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Occurrence of Beta- Lactamase Resistance among Isolates from Cancer Patients in Lagos, Nigeria

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Abstract: Bacteria infections associated with multidrug resistance have been implicated in the high mortality and morbidity reported among cancer patients. In recent years gram negative organisms isolates from patients with neoplasia have been found to produce beta-lactamases (β-lactamase) and this is of interest in developing countries where it is unreported or underreported. This study determined beta-lactamase mediated resistance in gram negative bacteria isolates from patients attending the radiology and Oncology clinic of Lagos University Teaching Hospital between April and November 2006.One hundred and nineteen samples were analyzed and sixty one gram negative isolates were recovered.The isolates were characterized with the analytical profile index(API) tests and antimicrobial susceptibility testing was determined using the disk diffusion method according to CLSI standard. Production of beta-lactamase and extended spectrum beta-lactamase (ESBL) were investigated using the nitrocefin stick and double disk synergy test (DDST) respectively. Plasmid analysis was done on each bacteria isolate showing multidrug resistance. Of the sixty one gram negative isolates, 55((90.2%) produced beta- lactamases; 20(32.8%) were found to be ESBL producers while 14(23%) showed AmpC enzyme production. Twenty five out of twenty seven strains harbored plasmids of sizes ranging between 3.0-4.9kb. Statistical analyses showed occurrence of ESBL and AmpC production to be significant. The result of this study has shown a high occurrence of beta-lactamase mediated resistance among clinical isolates from cancer patients. Many of these harbored plasmids which may encode genes for antibiotic resistance or virulence factors which are becoming persistent problems in the health care sector. **[**Researcher.2009;1(6):1-6].(ISSN:1553-9865).

Keywords: ESBL, AmpC, Beta-lactamase, Cancer, multidrug resistance.

1. Introduction

Of the various mechanisms of acquired resistance to β-lactam antibiotics, resistance due to β-lactamases is the most prevalent. Gram negative bacteria resistant to agents such as extended spectrum cephalosporin, monobactams, carbapenems and β-lactam-β-lactamase inhibitor combinations have emerged through the production of a variety of β-lactamases(Pitout *et.al*, 1997; Wood ,AJ. 1996) Emergence of resistance to these agents has resulted in a major clinical crisis(D'agata,FEMC.2000).

Bacteria infections associated with multi-drug resistance in cancer patients have been reported as high (Figuera *et.al*, 2006) and this is caused mostly by the effect of the cytotoxic chemotherapy and radiotherapy which lowers the immunity (Rice *et.al*, 1990). With the increased use of β-lactams among these patients an increase in bacteria resistance has developed. Extended spectrum beta-lactamase resistances are now a problem among patients with chronic cases and carcinomas. (Naumovski *et al.* 1992). In this study, we examined the occurrence of different β-lactamases among gram negative isolates from patients with cancer of the breast and cervix. We also determined the plasmid profiles of isolates producing extended spectrum beta-lactamase.

2.Materials and methods

2.1 Isolation and Identification

A total of 61 gram negative bacteria isolates from 119 breast and cervical cancer patients' samples who attended the Radiology and Oncology Clinic of the Lagos University Teaching Hospital, Nigeria between April, 2006 and November, 2006 have been included in this study. These isolates were from the urine, breast wound swabs and cervical swabs of non-hospitalized cancer patients attending the Radiology and Oncology Clinic of the Lagos University Teaching Hospital.

The strains were identified by conventional methods and confirmation to the species level was done biochemically with the use of API 20E and 20NE system. (API bio merieux, Nurtingen, Germany).

2.2. Beta-lactamase was determined using a chromogenic cephalosporin method (Nitrocephin-stick oxoid, UK) and positive control of *Staphylococcus. aureus* ATCC 29213.

2.3. Susceptibility testing was performed using the standard agar disc diffusion method as described by the NCCLS standard. (2000). ESBL production was detected using the double disk synergy test as described by Jarlier *et.al*. (1988) with modification by Thomson and Sanders. (1992). All strains showing resistance to third generation cephalosporins was screened fro ESBL production. An amoxicillin-clavulanate disk was placed at the centre of inoculated plate and disks containing ceftazidime (30µg), cefotaxime (30µg) ceftriaxone (30µg) and aztreonam (30µg) were placed 20mm apart (center to center) from the amoxicillin-clavulanate disk. Enhancement of the zone of inhibition of the oxyimino-β-lactam caused by the synergy with the clavulanate in the amoxycilin clavulanate disk was considered as an evidence of ESBL production. (Jarlier *et.al*.1988., Thompson and Sanders., 1992). *E.coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as control strains.

2.4. Presumptive Phenotypic Determination of CTX-M ESBL: Bacteria isolates resistant to cefotaxime, ceftriaxone and aztreonam were presumptively identified as producers of CTX-M ESBL (Livermore and Brown., 2001)

2.5. The inducibility of AmpC β-lactamase production was performed by disk antagonism tests in which disks containing an inducing agent, cefoxitin (30µg) and ceftazidime (30µg), were placed on Mueller Hinton agar plates. Blunting of the ceftazidime zone at adjusted distance between the disks and resistance to cefoxitin was observed. (Livermoreand Brown., 2001)

2.6. Plasmid DNA of isolates producing ESBL was extracted using the alkaline phosphate method of Birnboin and Doly(1979). Agarose gel electorphoresis was used to determine the profiles of the plasmid. The profiles were compared to a DNA of known molecular weight plasmids.

