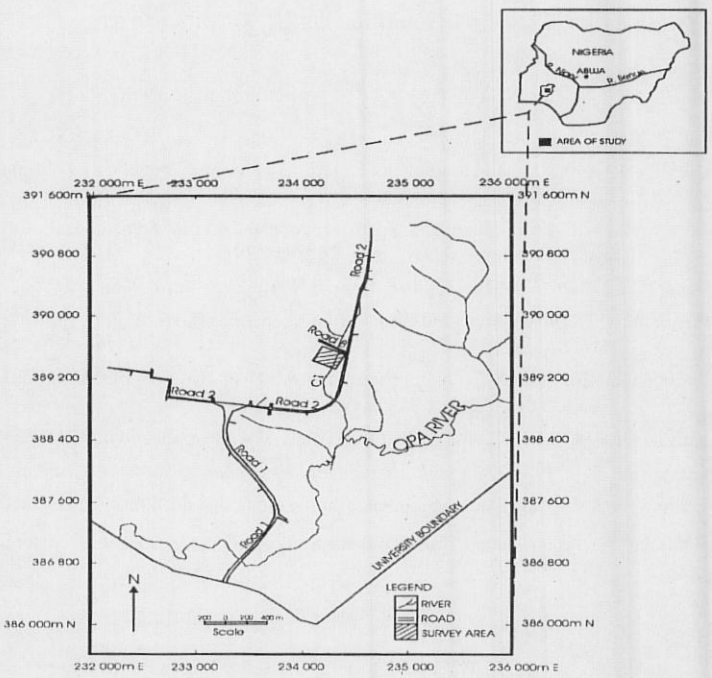

Figure 1: Location map of Obafemi Awolowo University Ile-Ife, showing the location of the study area.

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The geophysical investigations were carried out in the target area to delineate the subsurface layers beneath the sounding stations and determine their geoelectric parameters. It was also aimed at identifying the aquifer unit, determining its depth and lateral extent and identifying possible subsurface fault/fracture planes in the study area. The field layout of the geophysical traverses and measurement stations are shown in Figure 2.

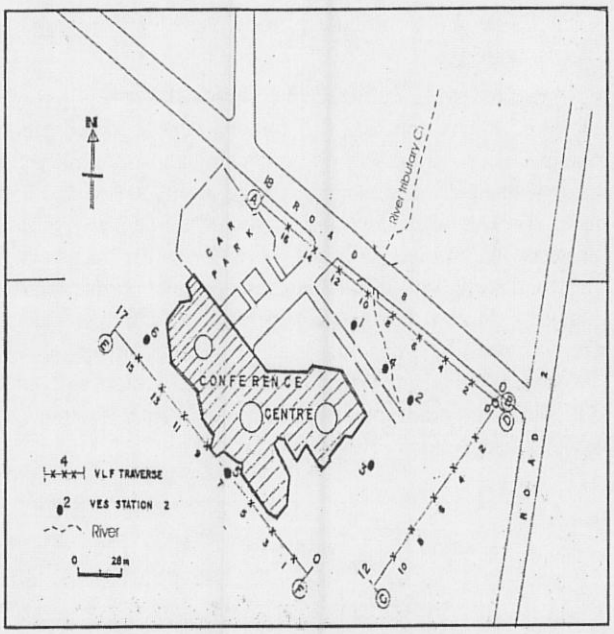


Figure 2: Geophysical survey map showing the field layout of the geophysical traverses

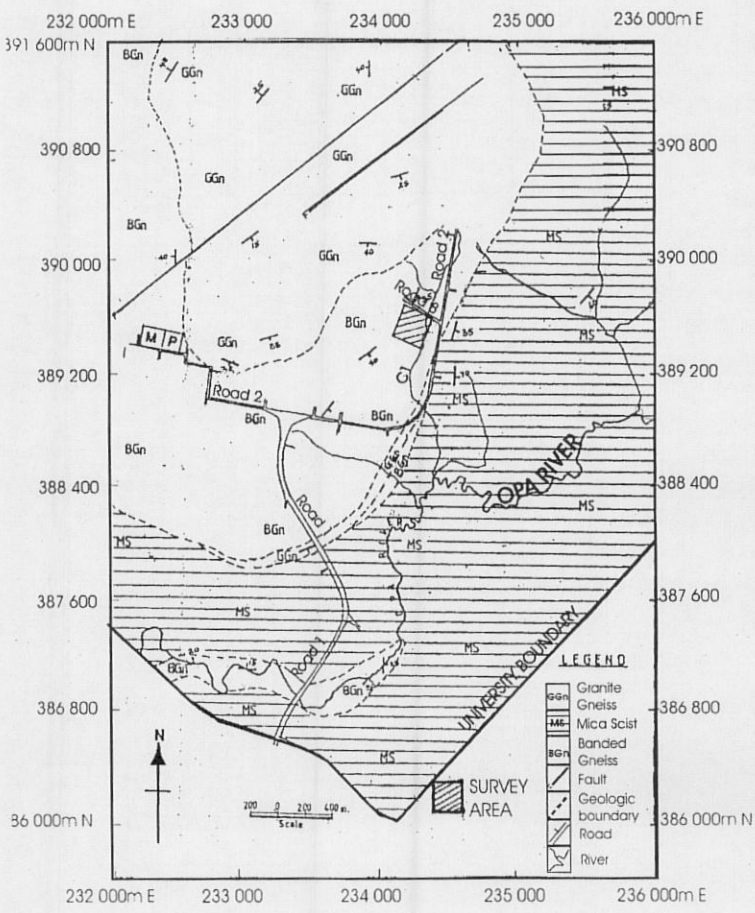


Figure 3: Geological and drainage map of the study area

2. Geomorphology and geology of the study area

The survey site is located on a lowland area east of Hill 1 on the Obafemi Awolowo University Campus. The terrain of the survey site is relatively flat. It slopes gently south-eastward into a river valley. The topographic elevation is less than 300m above sea level.

The site is underlain by the Precambrian Basement Complex rock of Nigeria. The main geological unit in the area is the grey or banded gneiss. The grey or banded gneiss rock belongs to the migmatic-gneiss complex which constitutes one of the major rock units of the Precambrian Basement of southwestern Nigeria. Figure 3 shows the geological and the surface drainage map of the study area. The main river in this area is the Opa river whose perennial tributary C1 flows North-South through the study area.

In a basement complex terrain, groundwater is confined within weathered layer and or fractured/jointed or sheared basement columns. Groundwater yield in such geological environment is a complex function of the weathered layer thickness, its clay content and the density of fractures.

The geophysical survey

Electromagnetic VLF survey

The electromagnetic VLF (Very Low Frequency) geophysical method provides a quick and powerful tool for the study of shallow conducting lineament features in the near-surface earth (Telford *et al.,* 1977). The method is based on measurement of the secondary magnetic field induced in local conductors by primary EM fields generated by powerful naval radio transmitters in the very low frequency range (15-25kHz). The instrument employed for the VLF survey was the EM-16 (VLF-EM) which measures the in-phase and quadrature components of the induced vertical magnetic field as a percentage of the horizontal primary field (GEONICS 1979). These measurements are equivalent respectively to the tangent of the tilt angle and the ellipticity of the polarization ellipse. The main fracture directions in this locality is approximately north-south, which approximate local directions to GBR (Rugby, England), FUO (Bordeaux, France) and NAA (Cutler, Maine) VLF stations. At the time of the survey, GBR (Rugby, England) was well received.

The VLF measurements were made along three traverses with station intervals of 10m (Fig. 2). The EM data are presented as profiles. The real VLF data were converted to filtered real by applying a filtering operator **Q** which transforms true VLF anomaly inflections to peak positive anomalies and false VLF anomaly inflections to peak negative anomalies. The filter operator is given by

$$\mathbf{Q} = [(\theta_3+\theta_4) - (\theta_1+\theta_2)] \qquad (1)$$

where **Q** is the filter operator and θ_1, θ_2, θ_3 and θ_4 are the readings of the measured real at stations 1, 2, 3 and 4. Figures 4, 5 and 6 show the real and filtered real VLF anomaly curves obtained along traverses A-B, C-D and E-F respectively.

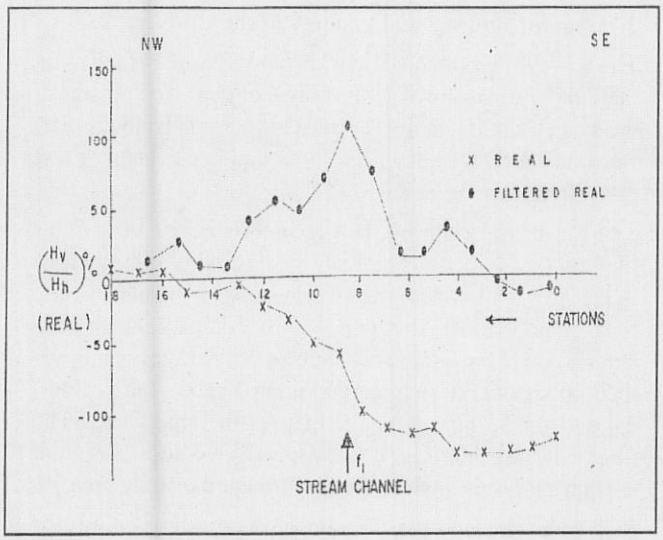


Figure 4: VLF anomaly curve (real component) along traverses A - B

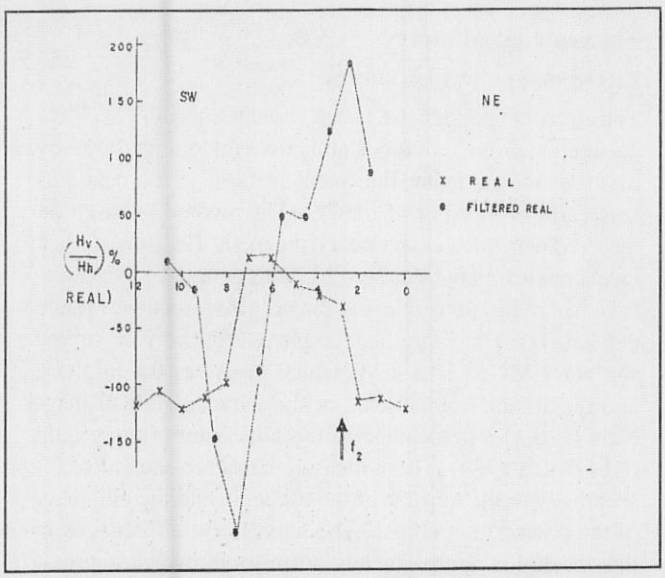


Figure 5: VLF anomaly curve (real component) along traverse C - D.

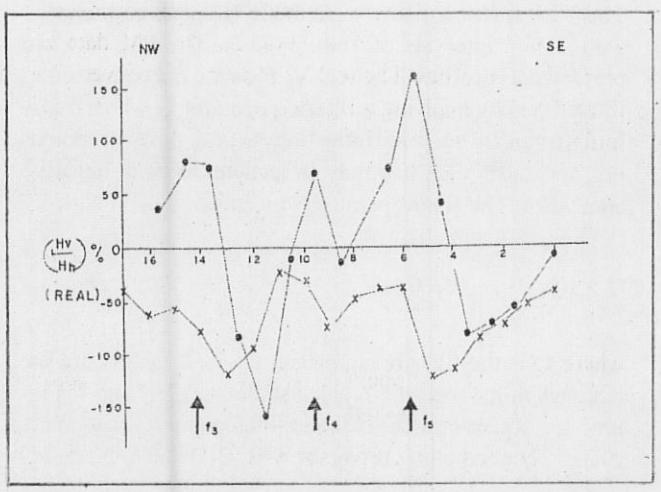


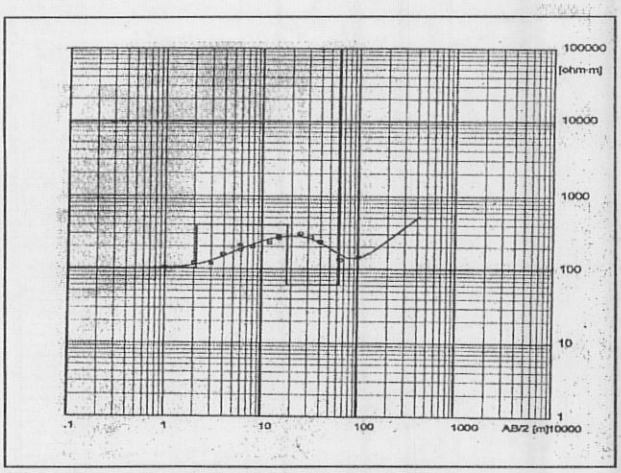
Figure 6: VLF anomaly curve (real component) along traverse E - F.

The imaginary component of the secondary electromagnetic field was noisy and therefore unreliable for qualitative interpretation.

Electrical Resistivity Survey

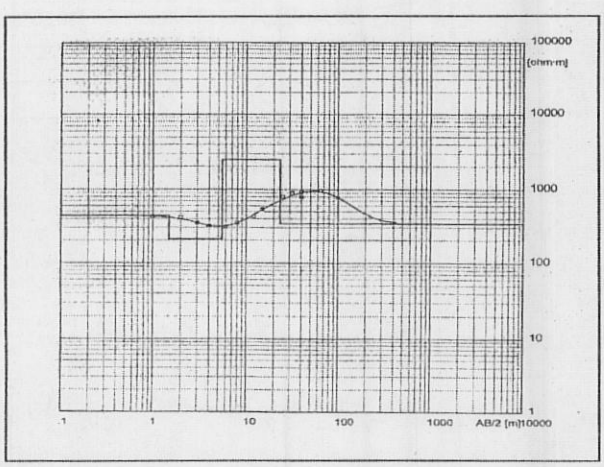
The electrical resistivity method involves passage of current into the ground through two current electrodes while the resulting potential difference is measured between another pair of potential electrodes which may or may not be located within the current electrode pair, depending on the electrode array.

The present survey utilized the Vertical Electrical Sounding (VES) technique. Six VES stations were occupied within the premises of the survey area using the Schlumberger array with electrode spacings (AB/2) varying from 1 to 100m (Fig. 2). The VES data are presented as depth sounding curves. Quantitative (1-D) interpretation of the VES curves involved partial curve matching and computer iteration techniques. Figures 7, 8 and 9 show typical Schlumberger curves from the study area and their interpretation using W-Geosoft's WinSev 5.1 resisitivity interpretation software. The depth sounding interpretation results are presented as geoelectric sections (Figs. 10 and 11).



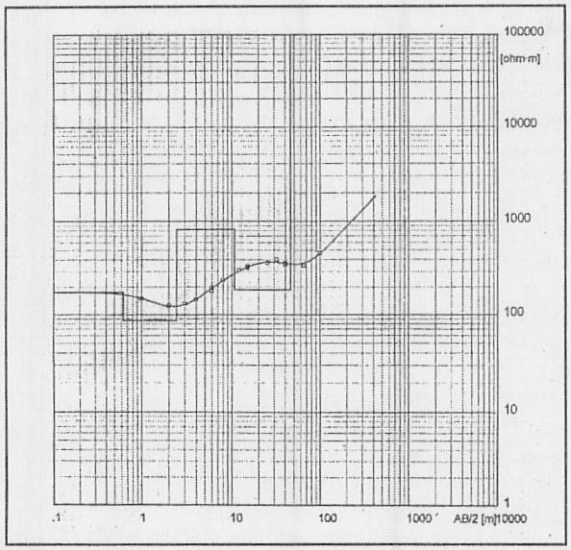
Model Resistivity (ohm-m)	102	397	61	100000
Thickness (m)	2.1	16	45	
Depth (m)		2.1	18	63

Figure 7 : Typical Schlumberger curve and its interpretation for VES station 1



Model Resistivity (ohm-m)	439	209	2502	338
Thickness (m)	1.5	4	18	
Depth (m)		1.5	5.5	24

Figure 8 : Typical Schlumberger curve and its interpretation for VES station 2



Model Resistivity (ohm-m)	175	86	819	187	100000
Thickness (m)	0.63	1.8	8.3	35	
Depth (m)		0.63	2.4	11	46

Figure 9 : Typical Schlumberger curve and its interpretation for VES station 4.

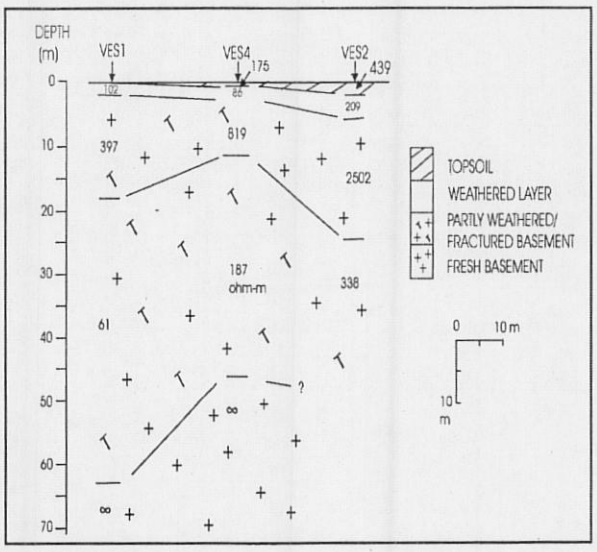


Figure 10: Geoelectric section relating VES stations 1, 4 and 2.

3. Results and Discussion

EM profiles

Geological interfaces ($f_1 - f_5$) were delineated using characteristic features of coincident inflections on real component anomaly curves and positive peaks of filtered real anomaly curves (ABEM, 1990; Olorunfemi *et. al.*, 1997) (see Figs. 4, 5 and 6). Interface f_1 coincides with the river course (compare Figs. 2 and 4). This is strongly indicative of structurally (fault) controlled river course. The other interfaces ($f_2 - f_5$) are near-surface/ sub-surface fractures in the basement complex rock. When such linear features are water saturated, they constitute good aquifer units.

Geoelectric Sections

The depth sounding interpretation results are presented as geoelectric sections (Figs.10 and 11). Four (4) subsurface layers were delineated as shown below:

1st Layer: Topsoil of sandy clay/clayey sand.
Resistivity : 102-469 ohm-m; thickness: 0.6-1.5m

2nd Layer: Weathered Layer. This layer is composed of clay/sandy clay/clayey sand
Resistivity : 86-287 ohm-m; thickness: 1.8-4.7m

3rd Layer: Partly weathered/Fractured Basement.
Resistivity: 61-338 ohm-m; thickness: 15.7- 67.3m (where the bottom of the layer was delineated)

4th Layer: Fresh Basement.
Resistivity: 819 - ∞ ohm-m; depth to rock head: 0.6-5.8m

The above shows that the survey area is characterized by a relatively thin (< 10m) weathered layer with limited hydrogeological significance. The partly weathered/fractured basement unit is however significantly thick and extensive with tendency for large storage capacity and significant groundwater yielding capacity.

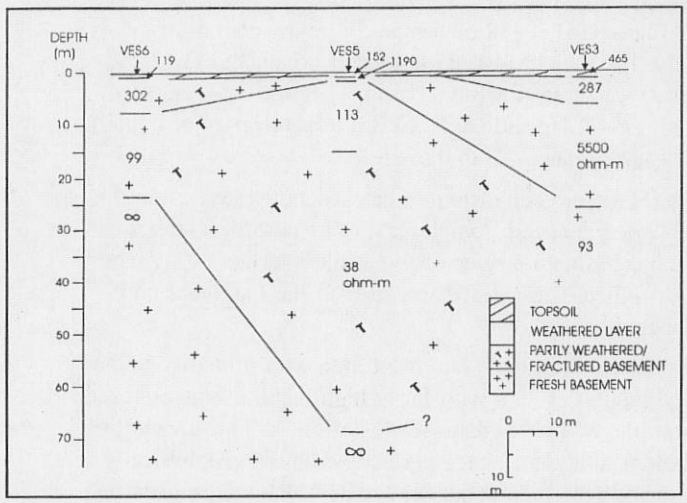


Figure 11: Geoelectric section relating VES stations 6, 5 and 3.

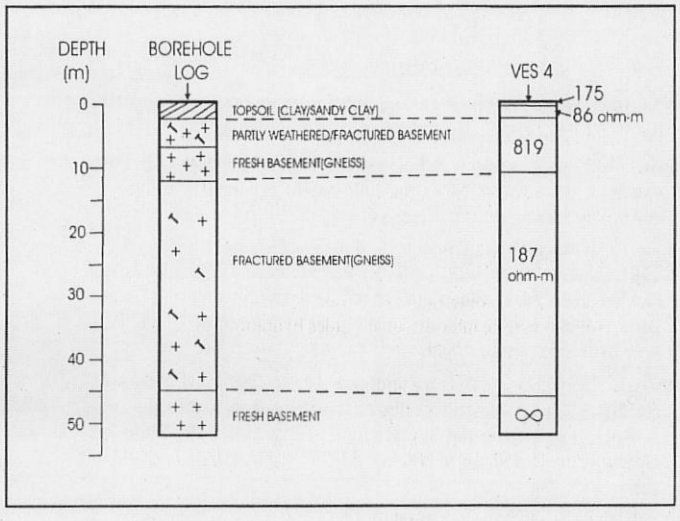


Figure 12: Correlation of borehole lithological log with vertical electrical sounding (VES 4) interpretation results.