Statistical analysis using analysis of variance (ANOVA) as test of significance was done. All statistical analysis was performed at 95% confidence interval and significant p values less than 0.05 were considered significant.

3.0 RESULT

Sixty one gram negative bacteria made up of seventeen (17) different species were recovered from one hundred and nineteen (119) clinical samples. Amongst them were *Klebsiella pneumoniae*, *Citrobacter amalonaticus, E.coli, P. mirabilis, A.baumanii, Serratia liquefaciens* and *Stenotrophomonas maltophilia*. Out of the sixty one (61) isolates 55, (90.2%) produced beta-lactamase (TEM/SHV like type), 20 (32.8%) and 14 (23%) were found to be extended spectrum beta-lactamase and AmpC producers respectively (Table 1). Most of the ESBL-producers were multi-resistant to the fluoroquinoles and aminoglycosides, 93.4% of isolates were found to be sensitive to imipenem and all isolates showed resistance to tetracycline (100%) (Table 2). Plasmid analysis was performed on each bacteria showing multi-drug resistance (Table 3). Twenty five (25) of twenty seven (27) strains harboured plasmids of sizes ranging between 3.0 – 4.9kb. (Table 4)

Statistical analysis showed occurrence of ESBL and AmpC production among isolated strains from cancer infections to be significant.

Table 1:Beta-lactamases detected in organisms isolated

Organisms	Beta-lactamase enzyme detection using nitrocephin.n=55	ESBL enzyme detection using double disk synergy test (DDST).n=20	AmpC (enzyme detection using cefoxitin resistance.n=14
E.coli (16)	16 (29%)	11 (55%)	6 (42.9%)
Enterobacter aerogenes (2)	2 (3.6%)	0%	0%
Enterobacter agglomerans (1)	1 (1.8%)	0%	0%
Enterobacter cloacae (2)	2 (3.6%)	0%	2 (14.3%)
Citrobacter amalonaticus / koserii (3)	3 (5.5%)	1 (5%)	1 (7.2%)
Citrobacter freundii (1)	1 (1.8%)	1 (5%)	0%

Klebsiella pneumoniae (6)	6 (10.9%)	4 (20%)	2 (14.3%)
Klebsiella planticola (6)	3 (5.5%)	2 (10%)	2 (14.3%)
Klebsiella oxytoca (4)	4 (7.3%)	0%	0%
Klebsiella rhinoscleromatis (1)	1 (1.8%)	0%	0%
Klebsiella ozaene (3)	1 (1.8%)	1 (5%)	0%
Pseudomonas aeruginosa	9 (16.4%)	0 (0%)	0%
Acinetobacter iwofii (1)	1 (1.8%)	0%	0%
Acinetobacter Baumanil (1)	1 (1.8%)	0%	0%
Proteus mirabilis (3)	2 (3.6%)	0%	0%
Serratia liquefaciens (1)	1 (1.8%)	0%	1 (7.2%)
Stenotrophomonas maltophilia (1)	1 (1.8%)	0%	0%

Table 2: Resistance Pattern of Isolates

Isolates	Antibiotic Resistance Profile
E. coli	Tzp, Caz, Cro, Ctx, Atm, Pef, Ofx, Cip, Amx, Gen, Nit, Cot, Cxm, Aug, Tet.
Enterobacter spp	Amx, Cxm, Aug, Tet
Serratia liquefaciens	Tzp, Fox, Ak, Gen, Cot, Aug, Tet,
Klebsiella spp	Tzp, Ctx, Cip, Amx, Gen, Nit, Cot, Cxm, Aug, Tet.
Proteus mirabilis	Ak, Pef, Ofx, Cip, Gen, Nit, Cxm, Tet,
Pseudomonas aeruginosa	Tzp, Fox, Caz, Cro, Atm, Ak, Pef, Ofx, Cip, Amx, Gen, Nit, Cot, Cxm, Aug, Tet.
Stenotrophomonas maltophilia	Tzp, Pef, Ofx, Cip, Amx, Gen, Nit, Cot, Tet.
Citrobacter spp	Tzp, Fox, Ctx, Atm, Pef, Cip Amx Gen, Nit, Cot, Cxm, Aug, Tet.
Acinetobacter spp	Tzp, Ctx, Imp, Amx, Gen, Nit, Cot, Cxm, Aug, Tet.

Key: Tzp(Tazobactam-pipercillin), Caz(Ceftazidime),Cro(ceftriaxone),Ctx(cefotaxime), Atm(Aztronam), Pef(pefloxacin),Ofx(ofloxacin),Cip(ciprofloxacin), Fox (Cefoxitin),Ak(Amikacin),Amx(Amoxicillin), Gen(Gentamycin),Cxm(Cefuroxime),Aug(Amoxicillin-clavulanic acid),Cot(Cotrimoxazole) Tet (Tetracycline),Gen(Gentamycin),Imp(Imipenem),Nit(Nitrofurantoin).