The basement rock as inferred from the electromagnetic VLF profiles and geoelectric sections is fractured beneath VES station 4 at depth ranges 2.5 - 7m and 12.0 - 45.0m. The fractured basement column is confined by an estimated 5m thick fresh basement column. On the basis of our result, a water borehole was recommended for drilling at VES station 4. Figure 12 shows a comparison of obtained geophysical results and drill-hole log. The VES interpretation result predicted depth to fresh basement bedrock to be 46m whilst drilling result confirmed 45m showing very good correlation. The test borehole was productive with groundwater yield of about 2.0 L/s which indicates adequate recharge. The static water level remains at about 1.2m.

The borehole log corroborated predictions from both VLF and VES interpretation results. It also confirms an earlier inference that the adjoining stream is structurally (fault) controlled.

4. Conclusions

Four major subsurface layers were delineated from the VES interpretation results. These include the topsoil, weathered layer, partly weathered/fractured basement and the fresh basement with resistivity ranges of 102 – 469 ohm-m, 86 – 287 ohm-m, 61 – 338 ohm-m and 819 - ∞ ohm-m respectively. The thicknesses of the topsoil, weathered layer and partly weathered/ fractured basement range between 0.6 – 1.5m, 1.8 – 4.7m and 15.7 – 67.3m respectively. The depth to rockhead ranges from 0.6 – 5.8m.

Five (5) major geological interfaces identified as f_1, f_2, f_3, f_4 and f_5 were inferred from the EM (VLF) profiles. Interface f_1 coincided with a river course while interfaces f_1 –f_5 are near-surface/ sub-surface fractures in the basement complex area.

The above indicates a basement area with primarily fractured aquifer system with fairly high depth extent. A test borehole was drilled at VES station 4. The borehole lithological log correlated perfectly with the geophysically predicted geological sequence and its structural disposition. The confined fractured basement column was identified as predicted by both the electromagnetic VLF and resistivity depth sounding surveys.

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DETERMINATION OF IODINE IN HUMAN MILK AND URINE

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Abstract

Human milk and urine samples were collected from 84 healthy volunteer nursing mothers living in Kano, Nigeria. The samples were analyzed for their Iodine content using the Iodine-catalyzed reduction of Ceric ion by Arsenous acid. Separating the Iodine by solvent extraction eliminated interferences. Physiological concentrations of iodine were determined in milk and urine. Recovery studies are reported along with results for the analysis of milk and urine samples. Iodine contents ranged from 10 - 110 (mean 52.88 ± 22.60μg/l) and 10 - 90 (mean 27.64 ±16.70) g/l in milk and urine respectively. A significant difference is indicated between the mean iodine in milk and urine. Iodine in milk and urine show a progressive decrease from 60±19.35μg/l in colostrums to 45±21.21μg/l in the mature milk.

Keywords: Iodine, human milk, urine, catalytic determination.

1. Introduction

Iodine is an essential component of the thyroid hormones in man and animals (Darrell, 1991). The total body content of iodine is estimated at 10 - 50 mg for an adult (Pennington, 1988). It is present in all body tissues and fluids but 70 - 90% is located in the thyroid gland which has iodine concentration of about 0.4 - 1.0μg/kg net weight. Iodine exists in blood in inorganic and organic forms. The normal plasma concentrations of inorganic iodine range from 0.08 - 0.6μg/l with values less than 0.08μg/l suggesting iodine deficiency (Hetzel and Maberly, 1986). Iodine concentrations of other tissues do not exceed 0.2 mg/kg. The daily requirement for iodine is 1 - 2 μg/kg of body weight. The daily intake of 50 -1000 μg/kg is considered safe (Food and Nutrition Board, 1970).

Children of mothers consuming less than 25μg/day of iodine are often afflicted with cretinism as a result of in-utero iodine deficiency, which may result in neurologic cretinism; characterized by mental deficiency, deaf mutism and the myxadematous type characterized by hypothyroidism and dwarfism (Hetzel and Dunn, 1989). Iodine deficiency may also result in miscarriages, stillbirths and congenital abnormalities (Hetzel and Mano, 1989). While iodine is of biological interest, it occurs as a trace element in human milk and urine and is difficult to determine by the conventional laboratory techniques. Catalytic methods are sensitive for trace analysis, though hampered by selectivity. By judicious coupling with separation procedures it is possible to apply the technique to the analysis of complex biological samples (Ayodele, 1996; Ayodele and Ogunlesi, 1998).

Byrne (1984) described a radiochemical separation technique followed by the extraction of iodine into carbon tetrachloride with a clean up based on a selective redox-stripping cycle using nitric and sulphuric acids. Gvardjancic *et al.* (1988) described a neutron activation procedure for the determination of iodine in milk in which a pre-irradiation separation procedure was employed. The iodine catalyzed reaction between Ce (IV) and As (III) has been used in the determination of iodine at the trace level in biological materials (Sandell and Koltholff, 1934; Kambhapati *et al.*, 1989). Rogina and Dubrovcic (1953) described a catalytic procedure for the determination of iodine, which possesses the sensitivity required for its determination in biological fluids. The method was adapted for the analysis of iodine in milk and urine. In our desire to exploit these differences and to obtain selectivity for the determination of iodine in biological fluids, this paper reports the level of iodine in human milk and urine samples from Kano –Nigeria.

2. Materials and Methods

Analytical reagent grade chemicals were used whenever possible. Glass distilled water was used throughout. Absorbance measurements were made with Cecil Model CE 373 Linear grating Spectrophotometer at 525nm in a 100mm cell.

Milk and urine samples were collected from 84 healthy volunteers 17-37 years old from Kano-metropolis in Kano State under standardized conditions. Since trace element concentration is a function of the stage of the feed, (Sanner and Dubrovic, 1984) donors were requested to express their milk ($10cm^3$) manually during feeding alternating between right and left breast. The sample from each donor represented the accumulated milk collection per feed. The samples were collected in collection vials and stored as reported earlier (Ayodele and Na'abba, 1993; Ayodele *et al.*, 1999).

Pathological histories like age of the donor, period of lactation, etc. were noted. Anamnestic data were collected with regards to the smoking of tobacco, drinking of alcohol,

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nutritional habits, medication and use of hormonal contraceptives (Muller, 1987).

Casual urine samples (25cm^3) of donors were collected in separate vials. Based on the interference studies of several authors (Sandell and Kolthoff, 1934; Chaney, 1940 and 1950; Moran, 1952) a separation procedure for the iodine was carried out. The separation employed was a combination of Fang *et al.* (1944) and Garvin *et al.* (1994). To 5cm^3 milk and 10cm^3 urine was added 0.1cm^3 of 2.8M KOH with heating at 100°C for 1hr or at 55°C for 2hr .The ashed sample on cooling was mixed with 5cm^3 water sonicated for 30min and then centrifuged for 10min at 900rpm.The supernatant was mixed with 0.5cm^3 of 1% ascorbic acid and 2g of Type 732 cation exchange sulphonic resin. After 2hr. the supernatant was neutralized with 0.1M NaOH and the resulting solution reduced to 2cm^3 was assayed for iodine.

Standard iodine solution 100µg/l was prepared by dissolving 261.60 mg of the iodine crystals and 1.0g of potassium iodide in water in a 250 cm^3 volumetric flask. By successive dilution, standard iodine solution with an iodide concentration of 1µg/cm^3 was prepared.

3. Procedure

Into one arm of a two-limbed reaction vessel was pipetted 10cm^3 of the sample solution. 1cm^3 of a solution of sodium chloride (200mg/cm^3) was added followed by 5.0cm^3 of the arsenious solution and 1cm^3 of 5M sulphuric acids. Into the other limb of the reaction vessel was pipetted. 5cm^3 of 0.02M ceric ammonium sulphate. Enough water was added to both arms of the vessel to bring the total volume to 50cm^3. The vessel was placed in a 25°C water bath. After 30 mins the vessel was removed and the solutions in the two limbs were allowed to mix by inversion while at the same time starting the stopwatch. On mixing the tube was returned into the bath. After 15 mins, 1cm^3 of 0.04 M ferrous ammonium sulphate was added with mixing followed by 1 cm^3 of 0.4% potassium thiocyanate solution. The absorbance was immediately read at 525nm. The amount of the iodide present in each sample was established from the calibration curve.

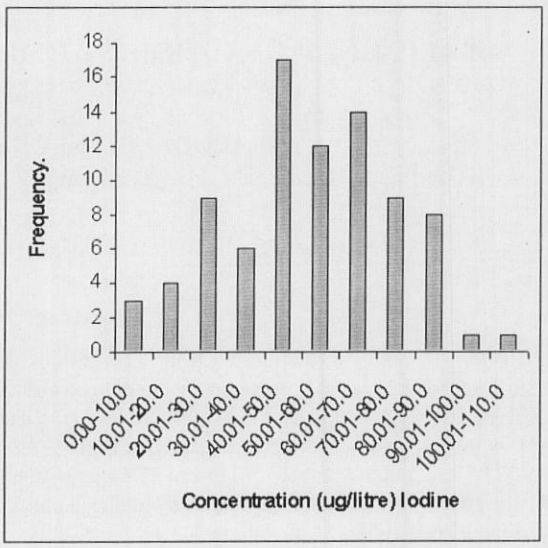

Figure 1: Frequency distribution of iodine in human milk.

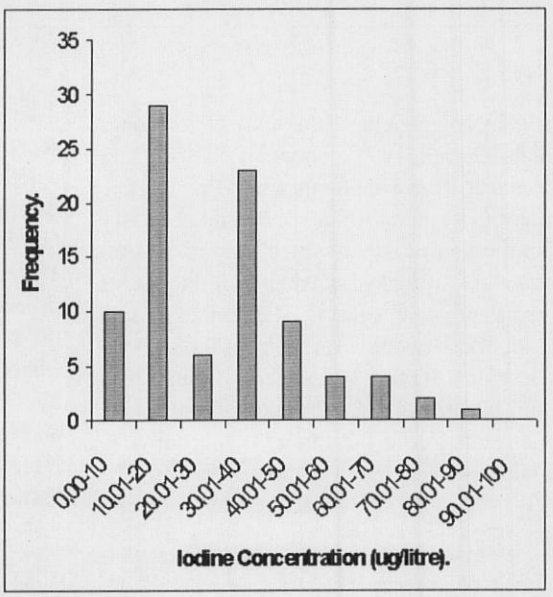

Figure 2: Frequency distribution of iodine in human urine.

4. Results

Table 1 summarizes the recovery of 0.2 g of iodine added to 2.0 and 10.0 cm^3 milk and urine using the recommended procedure. Recoveries were quantitative within experimental error. These results illustrate that interferences were negligible and that iodine could be determined in whole milk and urine. Figs. 1 and 2 summarise iodine distribution in the milk and urine samples investigated. The iodine levels fall within a range 10 - 110 and 10 - 90 µg/litre for milk and urine respectively. The mean values of 52.88 ± 22/60 and 27.62 ± 16.70/µg/l for milk and urine are in close agreement with the dietary allowance reported by WHO (1973), NAS (1980), Food and Nutrition Board (1970), Joerin (1975), Hetzel and Maberly (1986) but in contrast to other authors who reported higher values (Bruhn and Franke,1983; Gushurst, 1983; Muramatsu *et al.*, 1983; Kosta *et al.*, 1988) (Table 2). A significant difference was indicated between the mean iodine concentrations in milk and urine. Kosta *et al.* (1983) reported a mean of 88 ± 83 µg/l in mature milk from Yugoslav subjects. Muramatsu *et al.* (1983) reported much higher levels range 80 - 700 µg/l in human milk specimens from Japanese subjects who consumed dietary algae. Studies conducted under the auspices of IAEA (Iyengar, 1982) using similar analytical quality control procedures support the reported values in our study.

Fig. 3 summarizes iodine distribution in milk and urine. With respect to the period of lactation, longitudinal studies extending up to several months following delivery provided evidence of a progressive decrease of iodine in milk and urine as iodine levels showed a progressive decrease from

Table 1: Recovery of 0.2μg iodine added to milk and urine.

	Sample	Iodine added (μg)	Iodine Found (μg)	Iodine Recovered (μg
1.	2cm^3 milk	-	0.1	-
	2 cm^3 milk	0.20	0.31	0.11
2.	5 cm^3 urine	-	0.15	-
	5 cm^3 urine	0.2	0.36	0.15
3.	2 cm^3 milk	-	0.1	-
	2 cm^3 milk	0.3	0.39	0.09
4.	5 cm^3 urine	-	0.14	-
	5 cm^3 urine	0.3	0.45	0.15
5.	2 cm^3 milk	-	0.11	-
	2 cm^3 milk	0.4	0.52	0.12
6.	5 cm^3 urine	-	0.14	-
	5 cm^3 urine	0.4	0.54	0.14

Table 2: Typical reported values of iodine in human milk.

Country	Units	No of samples	Mean	Range	References
New Zealand	μg/day	-		0-0.04	Joerin [1975]
U.S.A	μg/day	-	40	--	NAS [1980]
U.S.A	μg/litre	8	142±81	68-296	Bruhn&Franke[1983]
U.S.A	μg/litre	61	178	29-490	Gushurst [1983]
Yugoslavia	μg/kg	-	-	132-510	Kosta et al[1983]
U.S.S.R	μg%	16	7.71±0.5	-	Borbiev [1982]
Turkey	μg/cm^3	26	109±0.50	45-208	Gokmen&Dagh[1995]
Europe	μg/cm^3	-	80	20-330	Delange et al [1978]
Guatemala	μg/cm^3	84	38	17-74	Iyengar [1982]
Hungary	μg/cm^3	71	43	17-66	Iyengar [1982]
Nigeria	μg/ cm^3	18	40	10-73	Iyengar [1982]
Philippines	μg/cm^3	63	75	33-104	Iyengar [1982]
Sweden	μg/cm^3	32	14	8-17	Iyengar [1982]
Zaire	μg/cm^3	60	3.2	3.5	Iyengar [1982]
Japan	μg/litre	-		80-700	Muramatsu et al [1983]
Finland	μg/litre	40	219	-	Koiranen&Stabel-Taucher [1976]
WHO	μg/ day	84	52.9	150-200	WHO [1983]
Nigeria	μg/ litre	84	52.9	10-110	This study

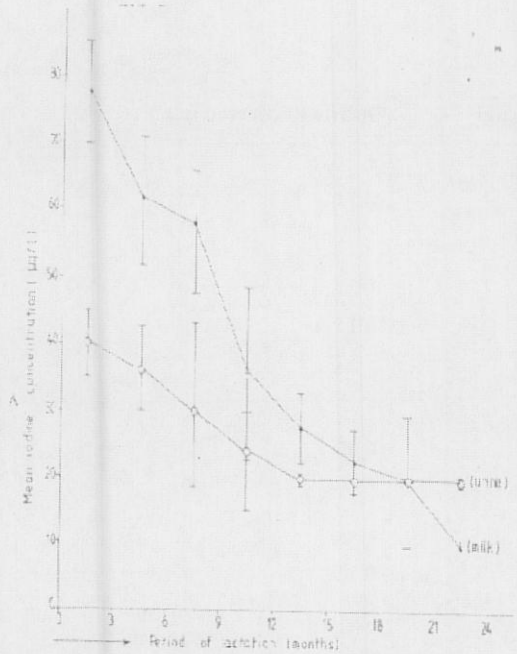

Figure 3: Mean Milk and Urine iodine concentrations at various stages of lactation

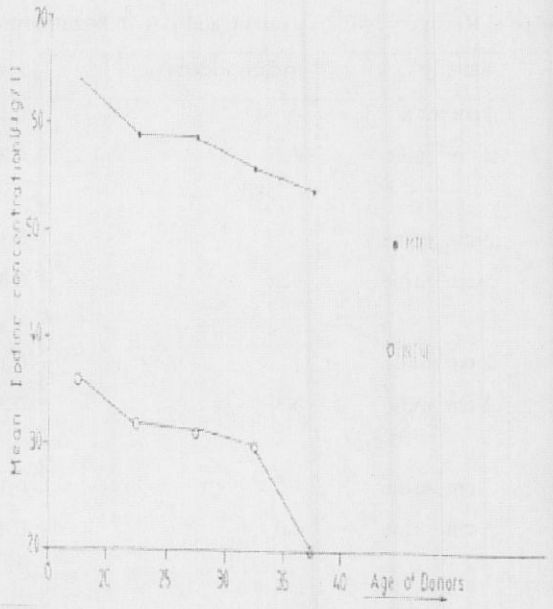

Figure 4: Mean iodine content in milk and urine versus the age of the lactating mothers.

60±19.35 µg/l in colostrums to 45± 21.21µg/l in the matured milk such that a significant difference was indicated between the two stages.

Fig. 4 summarizes iodine distribution in milk and urine with respect to the age of the lactating mothers. Young mothers' milk and urine appear richer in iodine than their older counterparts. However, the iodine level in the older mothers was within the daily requirements considered safe (Food and Nutrition Board, 1970; Bruhn and Franke, 1983).

5. Discussion

There have been reports of socio-economic and seasonal differences in elemental concentrations in milk (WHO, 1985; WHO, 1989). In our studies there were no signs of systematic differences between samples collected from donors of different socio-economic status. However this study was not designed to intercept such variance and the existence of such difference cannot be excluded. The nutritional status of the mother as reflected by their socio economic status does not appear to influence the concentration of iodine in milk. However, the ranges obtained may be useful in determing the desirable concentration of iodine in milk substitutes following the recommendation of a WHO Expert Committee (1973) that milk formula products should contain all the minor and essential trace elements at least in those levels that are present in human milk.

The composition of human milk is by no means constant and several factors both physiological and non-physiological have been responsible for the observed variations (Lonnerdal *et al.*, 1976a and b; Hibber, 1982; Hartmann and Prosser, 1984; Helsing and King, 1985; Muller, 1987). Physiological factors include the stage of lactation, the time of day, the time of sampling and the nutritional status of the mother. In addition, there may be effects caused by disease, medication and the use of hormonal contraceptives. Non-physiological factors include geochemical and other environmental aspects as well as the impact of certain habits such as tobacco smoking and drinking of alcohol (Picciano and Guthrie, 1977; Underwood, 1977; Siimes *et al.*, 1979; Borbiev, 1982; WHO, 1985; Muller, 1987).

Urinary iodine excretion is often used as an indicator of iodine status. Excretion of 50mg of iodine/kg is marginal and excretion of less than 25mg/kg is indicative of serious iodine deficiency (Querido *et al.*, 1974). There were no significant differences between the mean concentrations for the various age groups. Therefore concentrations of iodine in human milk and urine are probably controlled by homoecstatic mechanism, which accounts for the small variations. The recommended adult allowance of 150µg/day for both sexes provides a margin of safety (Food and Nutrition Board, 1970). An additional allowance of 25µg/day is recommended during pregnancy to meet the needs of the fetus and additional 50µg/day are recommended during lactation to meet the needs of the infants.

None of the volunteered mothers were smokers and only two were on medication /contraceptives. The results point out no differences between these sub-collectives. Also no final statement can be made about other possible correlations: such as duration of suckling, number of children, etc.

Although seasonal variations in the concentrations of some trace elements have been reported (Iyengar, 1982; WHO, 1985), neither a systematic effect nor correlation with a particular season was observed in this study as all samples were collected within a season. Differences in the concentrations of some minor and trace elements have been reported between urban well to do, urban poor and rural groups. However, no consistent pattern was identified in this study.

6. Conclusion

Determination of iodine in human milk and urine was achieved using separation and catalytic procedures. The current intake of iodine by infants fed on human milk in this study group are within the WHO recommended value. It may then be concluded that there is little difference in the iodine levels for the different age groups of the mothers. Concentrations of iodine in human milk and urine may probably be controlled by homeostatic mechanism, which accounts for the small variations.