Table 3: Antimicrobial resistance pattern of isolates in relation to plasmids.

Isolates	Antimicrobial Resistance Pattern	Number of Isolates	Number with Plasmids.
Pseudomonas aeruginosa	Tzp, Fox, Caz Cro, Atm, Ak, Pef, Ofx, Cip, Amx, Gen, Nit, Cot, Cxm, Tet.	4	4
Citrobacter spp	Tzp,Fox,Ctx,Cot,Atm, Pef, Cip, Amx, Gen, Nit, Cxm, Tet.	2	2
E.coli	Tzp,Caz,Ctx,Atm, Pef, Ofx, Cip, Amx, Gen, Nit,Cot,Cxm,Aug,Tet.	11	11
Klebsiella spp	Tzp, Ctx, Cip, Amx, Gen,Nit,Cot,Cxm,Aug, Tet.	7	7
Acinetobacter spp	Tzp, Ctx, Imp, Amx, Gen,Nit,Cot,Cxm,Aug, Tet.	1	1
Enterobacter spp	Amx,Cxm,Aug,Nit, Tet.	2	0

Table 4: plasmid screening results of isolates

Isolates	Source	Number with Plasmid	Plasmid Sizes (kb)
E .coli (1)	Urine	1	4.4
E. coli (2)	Urine	1	3.0,3.6, 4.4
E. coli (3)	Urine	1	4.3
E. coli (4)	Urine	1	4.6
E. coli (5)	Urine	1	4.5
E. coli (6)	Urine	1	4.8
E. coli (7)	Urine	1	4.6
E. coli (8)	Urine	1	4.6
E. coli (9)	Urine	1	4.9
E. coli (10)	Cervical swab	1	4.7
E. coli (11)	Cervical swab	1	4.6
Citrobacter spp (1)	Urine	1	4.4
Citrobacter spp (2)	Urine	1	4.6
K. pneumoniae (1)	Urine	1	4.5
K. pneumoniae (2)	Urine	1	4.4
K. pneumoniae (3)	Urine	1	4.7
K. pneumoniae (4)	Urine	1	4.7
K. planticola (1)	Urine	1	4.1, 4.7
K. planticola (2)	Swab	1	4.4
K ozaenae (1)	Urine	1	4.6
P. aeruginosa (1)	Urine	1	4.6
P. aeruginosa (2)	Urine	1	4.6
P. aeruginosa (3)	Urine	1	4.6
P. aeruginosa (4)	Swab	1	4.6
Acinetobacterspp(1)	Urine	1	4.6

4. Discussions

Chemotherapy and radiotherapy methods of treatment adopted for patients with carcinoma of the cervix and breast have been implicated as an agent of immunosupression. Hence, it's been known to contribute to infections in patient with an underlying debilitating effect of cancerous growth. (Rice *et.al.,* 1990) With the emergence and increase in bacterial resistance, surveillance of the prevalent pathogen and their resistance pattern is of utmost importance to reduce the mortality rate due to bacterial infections and also improve the quality of life of affected patient. (Figuera *et. al*., 2006).

Reports of ESBL producing strains have been appearing for about a decade among outpatients and in patients in this environment (Aibinu *et.al*., 2003) but no data exists on ESBL bacteria isolates from cancer patients in this environment. Therefore, it is noteworthy that this study isolated 61 gram negative pathogens and out of these 32.8% were found to be ESBL producers while 23% showed AmpC enzyme.

This result records so far the highest number of gram negative species to be recovered from cancer patients compared to previous studies on isolates from this environment that studied anaerobes and gram positive organisms. (Rotimi *et.al*., 1984., Oduyebo *et.al*., 2001)

Of the 61 isolates recovered from urine, and swabs of these patients, 49 (80.3%) were from urine. *Klebsiella* species had the highest occurrence in urine among these cancer patients which is in agreement with Podschunn and Ulmann (1998) that reported *Klebsiella* species as an opportunistic pathogen, which primarily attack immunocompromised individuals in the United States. On the other hand, *Proteus mirabilis* was the most frequently isolated organism from wound swabs of these cervical and breast cancer patients. This was followed by *Enterobacter* spp (E. *aerogenes* and *E. cloacae*); *Pseudomonas aeruginosa* and *Escherichia coli*. All these organisms have been implicated in wound infections (Goosens, H. 2005). *Klebsiella planticola* was the only species of *Klebsiella* isolated from wound swabs in this study. This is in agreement with the report of Podschunn and Ullmann (1998) which reported *K. planticola* to have a high frequency of recovery from clinical samples of wound. This findings highlights the organisms most commonly implicated in wound swabs of cervical and breast cancer and the organisms most commonly implicated

in infections among cancer patients in this environment.

Multi-drug resistance was found among the 9 groups of organisms isolated. The best coverage against these organisms was obtained with imipenem (100%), except for *Pseudomonas aeruginosa* and *Acinetobacter* species which showed resistance to the carbapenem with 33% and 50% resistance respectively.