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ENZYMATIC SACCHARIFICATION OF SOME AGRO-INDUSTRIAL CELLULOSIC WASTES BY CELLULASE PRODUCED FROM A MIXED CULTURE OF *ASPERGILLUS NIGER* AND *SACCHAROMYCES CEREVISAE* GROWN ON SORGHUM POMACE

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Abstract

Production of cellulase by mixed culture of Aspergillus niger and Saccharomyces cerevisae using sorghum pomace as nutrient source was investigated. Sorghum pomace was further supplemented with mineral elements to evaluate its effect on cellulase production. All the sorghum pomace media recorded significantly ($P<0.05$) higher level of cellulase enzyme (2.06-4.06 units/ml) than that of carboxymethyl-cellulose medium (1.72 units/ml). Mixed culture fermentation significantly ($P<0.05$) enhanced higher cellulase production than mono culture media. However, mineral supplementation significantly ($P<0.05$) suppressed cellulase production. Cellulosic substrates were significantly ($P<0.05$) more susceptible to crude enzyme from sorghum pomace than that of carboxymethyl cellulose

1. Introduction

Cellulases are undefined extracellular enzyme mixture produced by various microbes (fungi and bacteria), insects and lower animals which can hydrolyse cellulose (Shin and Yan, 1996). Cellulase activity in vivo is not mediated by a single enzyme, but rather by a complex system of several enzymes which act synergistically (Eveleigh, 1987; Lamed *et al.*, 1987; Gbelekeloluwa and Moo-Young, 1991). The cellulase complex forms a unique structure which has been called "cellulosome" and appears to be ubiquitous among cellulolytic micro-organisms (Lamed *et al.*, 1987). Cellulosome associated with fungi are Endo I, 4-β-glucanase, Exo-1, 4-β-glucanase and β-glucosidase (Eveleigh, 1987; Coughlan, 1990).

Cellulase is an industrially useful enzyme in brewing, chemical, textile, paper, food and pharmaceutical industries (Brown *et al.*, 1987). Despite the wide application of cellulases it is heavily imported into Nigeria.

Use of local substrates for cellulases production has been actively investigated (Udotong, 1997; Raji *et al.*, 1998; Abu *et al.*, 2001, 2003) and a number of agro- industrial wastes have been shown to be good substrates for cellulase production. However there is a dearth of information on the application of the enzyme produced (Udotong, 1997).

This paper reports on the preliminary investigation of the application of crude cellulase to cellulosic hydrolysis and the effect of mixed culture and mineral supplementation on cellulase production and cellulose hydrolysis.

2. Materials and Methods

Organism

Aspergillus niger sl.1 and *Saccharomyces cerevisae* used were isolated from soil and rotten cassava respectively. They were purified, characterized and identified by the Department of Microbiology, Ahmadu Bello University Zaria-Nigeria.

Cellulosic Substrates

Corn bran, corn cob, rice bran, wheatbran and sorghum pomace were obtained from harvest dump or households in Zaria, Kaduna State.

Culture Media

Aspergillus niger sl.1 inoculum was prepared in yeast peptone soluble starch (YPS) agar medium containing the following in g/l; yeast extract, 5; peptone 10; soluble starch, 10; and agar 10. The culture medium was incubated for 96 hours at 30°C. The medium for *S.cerevisae* consisted of the following in g/l; sucrose, 50; yeast extract 10; peptone 5; KH_2PO_4, 1; $(NH_4)_2SO_4$, 2 and M_gSO_4 $7H_20$, 1. The medium was incubated for 96 hours at 30°C.

Fermentation for enzyme production

The fermentation medium comprised the following in g/l; yeast extract, 0.5; $(NH_4)_2SO_4$, 10.5; KH_2PO_4 10, $M_gSO_4.7H_2O$, 0.3; $CaCl_2$, 0.5; $FeSO_4.7H_2O$, 0.013; $MnSO_4.7H_2O$, 0.004; $ZnSO_4.7H_2O$, 0.004; $CoCl_2.6H_2O$, 0.0067 and carboxymethyl cellulose, 40. For studies on the use of sorghum pomace and mineral supplementation, carboxymethlycellulose was substituted with sorghum pomace (40g) in mineral salt media while whole pomace was used as the sole nutrient source in non-mineral supplemented media.

The media were sterilized for 15 minutes at 121°C in an autoclave and the pH was adjusted to 5.0. with 0.1M hydrochloric acid or sodium hydroxide. Monoculture media (including

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(including that of carbohymethlcellulose CMC) were inoculated with a spore suspension (3.62 x 10^5 spores) of *A.niger* sl.1 while those for mixed culture were inoculated with 1.81 x 10^5 spores each of *A.niger* sl.1 and *S.cerevisae*. The media were incubated at 30°C in an orbital shaker (CAT No. 14460; APP No. IB, 2621 Cuo Gallen KAMP) set at 100 rpm for 72 hours. Three replicate fermentation was carried out for each culture.

Enzyme Assay

Cellulase activity was assayed according to the method described by Ali *et al.*, 1991). The reaction mixture consisted of 1ml each of 0.1M acetate buffer (pH 5.0), crude enzyme and 1% CMC solution. The mixture was incubated at 30°C in a test-tube for 10 minutes and total reducing sugar was determined by the Dinitrosalicylate (DNS) method (Miller, 1959).

One unit of cellulase activity was defined as the amount of enzyme which released 1µmole glucose min^{-1}.

Saccharification of Cellulosic Substrates

Evaluation of substrates for enzymatic hydrolysis was carried out with 5g (dry weight) of substrate, 35ml 0.1M acetate buffer pH, 5.0 and 60ml of crude enzyme in 250ml conical flasks and incubated in a water bath at 30°C for 1 hour. The reducing sugar was measured by the dinitrosalicylate (DNS) procedure in aliquots of the suspension. Percentage saccharification of this 5% slurry was calculated as the product of milligram glucose and 1.8 (Mandels *et al.*, 1974).

Effect of pH on crude enzyme activity

Acetate buffer of 0.1M was used for pH 3.0-7.0 while 0.1M phosphate buffer was used for pH of 8.0 and 9.0 to study the effect of pH on cellulase activity.

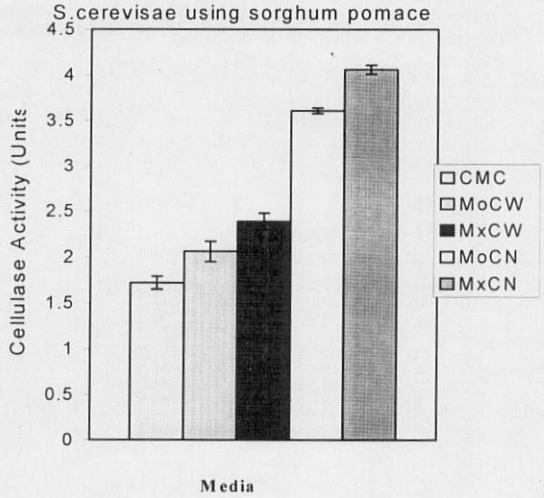
Statistical Analysis

Statistical analyses were by analysis of variance (ANOVA). Turkey test was used to identify means that differed significantly.

3. Results and Discussion

Cellulase production using different sorghum pomace media compared to carboxymethyl cellulose medium is shown in Figure 1. All the sorghum pomace media recorded significantly ($P<0.05$) higher level of cellulase activity than that of CMC medium. This result is encouraging because until now carboxymethl cellulose has been regarded as the conventional substrate for cellulase production. Cellulase production in mixed culture media (with or without mineral supplementation) was significantly ($P<0.05$) higher than the corresponding monoculture media (Figure 1). This could be due to kinetic advantage of symbotic relationship between fungus and yeast in the mixed culture medium. End-product inhibition of cellulases produced by fungus is well-known (Ladish *et al.*, 1983; Holztapple *et al.*, 1990) but periodic removal of product sugars were found to be

Figure 1: Cellulase Production (Units/ml) by mixed culture of A.niger and S.cerevisae using sorghum pomace



LEGEND
CMC = Carboxymethylcellulose
M_oCW = Monoculture of *A.niger* with mineral supplementation
M_xCW = Mixed culture of *A.niger* and *S.cerevisae* with mineral supplementation
M_oCN = Monoculture of *A.niger* without mineral supplementation
M_xCN = Mixed culture of *A.niger* and *S.cerevisae* without mineral supplement

effective at reducing end product inhibition (Gregg and Saddler, 1996). The fermentation of sugars to ethanol by *S.cerevisae* reduces the end-product inhibition of cellulases produced by *A.niger*. This could be responsible for increased cellulase production in the mixed culture media. (Szczodrak and Targonski, 1989; Phillipidis, 1994).

Supplementation of sorghum pomace with mineral elements was found to suppress cellulase production by over 40% in both monoculture and mixed culture media as shown in Figure 1. This implies that sorghum pomace has adequate minerals nutrients required for growth of the organism as well as enzyme production. Excess levels of some mineral ions in the medium has been reported to have inhibitory effect on growth and activity of micro-organisms (Mbanefo, 1991).

The results of the effect of crude enzymes on enzymatic hydrolysis of the cellulosic substrates are illustrated in Table 1. It can be observed that wheatbran and ricebran are generally more susceptible to enzymatic hydrolysis than sorghum pomace, corn bran or corn cob. Even though sorghum pomace was used as substrate for crude cellulase pro-

Figure 2: Total Saccharification of Cellulose by Crude Enzyme Extract

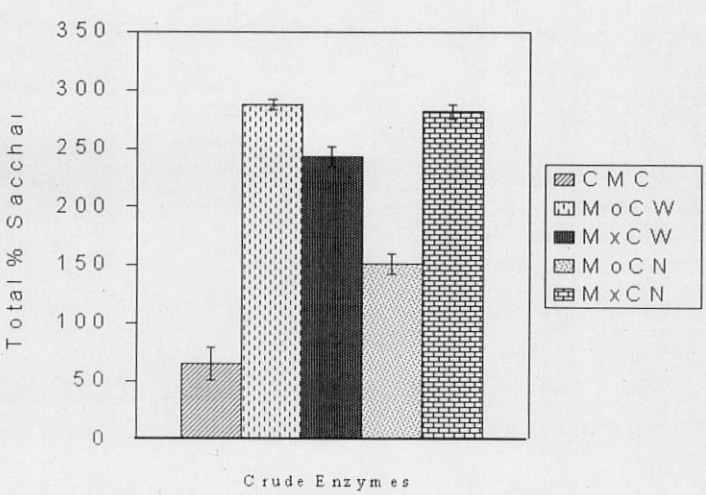


Table 1: Effect of Crude enzyme Extract on the Saccharification (%) of Cellulosic Substrates

SUBSTRATES	CMC	M_OCW	M_XCW	M_OCN	M_XCN	Total % Saccharification
Corn bran	4.05 ± 0.65^c	52.65 ± 4.07^a	52.65 ± 2.71^a	25.65 ± 2.05^b	49.95 ± 2.61^a	184.95 ± 21.63
Corn cob	4.05 ± 0.76^e	58.05 ± 3.89^a	47.25 ± 3.77^c	22.95 ± 3.91^d	52.65 ± 2.82^b	184.95 ± 22.78
Rice bran	17.55 ± 1.66^c	54.00 ± 2.11^a	55.35 ± 2.11^a	39.15 ± 2.67^b	58.05 ± 3.33^a	224.10 ± 16.93
Wheat bran	36.45 ± 2.89^c	62.10 ± 1.04^b	33.75 ± 4.01^d	40.50 ± 3.62^c	66.15 ± 2.69^a	238.950 ± 15.17
Sorghum pomace	2.70 ± 0.71^d	60.75 ± 2.06^a	54.00 ± 3.92^b	22.95 ± 2.74^c	55.35 ± 2.14^b	195.75 ± 25.20

Values are means ± SD of three replicate cultures
Values not followed by the same superscript in a row are significantly different ($P<0.05$).

LEGEND

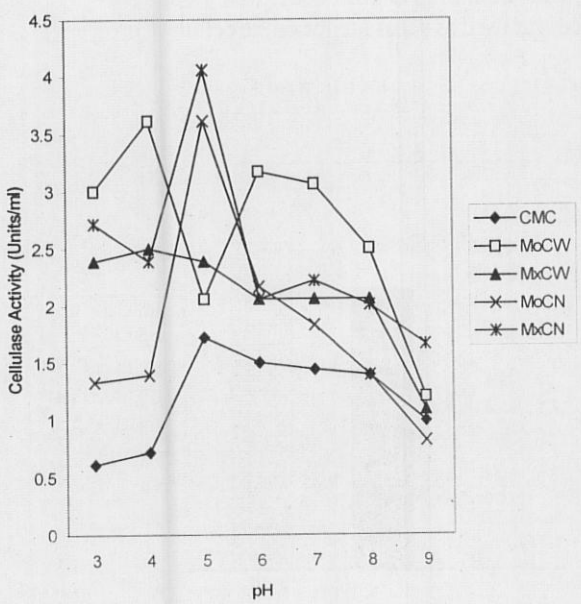
CMC	=	Carboxymethylcellulose
M_OCW	=	Monoculture of *A.niger* with mineral supplementation
M_XCW	=	Mixed culture of *A.niger* and *S.cerevisae* with mineral supplementation
M_OCN	=	Monoculture of *A.niger* without mineral supplementation
M_XCN	=	Mixed culture of *A.niger* and *S.cerevisae* without mineral supplementation

duction, no correlation existed between the capacity of sorghum pomace to induce cellulase enzyme and its susceptibility to enzymatic hydrolysis from its own crude enzyme (Okolo *et al.*, 1995). Hence cellulase produced using sorghum pomace could have a broad spectrum of substrate for hydrolysis. The results further show that the susceptibility of the cellulose substrates depends on the crude enzyme source (see Figure 2). Cellulosic substrates, based on Fig. 2, were more resistant to crude enzyme from CMC than those from sorghum pomace media. However mixed culture fermentation or mineral supplementation had no significant ($P>0.05$) influence on the hydrolytic capacity of crude enzyme extracts (Figure 2).

The activity of the crude enzyme extract was evaluated at various pH values (Figure 3). Optimum pH for enzyme activity, which varied from 4.0 to 6.0 depended on the media source. The optimum pH values for cellulase activities from fungal species has been reported to vary from species ranging from 3.0 to 6.0 (Ali *et al.*, 1991; Shambe, 1999; Abu *et al.*, 2003). Two pH optima were discernable for MoCW and M_XCN enzymes. Okolo *et al.* (1995) reported similar pH optima for raw starch digesting amylase using 0.1M acetate buffer of pH 3.0-7.0 when *A.niger* was grown on native starch.

This presumably suggests two or three distinct cellulolytic activities in these media. Cellulase has been reported to be composed of at least three different components which act synergistically (Eveleigh, 1987; Coughlan, 1990). Single pH peak in some other media (CMC and MoCN in Fig. 3) shows the closeness of the optimal pH for different cellulase components (Ueda, 1984). pH insensitivity observed over a wide pH range 3.0 – 8.0 in these media could be a reflection of the pH relationship of the synergistic interaction of the various cellulases (Coughlan, 1990).

Figure 3: Effect of pH on Cellulase Activity (Units/ml)



4. Conclusion

In conclusion, cellulase which demonstrates high level hydrolytic capacity can be produced by mixed culture of *A.niger* and *S.cerevizae* using sorghum pomace. Mixed culture of *A.niger* and *S.cerevizae* significantly ($P<0.05$) enhanced cellulase production but further supplementation of pomace with mineral nutrients suppressed enzyme production. Sorghum pomace is preferred to carboxymethyl cellulose for cellulase production. The most pressing requirement for further development and practical application of enzymatic saccharification of cellulose using crude enzyme from this source, is a thorough elucidation of the economics of the complete process through large scale studies. Nevertheless, the local availability and the status of sorghum pomace as food processing by-product could be of a great economic value to developing nations like Nigeria.

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RESEARCH NOTE

ENERGY DISPERSIVE X-RAY FLUORESCENCE ANALYSIS TECHNIQUE FOR GEOLOGICAL, BIOLOGICAL AND ENVIRONMENTAL SAMPLES

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Abstract

Energy Dispersive X-Ray fluorescence (EDXRF) technique for the analysis of geological, biological and environmental samples is described. The technique has been applied in the analysis of 10 (geological, biological, environmental) standard reference materials. The accuracy and precision of the technique were attested to by the good agreement between our measured results and the certified values of these standards. The measured and the certified values were generally within 5% of each other except for a few cases that were within 15% of each other for the geological samples.

1. Introduction

Qualitative and quantitative determination of elements in given samples are very important tasks in geological, mineralogical, environmental, medical, industrial and other fields (Ertugrul *et al.*, 1996). Accurate and precise determination of major, minor and trace elements in mineral ores is very important in their geochemical studies (Ossaka *et al.*, 1994). In the food industry, it is known that a knowledge of the chemical composition of foods is essential in most quantitative studies of human nutrition and in dietary treatment of diseases (McCance, 1960). Also elemental characterisation of environmental samples - soil, water and aerosols, is very crucial in pollution monitoring and control.

With the large number of samples involved in the various fields requiring elemental analysis, there is a need for a fast, reliable and multi-elemental technique for sample analysis. The Energy Dispersive X-Ray Fluorescence (EDXRF) technique is one such technique. It is non-destructive and can be used for the analysis of a wide variety of samples (Johnson *et al.*, 1996; Leenanupan and Srichom, 1996; Nwachukwu *et al.*, 2000; Obiajunwa, 2001). The EDXRF technique is capable of measuring elemental concentrations from trace levels of parts per million (ppm) up to 100% levels for major components. Solid samples for EDXRF analysis, in general, need little preparation. They only need to be presented in a homogeneous and reproducible form to the spectrometer (Obiajunwa, 2001).

In the present work, we demonstrate the capability of the EDXRF spectrometer to give accurate and reliable analysis of geological, biological and environmental samples.

2. Experimental

Samples

The samples analysed in this work consisted of (i) six geological standards, namely: NBS 278 (obsidian), BE-N (basalt), Mica-Fe (biotite), Mica-Mg (phlogopite), AC-E (granite) and GSR5 (shale); (ii) one environmental standard - MAG-1 (marine mud), obtained from the National Institute of Standards and Technology (NIST); (iii) three biological standards - CRM 062 (olive leaves), CRM 063 (natural skim milk), both from the European Community Bureau of Reference Standards, and A-11 (milk standard) from the International Atomic Energy Agency (I.A.E.A). These standards were pressed into thick pellets of 13 mm diameter in Spec-Caps (Obenauf, 1991) with no binders.

Analysis

The elemental analysis of the samples was performed using the Energy Dispersive X-Ray Fluorescence (EDXRF) spectrometer set-up at the Centre for Energy Research and Development, Obafemi Awolowo University, Ile-Ife. The spectrometer consists of a Siemens FKO-04 tube with Mo anode, a Kristalloflex 710H X-ray Generator, a Canberra series 7300 Si(Li) detector (resolution of 165eV at 5.9keV), a Canberra Model 1510 Integrated Signal Processor, and a Canberra S 100 MCA card interfaced to a 486 IBM/PC. The equipment runs under QXAS (Quantitative X-ray Analysis System) (IAEA, 1993) which includes facilities for data acquisition, spectrum analysis and interpretation and quantitative analysis. Each pellet was irradiated for 20 minutes at fixed tube operating conditions of 25kV and 6mA. The unfiltered Mo-$K_{\alpha,\beta}$ excitation allows determination of elements with characteristic K- or L- lines in the energy range 3.3 - 16 keV. A parameterless smooth-filter model in the AXIL program of the QXAS package was used for fitting the spectra over the energy region of interest. The AXIL program was also used to obtain the quantitative data on the samples, using the "Direct Comparison of Count rates" procedure. This procedure very much reduces the matrix effects when standards of similar composition to the analysed samples are used for the program implementation. The following standards were used of the quantitative analyses – AD2000 (Obsidian), G2 (Granite),

(email: eobiajun@oauife.edu.ng)

GSR3 (Basalt), from NIST, and IAEA-V-10 (Hay powder), from the International Atomic Energy Agency, and BCR 151 (contaminated milk powder) from the European Community Bureau of Reference Standards.

3. Results and Discussion

Figures 1a-1c show typical XRF spectra for a geological (shale) sample, an environmental (marine mud) sample and a biological (olive leaves) sample respectively. The continuous background lying under the characteristic x-ray lines is an inherent feature of the photon excitation. It is due mainly to the Compton scattering of x-rays in the target and in the detector (Benyaich *et al.*, 1997).

The results of the EDXRF analysis for the geological standards and the certified values are presented in Table 1, while those of the environmental and biological standards and the certified values are presented in Table 2. All data are the results of an average of three measurements on each sample with a relative standard deviation of less than 10%.