High resistance to amoxicillin clavulanic acid, a beta-lactam beta- lactamase inhibitor was observed among the isolates and all other beta-lactam agents. The third generation cephalosporin were effective against *S. maltophilia, Serratia* spp with over 75% sensitive, multi-drug resistance occurred in E*. coli, Pseudomonas aeruginosa, Acinetobacter* spp, *Serratia liquefaciens* with complete resistance to over 7 antibiotics.

Most of the ESBL isolates were multi-resistant but susceptibility to imipenem and amikacin (>60%) was high. However, imipenem is not easily available in Nigeria because of its high cost. Resistance to the quinolones (ofloxacin, ciprofloxacin, pefloxacin) was detected in most of the ESBL and AMPC producers (>50%). This further limits the choice of effective antibiotics among these patients.

Emergence of these resistance and production of ESBLS and AmpC is of concern among these patients. Plasmid profiles showed that all the strains were diverse in nature with respect to transmission of antibiotic resistance except for *P.aeruginosa* isolates which had all strains harbouring only one plasmid of same molecular weight. It was also observed that *Enterobacter cloacae* showing AmpC did not harbour plasmid suggesting that the resistance may be chromosomally borne.

This emergence of ESBLs and stable derepressed mutants that hyperproduced chromosomal beta-lactamases have the potential to diminish the activity of all extended spectrum cephalosporins (Goosens.,2005) and these important pathogens are currently on the rise in critically ill group of patient (Patterson and Bonomo2005).

It is of concern in this study that proliferation of beta-lactamase resistance among strains may have been due to misuse of antibiotics, proliferation of multiply resistant clones, transfer of resistance-carrying plasmids and inability to detect emerging phenotypes in developing countries as stated by Croft *et.al.*, 2007.

This study has been able to show an emergence of different strains of multi-resistant bacteria flora producing beta-lactamases among clinical isolates of cancer patients. Many of these multiresistant species harboured plasmids which may encode genes for antibiotic resistance or virulence factors and may predispose to high morbidity and mortality of the disease. The resistance has paralleled the introduction, administration misuse and overuse of broad spectrum of antibiotics. All physicians should be obligated to prescribe antimicrobial agents more deliberately. Also, there is need for antibiotic surveillance in this population of patients to ensure judicious use of antibiotics.

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Evaluation of the Protective Effect of L-Carnitine on Radiation Induced Free Oxygen Radicals and Genotoxicity in Male Mice

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Abstract: The aim of the study was to evaluate the potential protective effect of L-carnitine on radiation induced free radicals in mice .To achieve the ultimate goal of this study, 122 adult Swiss male mice were randomly divided into 4 groups. Group 1: Did not receive L-carnitine or irradiated and served as control group. Group 2: Daily injected subcutaneous with L-carnitine (200 mg/Kg) for 3 successive weeks. Group 3: Irradiated with fractionated dose of 6 Gy where the dose was 2 Gy once per week for 3 successive weeks. Group 4: Received a daily subcutaneous injection of L-carnitine (200 mg/Kg) for 7 consecutive days before the first dose of irradiation and continuous together through the experimental period. The animals were sacrificed at 1, 7 and 14 days after last dose of irradiation. The samples were taken from the blood and some organs, heart, spleen, testes and bone marrow for the biochemical and cytogenetics analysis. In the irradiated group, there were a significant decrease in RBCs ,WBCs, Hb, Hct, plasma levels of testosterone and ferritin. Also, significant increase in TBARS in both cardiac and spleen homogenates tissues which accompanied with significant decrease in reduced GSH content in both cardic and spleen tissues as compared to control group. We analysed micronuclei in bone marrow cells and chromosome aberrations in spermatocyte cells, which represent an appropriate cytogenetic model to study compounds that enhance cell protection against externally induced DNA damage. Our results showed that L-carnitine has the ability to reduce the haematological, some biochemical changes and chromosomal damage induced by γ-rays. In conclusion, L-carnitine has a potential effect for scavenging ROS induced by radiation.
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Key words: L-carnitine, γ-irradiation, Haematopiotic, GSH, Testosterone, Genotoxicity, Oxidative stress, Mice

1. Introduction

L-carnitine is a vitamin like substance that is structurally similar to amino acids. Most carnitine is obtained from diet. It can also be synthesized endogenously by skeletal muscle, heart, liver, kidney and brain from the essential amino acids lysine and methionine **(Rebouche and Seim, 1998)**. It is known that L-carnitine and its derivatives prevent the formation of reactive oxygen species (ROS) and protect cells from per oxidative stress **(Sener *et al,* 2004**; **Dokmeci *et al,* 2006).** The dependence on carnitine uptake is evident from patients suffering from primary systemic carnitine deficiency (CDSP), an autosomal recessive disorder of fatty acid oxidation, caused by mutations in the *OCTN*2 gene encoding an organic cation/carnitine transporter **(Wang et *al,* 1999)**. It has been reported that carnitine protects the myocardium against ischemia (**Reznick *et al,* 1992**), myocardial infarction **(Singh *et al,* 1996)** and skeletal muscle myopathy in heart failure **(Vescovo *et al,* 2002)**.

The interaction of radiation with the component of living organs results in the generation of ROS which is responsible for many injurious in living cells, including gene mutations, cellular transformation and cell death. These effects have seen attributed to radiation induced DNA damage. However, the cytotoxicity may be delayed for up to six generations for cell replication **(Wright *et al,* 1998)**, and chromosomal aberrations due to destabilization of the genome (**Wang *et al,* 2000**). Ionizing radiation damage to cells is oxidant dependent and can be inhibited with exogenous antioxidant supplementations **(Reeves, 2003).**

So, this study was designed to detect the capability of L-carnitine to protect mammalian cells from oxidative stress induced haematological, biochemical and DNA damage as a consequence of gamma irradiation in male mice.

2. Material and Methods

2.1 Experimental animals

Male Swiss mice (28± 2 gm) obtained from the animal house of National Research Center, Dokki, Giza, Egypt. Animals were kept under standard conditions through the experimental period. The

mice were fed on pellet concentrated diet containing all the necessary nutritive elements. Liberal water intakes were available.

2.2 Radiation Facillity

Whole body gamma irradiation was performed using gamma cell Co-60 unit installed at the Middle Eastern Regional Radioisotopes center for the Arab Counteries (MERRCAC),Dokki,Giza,Egypt.Mice were exposed to fractionated gamma irradiation delivered as 2 Gy once per week up to cumulative dose of 6Gy through 3 successive weeks .

2.3 Chemical

L-carnitine was purchased from Arab Company For Pharmaceuticals and Medicinal Plants,Egypt. The product is provided as 350 mg capsules. A capsule content was dissolved in distilled water. All other chemicals used of analytical grade.

2.4 Experimental design

Animals were divided into 4 groups. Group 1:Mice were neither treated with L-carnitine nor irradiated and were considered as control. Group 2: Mice received daily subcutaneous injection of 200 mg /Kg body weight of L-carnitine for 21 consecutive days. Group 3: Mice were exposed to fractionated gamma irradiation 2 Gy once per week up to a cumulative dose of 6Gy. Group 4 : Mice were received a daily subcutaneous injection of L-carnitine (200 mg/Kg) for 7 consecutive days before the first dose of irradiation and continuous simultaneously through the experimental period. Mice from each group were slightly anaesthetized with ether and sacrificed at every experimental time intervals 1, 7 and 14 days after last irradiation dose.

2.4 Biochemical analysis

Heart and spleen were removed from six mice per each group to run biochemical analysis. Blood samples were obtained on EDTA for RBCs,WBCs,Hb and Hct.RBCs and WBCs determination using improved Neubauer chamber according to **Dacie and Lewis (1991**). Blood haemoglobin was determined calorimetrically as cyanmethaemoglobin in grams per deciliter using spectrum Diagnostic kit according to **Teitz (1990).** The ratio of erythrocytes to plasma in percent (Hct) was measured as the volume of erythrocytes per 100 ml blood after **Seivered (1964).** GSH content was determined according to **Beutler *et al* (1963**) in both cardiac and spleen homogenates. Lipid peroxide content was determined by quantifying the thiobarbituric acid reactive substances (TBARS) in cardiac and spleen tissue homogenates after the method described by **Yoshioka *et al* (1979).** Plasma levels of testosterone and ferritin were assayed by radioimmunoassay (RIA) techniques using commercial kits depending on solid phase RIA (Coat-A-Count) Diagnostic product corporation (DPC), Los Angles,USA.

2.5 Slide preparation and scoring for cytogenetic analysis.

2.5.1 Micronucleus test.

The micronucleus assay from mouse bone - marrow cells was performed following the standard procedure described by **Schmid (1973**). The significance of the experimental groups from control data was calculated using differences between 2 proportions **(Daniel, 1974),** in the case of PE's and the tables of **Kastenbaum and Bowman (1970)** for PE's with micronuclei.Five animals per group were examined for assessment of micronucleus test.

2.5.2 Chromosomal aberrations.

Chromosomal preparations from testes were made according to the technique developed by **Evans *et al* (1964)** and 100 well-spread diakinesis metaphase-I cells were analyzed per animal to assess abnormalities in five mice per group. Metaphases with translocations were recorded.

2.6 Statistical analysis

The significance of the difference between different treated groups versus control and between L-carnitin plus radiation against radiation group alone was calculated using the *t*-test according to **Snedecor and Cochran (1982).** The differences were considered significant at $P<0.05$.

3. Results

Table (1) showed that mean circulating RBCs, WBCs, Hb and Hct % exhibited significant decrease with irradiation compared to control group at ($P<0.05$) through the experimental period. Also, significant decrease in plasma testosterone and ferritin levels in irradiated group as compared to control was recorded. The treatment with L-carnitine revealed significant improvement in all the tested parameters.

Table (2) showed that fractionated gamma irradiation of 6Gy significantly depressed GSH content in both cardiac and spleen homogenates tissues. L-carnitine together with irradiation ameliorated that value through time intervals. Significant increase in TBARS levels($P<0.05$) above the control level is detected post gamma irradiation , treatment with L-carnitine alone and L-carnitine together with irradiation ameliorated that value through time intervals.

Table (1): Effect of l-carnitine on RBCs, WBCs, Hb and Hct% in mice exposed to fractionated dose of 6 Gy.