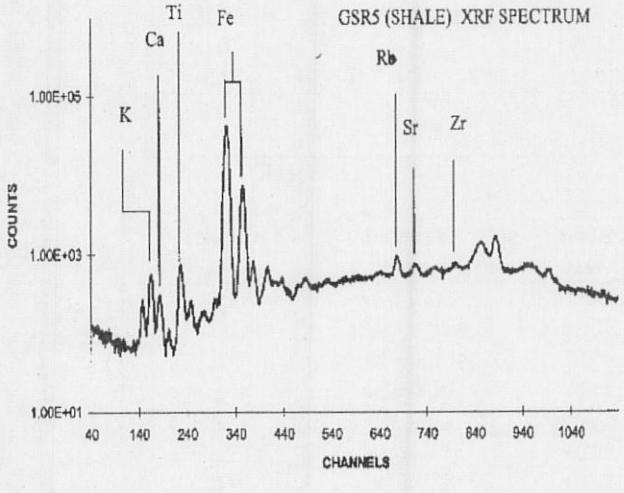

Figure 1a: XRF spectrum of GSR5 (shale) sample

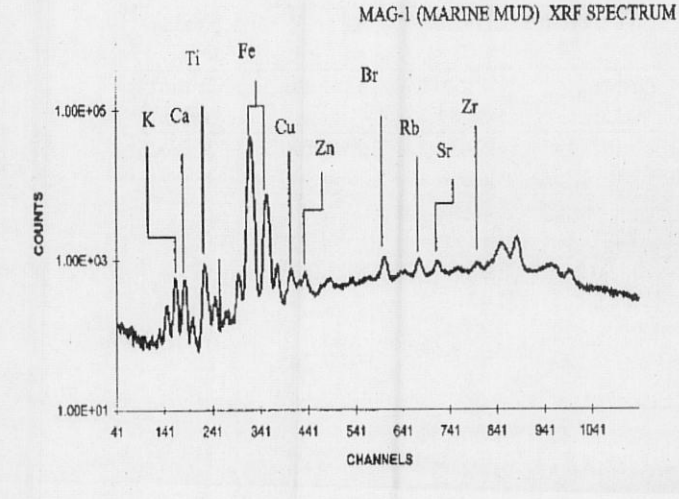

Figure 1b: XRF spectrum of MAG-1 (maritime mud) sample

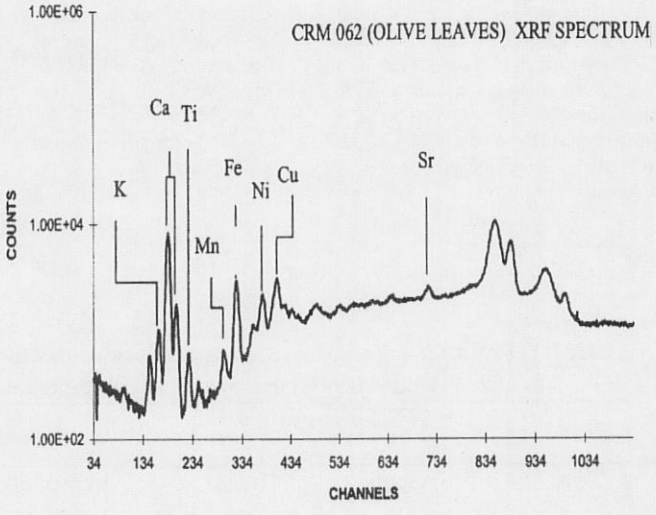

Figure 1c: XRF spectrum of CRM062 (olive leaves) sample.

The measured results are in good agreement with the certified values (Govindaraju, 1994; BCR, 1992; IAEA, 1989).

With the assured accuracy of the experimental values obtained from this EDXRF spectrometer, the system can be used on a routine basis for the analysis of geological, biological and environmental samples.

4. Conclusion

The EDXRF set-up of the Centre for Energy Research and Development, (CERD), Obafemi Awolowo University, Ile-Ife, has been used for the elemental analysis of geological, biological and environmental samples. The EDXRF technique is fast, reliable, multi-elemental and non-destructive. The concentrations of major, minor and trace elements in ten standard reference samples were determined. The measured elemental concentrations in the samples were in very good agreement with the certified values. The measured and the certified values were generally within 5% of each other except for a few cases that were within 15% of each other for the geological samples.

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Table 1: EDXRF results for the geological standards and their certified values. Concentrations of elements are given in µg/g except for those of major elements in %. Errors are due to counting statistics.

	NBS 278 (obsidian)		BE-N (basalt)		Mica-Fe (biotite)	
Element	Analysed value	Certified value	Analysed value	Certified value	Analysed value	Certified value
K	2.73 ± 0.12%	2.76%	1.3 ± 0.4%	1.154%	7.22 ± 0.87 %	7.26%
Ca	5774.0 ± 200.0	5620.0	11.08 ± 0.55%	11.8%	3550.0 ± 500.0	3070.0
Ti	1154.0 ± 102.0	1170.0	1.65 ± 0.15%	1.564%	1.65 ± 0.60%	1.499%
Mn	274 ± 16.0	322.2	1547.0 ± 60.0	1549.0	2905.0 ± 165.0	2711.0
Fe	1.14 ± 0.05%	1.141%	8.04 ± 1.02%	8.98%	16.8 ± 2.0%	17.94%
Zn	51.0 ± 12.0	44.0	138.0 ± 14.5	120.0	1105.0 ± 60.0	1300.0
Rb	101.0 ± 10.0	102.0	45.0 ± 7.5	47.0	2220.0 ± 170.0	2200.0
Sr	56.0 ± 10.0	50.8	1365.0 ± 60.0	1370.0		
Zr	265.0 ± 30.0	232.0	254.0 ± 26.0	256.0	793.0 ± 55.0	800.0

Table 1 (Contd.)

	Mica-Mg (phlogophite)		AC-E (granite)		GSR5 (shale)	
Element	Analysed value	Certified value	Analysed value	Certified value	Analysed value	Certified value
K	8.23 ± 0.87%	8.301%	3.75 ± 0.15%	3.727%	3.37 ± 0.25%	3.453%
Ca	629.0 ± 50.0	572.0	2329.0 ± 450.0	2430.0	4894.0 ± 900.0	4288.0
Ti	1.02 ± 0.13%	0.977%	625.0 ± 40.0	659.0	4151.0 ± 110.0	3956.0
Mn	2055.0 ± 150.0	2014.0	437.0 ± 20.0	449.0	180.0 ± 50.0	155.0
Fe	6.46 ± 0.55%	6.616%	1.82 ± 0.05%	1.769%	5.15 ± 0.5%	4.68%
Zn	293.0 ± 25.0	290.0	222.0 ± 35.0	224.0	57.0 ± 10.0	55.0
Rb	1302.0 ± 105.0	1300.0	126.0 ± 20.0	152.0	85.0 ± 10.0	90.0
Sr	32.0 ± 4.5	27.0			110.0 ± 20.0	96.0
Zr	23.0 ± 5.0	20.0	774.0 ± 30.0	780.0		

Table 2: EDXRF results for the environmental and biological standards and their certified values. Concentrations of elements are given in µg/g except for those of major elements in %. Errors are due counting statistics.

	MAG-1 (marine mud)		CRM 062 (olive leaves)		CRM 063 (natural skim milk)		A-11 (IAEA milk powder)	
Element	Analysed value	Certified value	Analysed value	Certified value	Analysed value	Certified value	Analysed value	Certified value
K	2.86 ± 0.25 %	2.947%	3194.0 ± 150.0	3070.0	1.76 ± 0.056%	1.78%	1.69 ± 0.040%	1.72%
Ca	9820.0 ± 115.0	9791.0	1.67 ± 0.20%	1.75%	1.26 ± 0.036%	1.26%	1.29 ± 0.040%	1.29%
Ti	4540.0 ± 70.0	4501.0	276.0 ± 40.5	239.8				
Mn	740.0 ± 50.0	759.0	57.0 ± 5.0	57.0	ND	0.226	ND	0.259
Fe	4.68 ± 0.30%	4.756%	293.0 ± 25.0	281.5	2.0 ± 0.15	2.06	3.4 ± 0.30	3.65
Zn	125.0 ± 20.0	130.0	25.0 ± 5.0	16.0	43.0 ± 3.80	42.0		
Rb	154.0 ± 12.0	149.0					ND	0.378
Sr	148.0 ± 12.0	146.0	120.0 ± 10.0				39.0 ± 2.45	38.9
Zr	120.0 ± 10.0	126.0					31.0 ± 1.80	30.8

ND: Not determined

RESEARCH NOTE

COMPARATIVE ANTIBACTERIAL ACTIVITIES OF OIL PALM-*ELAEIS GUINNEESIS* JACQ. (PALMAE) NUT AND COCONUT-*COCOS NUCIFERA L.* (PALMAE) SHELLS PYROLYSATES

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Abstract.

There are many industrial or plant waste products which can be examined for their probable use(s) medicinally.This work was carried out to examine the probable anti-bacterial effects of liquid pyrolysates obtained from oil palm nut and coconut shells. The antimicrobial activities liquid pyrolysates (obtained by destructive distillation), their fractions (obtained by successive partitioning of each pyrolysate with hexane, chloroform and ethyl acetate) were tested against clinical isolates and type bacteria. The anti microbial activities of the pyrolysates were compared with phenol.

All the organisms used showed remarkable sensitivities to the pyrolysates at 0.5ml (i.e.469mg of oil palm nut shell and 484mg of coconut shell). MIC and MBC of 0.02 ml (i.e.18.78mg of oil palm nut shell pyrolysate and 19.34mg of coconut shell pyrolysate) was obtained for the pyrolysates while their activities were observed to be comparable with phenol (0.01ml). The antibacterial activities of the samples appeared to be due to the components of their respective chloroform fractions which showed higher activities than the crude pyrolysates.

Thus, the pyrolysates obtained from oil palm kernel and coconut shells have a substantial antibacterial potential which are yet to be fully examined and made into appropriate useful products.

1. Introduction

Many agricultural waste products are usually regarded as useless and are therefore dumped or discarded indiscriminately in our society. If such materials are treated properly, it is possible to convert them to useful products, especially in formulating medicinal compounds. Oil palm nut and coconut shells are examples of such materials. With enormous oil palm plantations and palm oil production as well as extensive cultivation and use of coconut for various purposes in Nigeria, the annual yield of oil palm nut shell can be estimated at 2 million metric tones, while that on coconut shell is equally substantial. Although, palm kernel oil as well as coconut oil are well known to have pharmaceutical importance (Evans, 1989) while their shells are used by blacksmiths to generate energy to melt irons, the medicinal importance of the shells have received little or no attention.

Destructive distillation of these shells were reported to contain phenol and other phenolic derivatives like O-cresol, guaicol,2,6-dimethoxyphenol e.t.c. although in varying concentrations. (Chan *et al.*, 1976a,b, 1980). This present work was carried out to examine the effects of the pyrolysates yielded by these shells using destructive distillation equipment designed by Badmus (1999). The antimicrobial effects of the organic solvent fractions were also examined and compared with phenol.

2. Materials and Methods

After harvesting and processing of oil palm and coconut fruits, 1.81kg and 1.52kg of their respective shells were separately reduced to pieces by passing through a cracking machine. Each was subjected to destructive distillation by heating in a steel-made closed chamber for 1 1/4 hr using pyrotechnic distillation equipment designed by Badmus (1999) to yield charcoal and volatile compounds. These were collected after passing through a condenser. The condensates consisted of pyroligneous and tarry layers.