Parametes	Time post irradiation (days)	control	L-carnitine	Irrad.	L-carn. +irrad.
RBCs 10^6/cc ^{3}	1	6.49±0..32	6.82±0.24	4.09±0.25^{ab}	4.67±0.27 ^{ab}
	7	6.16±0.24	6.79±0.23	4.03±0.06^{ab}	4.95±0.06^{abc}
	14	6.44±0.30	6.96±0.09	4.92±0.24^{ab}	5.92±0.31^{bc}
WBCs 10^3/cc ^{3}	1	11.22±0.52	11.25±0.53	5.48±0.76^{ab}	9.07±0.49 ^{abc}
	7	11.88±0.87	12.02±0.40	5.13±0.18^{ab}	8.83±0.44 ^{abc}
	14	11.12±0.41	11.88±0.56	7.39±0.64^{ab}	10.65±0.44^{c}
Hb g/dl	1	14.11±0.31	14.19±0.16	12.83±0.28^{ab}	13.43±0.28^{b}
	7	14.21±0.19	14.55±0.27	11.81±0.17^{ab}	13.63±0.36^{c}
	14	14.16±0.16	14.15±0.19	11.56±0.29 ^{ab}	13.81±0.30^{c}
Hct %	1	52.67±0.85	54.50±0.52	41.00±0.48 ^{ab}	43.00±0.48 ^{abc}
	7	54.83±0.77	55.83±0.71	43.86±0.58^{ab}	49.25±0.67^{abc}
	14	55.75±0.67	56.08±0.62	50.83±0.74^{ab}	55.33±0.61^{c}
Testosterone ng/ml	1	1.62±0.11	1.50±0.05	0.66±0.14^{ab}	1.27±0.20^{c}
	7	1.58±0.12	1.47±0.17	0.98±0.06^{ab}	1.45±0.04^{c}
	14	1.88±0.15	1.85±0.08	1.27±0.12^{ab}	1.75±0.07^{c}
Ferritin ng/ml	1	0.58±0.01	0.57±0.03	0.40±0.02^{ab}	0.45±0.01 $^{ab\ c}$
	7	0.59±0.01	0.58±0.02	0.45±0.01 ^{ab}	0.49±0.001 $^{ab\ c}$
	14	0.57±0.01	0.59±0.03	0.41±0.02 ^{ab}	0.53±0.05^{c}

Number of animals/group 6±S.E. and are considered significant at P<0.05 a:significant different from control group.
b:significant different from l-carnitine group. c:significant different from irradiated group

Significant increase in TBARS levels (P<0.05) above the control level is detected post gamma irradiation, treatment with L-carnitine alone and L-carnitine with irradiation decreased the level of TBARS to near the control level.

Table (2): Effect of L-carnitine on reduced glutathione content (GSH) and lipid peroxidation(TBARS)in cardiac and spleen tissue in male mice exposed to fractionated dose of 6 Gy

Parameters	Time post irradiation (days)	Control	L-Carnitine	Irradiated	L-Carnitine + Irradiation
Cardiac GSH µg/ g wet tissue	1	13.38±0.31	13.22± 0.37	11.44± 0.17^{ab}	13.13± 0.34^{c}
	7	14.71±0.79	15.07± 0.74	12.29± 0.07^{ab}	13.41± 0.44^{c}
	14	13.26±0.32	13.41± 0.59	9.61± 0.36^{ab}	12.32± 0.43^{c}
Cardiac TBARS µmol/ g wet tissue	1	15.87±0.61	15.21± 0.55	26.92± 0.48^{ab}	20.18± 0.61^{abc}
	7	18.71±0.49	18.77± 0.66	24.43± 0.63^{ab}	21.79± 0.39^{abc}
	14	18.41±0.37	18.25± 0.57	27.91± 0.61^{ab}	19.64± 0.76^{c}
Spleen GSH µg/ g wet tissue	1	23.16±0.82	23.23±0.88	15.03± 0.76^{ab}	17.59± 0.79^{abc}
	7	23.08±0.87	25.05±0.66	16.56±0.48^{ab}	18.34± 0.57^{abc}
	14	23.25±0.77	24.75±0.35	16.51± 0.77^{ab}	21.39± 0.68^{bc}
Spleen TBARS µmol/ g wet tissue	1	20.29±0.42	20.01±0.36	43.63± 0.59^{ab}	38.46± 0.46^{abc}
	7	22.66±0.31	22.39±0.32	35.65± 0.55^{ab}	30.46± 0.48^{abc}
	14	21.02±0.57	21.010.27	30.59± 0.62^{b}	23.02± 0.84^{bc}

Legends as in Table 1

From the results presented in Table (3) we found that the percentage of micronuclei in polychromatic erythrocytes (MPCE) statistically significant after all treatment compared to control. The highest level reached after 1 day then declined at 7 and 14 days post irradiation. The protective effect of L-carnitine administered as successive dose for 3 weeks with γ-rays exposure reduced MPCE after 1, 7 and 14 days compared to irradiated group alone.

Fractionated dose of 6 Gy induced a significant elevation in the percentage of chromosomal translocations compared to the control. The maximum percentage observed after 1 day post-irradiation in mouse spermatocytes. It reached of 10.4 ± 0.52 then the percentage of observations decreased with longer duration after exposure. This maximum percentage decreased to 9.0±0.54 in the treated group received L-carnitine with irradiation (Table 4).

Table (3): Percentage of MPCE in mouse bone – marrow cells exposure to γ-rays and L-carnitine with γ-rays for 3 weeks.

Treatment	Time post irradiation (days)	No of PE with micronuclei	No. of PCE's	% MN (PCE's ± S.E.)
Control	—	14	2630	0.53 ± 0.35
L-carnitine (200 mg kg^{-1} b.wt.)	1	12	2421	0.49 ± 0.41
	7	12	2401	0.50 ± 0.53
	14	10	2385	0.42 ± 0.50
γ-rays 2 Gy once/week/3weeks	1	412	3011	13.68 ± 0.67 ^{ab}
	7	328	2978	11.01± 0.81 ^{ab}
	14	301	3112	9.67 ± 0.73 ^{ab}
L-carnitine + γ-rays	1	327	2851	11.46 ± 0.55 ^{abc}
	7	307	3272	9.38 ± 0.75 ^{ab}
	14	218	2601	8.38 ± 0.70 ^{ab}

Legends as in table 1

Table 4: Detailed results of translocations induced in mouse spermatocytes exposure to γ-rays and L-carnitine with γ-rays for 3 weeks.

Treatment	Time post irradiation (days)	No of abnormal cells	Chromosome aberrations				Translocations %± **S.E.**
			Chain IV	Ring IV	Chain VI	Ring VI	
Control	—	—	—	—	—	—	—
L-carnitine (200 mg kg^{-1} b.wt.)	1	—	—	—	—	—	—
	7	—	—	—	—	—	—
	14	—	—	—	—	—	—
γ-rays 2Gy once/week/3weeks	1	52	23	15	8	6	10.40±0.52 [ab]
	7	48	20	14	9	5	9.60±0.63 [ab]
	14	40	18	14	6	2	8.0±0.57 [ab]
L-carnitine + γ-rays	1	45	23	13	6	3	9.0±0.54 [ab]
	7	40	23	10	4	3	8.0±0.48 [ab]
	14	30	17	8	4	1	6.0±0.64 [ab]

No. of examined metaphases = 500

Legends as in table 1

4. Discussion

Radiation can have tremendous therapeutic benefits for humans. It is also associated with the risk of serious adverse effects **(Borek, 2004; Jagetia *et al,* 2006).** It produces reactive oxygen species (ROS) that damage proteins, lipids and nucleic acid **(Nair *et al,* 2001).** Haematopoietic system mainly bone marrow is known to be one of the most radiosensitive and its damage may be critical for the survival due to haematopoietic syndrome **(Tukov *et al,* 2002).**

The present study demonstrated that fractionated whole body gamma irradiation dose of 6Gy significantly affected red blood cells number ,haemoglobin content and haematocrit, which is a sign of anemia. This may be attributed to lysis of circulating RBCs due to a decreased production of erythropoietin **(Alfrey *et al,* 1997)** and haemorrhages caused by structural changes in membrane proteins ,modification in internal peptides as well as internal viscosity of RBCs (**Gwozdinski 1991**).Moreover,radiation induced haemodilution shown by decreased haematocrite value. The circulating WBCs were affected by fractionated whole body gamma irradiation dose of 6 Gy. It is known that lymphocytes are replication components that normally survive in the blood for 2-4 days and are highly radiosensitive **(Sado *et al,* 1998)**. Radiation induced depletion in lymphocytes is primarily due to apoptosis, although necrotic death occurs **(Kajioka *et al,* 2000).**

The obtained results observed that whole body gamma irradiation caused dramatic decrease in plasma testosterone levels. This finding is in accordance with **Jègou *et al* (1991) and Dygalo *et al* (1997)**. They reported that radiation has particularly sever adverse effect on gonads and therefore on fertility in both animals and man. Also, irradiated animals showed significant decrease in plasma ferritin level. Oxidative stress induced by irradiation causes damage in the liver of rats resulting in ferritin degradation ,increase in the intracellular free iron levels **(Atkinson *et al,* 2005**).However, the increase in iron level may be resulted from releasing iron from haemoglobin in irradiated animals and releasing iron from its macromolecular complexes ferritin and transferring **(Comporti *et al,* 2002).**

Exposure of animals to fractionated dose of 6 Gy affected seriously the biological cell membrane. **Gatsko *et al* (1990**), attributed the intensity of lipid peroxidation to the inhibition occurring in the antioxidant system caused by irradiation. Also, **Zheng *et al* (1996)** demonstrated that the increase in lipid peroxidation was related to the decrease in the biooxidase activities after irradiation. The anion radicals formed by ionizing radiation react with polyunsaturated fatty acids in biological membrane forming lipid peroxides which result in severe

damage to cellular membrane, organelles and their associated enzymes **(Poli *et al,* 1985)**. The observed decrease in GSH content after irradiation may be due to the diminished activity of glutathione reductase and to the deficiency of NADPH formed by glucose -6-phosphate –dehydrogenase which is necessary to change the oxidized glutathione to its reduced form **(Pulpanova *et al,* 1982).**