Partitioning of the pyrolysates with organic solvents

After a thorough mixing of the pyroligneous and the tarry layers, 500ml each of the pyrolysates (i.e. 469.3g of oil palm nut shell pyrolysate, 484g of coconut shell pyrolysate) was separately subjected to partitioning in succession using hexane, chloroform and ethyl-acetate.

The organic solvent fractions obtained were concentrated using rotavapor order to absolutely remove traces of the solvent. Each of them was measured, weighed and kept in a refrigerator until needed.

Bacterial isolates

All the bacteria used were obtained from Medical Microbiology laboratory, University of Benin Teaching Hospital. The organisms included *Pseudomonas aeruginosa* ATCC 27857, *Escherichia coli* NCTC 10418, *Staphylococcus aureus* NCTC6571, which were all type strains while *Klebsiella pneumoniae, P. aeruginosa, Salmonella typhimurium, E. coli and Bacillus subtilis* were isolated locally.

+ *corresponding author*

All the organisms were cultured on agar slant of buffered peptone water and sub cultured onto deoxychocolate citrate agar incubated at 37°C for 24h before use.

Determination of Antibacterial Activities

The agar-cup (7mm) diffusion method was used in determining the antibacterial activities of the samples using isosensitest agar medium. For sensitivity tests, 0.5ml each (939mg/ml for oil palm nut shell and 967mg/ml for coconut shell pyrolysates) of the pyrolysates were used to challenge the overnight cultures of the organisms while the MIC and MBC of the pyrolysates were determined using 0.01 – 0.16ml (0.01ml is equivalent to 9.39mg of oil palm nut shell pyrolysate and 9.67mg of coconut shell pyrolysate) and observed for 72hrs. The organic solvent fractions of the pyrolysates as well as their respective residues were tested at concentrations of 0.01, 0.015 and 0.02ml. For oil palm nut shell pyrolysate, 0.01ml is equivalent to 8.9mg of hexane fraction, 10.3mg of chloroform fraction, 9.9mg of ethyl acetate fraction and 8.7mg of residue. For the coconut shell pyrolysate, 0.01ml is 6.69mg of hexane fraction, 9.47 of chloroform fraction, 8.87mg of ethyl acetate fraction and 8.9mg of the residual fraction obtained after the partitioning. The antibacterial activities of the pyrolysates and the fractions were compared with phenol at the same concentrations.

3. Results and Discussion

At the end of the destructive distillation process, 1.81kg of oil palm nut shell yielded 654.0ml (613.8g, 36.09%) liquid pyrolysate while 1.52kg coconut shell produced 614ml (596.6g, 40.4%). It was observed that 0.5ml of each of the pyrolysates absolutely inhibited the growth of all the bacteria after incubation for 24h (Table 1). After 24h, a minimum inhibitory concentration of 0.02ml (i.e. 18.78mg of oil palm nut shell pyrolysate and 19.34mg of coconut shell pyrolysate) was observed for the two crude samples. This concentration was found to be same for the minimum bactericidal concentration observed after 72h (Tables 2 and 3).

Partitioning of samples with solvents of increasing polarities ensures separation of components of such samples based on their solubility potentials in the respective solvents used. Table 4 shows the effects of various fractions of the pyrolysates on the microorganisms. The hexane fractions as well as the residue obtained after the final partitioning with ethyl acetate did not exhibit antibacterial effects. In both samples, the chloroform fractions seem to be more potent than the crude pyrolysates since at a concentration of 0.01ml (i.e. 10.30mg of chloroform fraction of oil palm nut shell pyrolysate, 9.47mg of chloroform fraction of coconut shell pyrolysate) the fraction, particularly that of former, inhibited the growth of *P.aeruginosa* ATCC 27857, *E.coli* NCTC 10418, *S.aureus* NCTC6571 and *S. typhimurium*.

The antibacterial activities of the pyrolysates and their chloroform fractions were relatively similar to those observed in phenol 0.01 – 0.02ml. The higher activities observed in the chloroform fractions of the pyrolysates suggest that the bulk of the antibacterial components are contained in the fractions. However, the fact that the oil palm nut shell pyrolysate as well as its chloroform fraction showed higher activities than the coconut shell pyrolysate indicate the higher concentration of the antimicrobial constituents in the former than later. The similarities in the pattern of activities of the two pyrolysates as well as their organic solvent fractions suggest that the two of them contain similar constituents (as reported in literature) although the con-

Table 1: Antibacterial activities of oil palm nut shell (ons) and coconut shell (cs) pyrolysates at 0.5ml.

ORGANISMS	Oil palm nut shell pyrolysate (0.5ml of 939mg/ml)	Coconut shell pyrolysate(0.5ml of 967mg/ml)
Pseudomonas aeruginosa ATCC 27853	-	-
Escherichia coli NCTC 10418	-	-
Staphlococcus aureus NCTC 6571	-	-
Klebsiella pneumoniae	-	-
Staphlococcus aureus	-	-
Pseudomonas aeruginosa	-	-
Salmonella typhimurium	-	-
Escherichia coli	-	-
Bacillus subtilis	-	-

+ Growth at 0.5ml
- No growth at 0.5ml

Table 2: Minimum inhibitory concentrations (MIC) of crude oil palm nut shell (ons) and coconut shell (cs) pyrolysates.

ORGANISMS	Oil palm nut shell pyrolysate (939mg/ml)							Coconut shell pyrolysate (967mg/ml).						
	0.01	0.02	0.04	0.08	0.1	0.12	0.16	0.01	0.02	0.04	0.08	0.1	0.12	0.16
E.coli NCTC 10418	+	-	-	-	-	-	-	+	-	-	-	-	-	-
S. aureus NCTC 6571	+	-	-	-	-	-	-	+	-	-	-	-	-	-
P.aeruginosa ATCC 27853	+	-	-	-	-	-	-	+	-	-	-	-	-	-
**B.subtilis*	+	-	-	-	-	-	-	+	-	-	-	-	-	-
**P. aeruginosa*	+	-	-	-	-	-	-	+	-	-	-	-	-	-
**K.pneumoniae*	+	-	-	-	-	-	-	+	-	-	-	-	-	-
**S. aureus*	+	-	-	-	-	-	-	+	-	-	-	-	-	-
**E. coli*	+	-	-	-	-	-	-	+	-	-	-	-	-	-
**S.typhmurium*	+	-	-	-	-	-	-	+	-	-	-	-	-	-

* Clinical isolates.
\+ Growth
\- No growth

Table 3: Minimum bactericidal concentration (MBC) of oil palm nut shell and coconut shell pyrolysates after 72hrs .

ORGANISMS	Oil palm nut shell pyrolysate (939mg/ml)			Coconut shell pyrolysate (967mg/ml).		
	0.01	0.015	0.02	0.01	0.015	0.02
P.aeruginosa ATCC 27853	+++	+	-	+++	+	-
Esch. coli NCTC6571	+++	+	-	+++	+	-
S. aureus NCTC 6571	+++	+	-	+++	+	-
S. aureus	+++	+	-	+++	+	-
K. pneumoniae	+++	+	-	+++	+	-
S.typhimurium	+++	+	-	+++	+	-
P. aeruginosa	+++	+	-	+++	+	-
E. coli	+++	+	-	+++	+	-
B. subtilis	+++	+	-	+++	+	-

+++ Significant growth
\+ Insignificant growth
\- No growth

Table 4: Antibacterial effects of organic solvent fractions of oil palm nut shell (ons) and coconut shell (cs) pyrolysates.

Organisms	Conc. (ml)	Zones of inhibition (mm)										
		Crude		Hexane fraction		Chloroform fraction		Ethylacetate fraction		Residue		Phenol
		ons	cs	ons	cs	ons	cs	ons	cs	ons	cs	
P.aeruginosa ATCC 27857	0.01	-	-	-	-	15	-	18	-	-	-	21
	0.015	15	-	-	-	18	18	18	-	-	-	22
	0.02	20	20	-	-	20	20	12	-	-	-	24
E.coli NCTC 10418	0.01	-	-	-	-	15	10	-	-	-	-	23
	0.015	15	-	-	-	20	18	12	-	-	-	24
	0.02	20	20	-	-	22	22	18	20	-	-	25
S.aurens NCTC 6571	0.01	-	-	-	-	15	-	-	-	-	-	25
	0.015	15	-	-	-	18	10	15	-	-	-	27
	0.02	20	20	-	-	20	23	18	20	-	-	28
K.pneumoniae	0.01	-	-	-	-	-	-	-	-	-	-	22
	0.015	15	-	-	-	17	18	-	-	-	-	22
	0.02	20	18	-	-	20	20	-	16	-	-	23
S. aureus	0.01	-	-	-	-	-	-	-	-	-	-	25
	0.015	18	-	-	-	20	18	15	-	-	-	26
	0.02	22	20	-	-	22	22	18	20	-	-	28
P.aeruginosa	0.01	-	-	-	-	-	-	-	-	-	-	21
	0.015	10	-	-	-	10	10	-	-	-	-	22
	0.02	20	20	-	-	20	18	-	-	-	-	24
S.typhimurium	0.01	-	-	-	-	15	10	10	-	-	-	24
	0.015	-	-	-	-	18	18	15	-	-	-	25
	0.02	20	18	-	-	20	20	18	-	-	-	26
E.coli	0.01	-	-	-	-	-	-	-	-	-	-	20
	0.015	20	-	-	-	20	18	18	-	-	-	23
	0.02	22	20	-	-	22	22	18	20	-	-	25
B.subtilis	0.01	-	-	-	-	-	-	-	-	-	-	12
	0.015	-	-	-	-	-	18	-	-	-	-	14
	0.02	20	19	-	-	20	20	16	20	-	-	14

centrations of each of such components may vary. Many plants as well as their secondary metabolites have been reported to exhibit inhibitory effects on bacterial growth and proliferations. For instance, *Garcinia cola, Aframomum melegueta, Nauclea latifolia,* etc., have been investigated and confirmed to have significant inhibitory effects on many bacteria as well as fungi although the constituents responsible for the activities vary (Iwu *et al.*, 1999).

The antibacterial activities of the pyrolysates reported here are of importance as the shells from which they were obtained were hitherto not known to be of medicinal value. As their distillation requires simple locally made equipment, this research work suggests their probable incorporation into soaps and creams for dermatological infections. Further works are being considered to ascertain the actual nature of the constituents of both pyrolysates and their respective chloroform fractions.

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