In the present study animals injected with L-carnitine before irradiation had significant improvement in RBCs, WBCs, Hb and Hct as compared to irradiated group. This may be due to that L-carnitine had beneficial effects on stabilized cellular membranes prolongs their lives and raises red blood cell osmotic resistance **(Nikolaos *et al,* 2000 ; Matsumoto *et al,* 2001).** Also, L-carnitine induced elevation in GSH content in both cardiac and spleen homogenates which is accompanied with significant decrease in TBARS in the same tissue homogenates. However, GSH has a major role in the antioxidant defense mechanisms against irradiation injury **(Harun *et al,* 2006; Ibrahim *et al,* 2007).** Carnitine improves the turnover of fatty acids peroxidated by the free oxygen radicals produced during normal metabolism **(Rebouch and Seim, 1998)**. **Ronca *et al* (1992)** showed that L-carnitine suppressed hydroxyl radical production in the fenton reaction, probably by chelating the iron required for the generation of hydroxyl radicals. Furthermore, the preventive effect of L-carnitine on the formation of ROS due to the xanthine /Xo system has been demonstrated **by Di Giacomo *et al* (1993).**

In the absence of direct data on the genetic radio sensitivity of human germ cells, the assumption is made that, unless there is evidence to the contrary, the response of human germ cells is similar to those of the mouse **(Paul and Van Buul ,1973).** In recent years it has become clear that the biology of spermatogenesis plays a major role in determining yields of genetic damage from mammalian spermatogonial stem cells following γ-rays. This has been best demonstrated in the mouse using reciprocal translocations as the genetic end-point **(Cattanach and Crocker, 1980)**. The micronucleus test is found to be a sensitive marker for clastogenic and mutagenic effects to detect chromosomal damage caused by different types of mutagens **(Mavouronic *et al,* 1990).**

Our results showed that the frequency of micronuclei in polychromatic erythrocyte (MPCE) and chromosome translocation in spermatocytes increased significantly after 1 day post exposure then declined up to 14 days. The same results observed by **Cole *et al,* (1981)** and Jagetia **and Ganapathi (1994)** they found that the frequency of MPCE increased significantly at 12h post –exposure, reached the highest percentage after 24h, thereafter it declined. **Zahran *et al (*2004)** reported that the percentage of MPCE from rat bone marrow cells reduced from 1 to 10 days after exposure to 6 Gy gamma rays.

Winegar *et al* (1994) found that γ-rays at doses ranging from 0.1 to 14 Gy induced point mutations, deletions and micronuclei in lacI transgenic mice. The induction of MPCE of bone-marrow of Swiss mice exposure to γ-rays was dose dependent **(Frash *et al,* 1999)**. The induction of translocations in the spermatocytes of the mouse by radiation has been extensively studied because of its relevance to the induction of hereditary defects in man **(Leenhouts and Chadwick, 1981).**

The decreased in the frequency of MPCE and chromosome translocation with increasing the time after irradiation may be attributed to decline the stem cell killing levels gradually and for rapid stem cell repopulation **(Leenhouts and Chadwick, 1981).** Cytological evidence for rapid stem cell repopulation has indeed been found several days after radiation exposure in both rat and mice **(Huckins and Oakberg, 1978**; **Zahran *et al,* 2004).**

Our study observed that injection of L-carnitine with irradiation reduced DNA damage in mouse somatic and germ cells. The modulation of DNA repair by L-carnitine, as an alternative mechanism to counteract radiation-mediated clastogenicity, is supported by many studies suggest that L-carnitine could help the cells to repair single-strand breaks (SSBs) induced in DNA **(Boerrigter *et al,* 1993)** and to protect it from oxygen free radicals **(Vanella *et al,* 2000).** Other studies indicated that L-carnitine-dependent reduction of DNA single-strand breaks after *in vitro* treatment with an oxygen radical-generating system, in isolated human lymphocytes **(Garcia *et al,* 2006). Santoro *et al* (2005)** reported that L-carnitine have the ability to inhibition chromosomal damage induced by H2O2 as an oxidative stress in CHO cells. Pre-treatment of mice with L-carnitine 1h before exposure to magnetic field caused a significant recovery of mice testes damage induced by high magnetic field (**Ramadan *et al,* 2002). Berni *et al* (2008)** demonstrated that L-carnitine pre-treatment on oxidative DNA damage (tert-butyl-hydroperoxide) produced an enhancement of the rate and extent of DNA repair in *Ataxia telangiectasia* (A-T) patient's cell lines at early recovery time. Furthermore, a reduction of all types of chromosomal aberrations was observed, both in A-T and in wild-type cell lines.

It could be concluded that, the data obtained *in vivo* represent a possible strategy to reduce oxidative stress and protect mammalian cells from the damage caused by reactive oxygen species using a natural compound like L-carnitine.

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The Protective Role of Folic Acid, Vitamin B12 and Vitamin C on the Mutagenicity of the Anticancer Drug Daunorubicin

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Abstract: Nowadays genotoxicity of anticancer drugs to normal cells is one of the most serious problems of chemotherapy due to the possibility of inducing secondary malignancies. Daunorubicin (DNR) is an anthracycline antibiotic. It has been used for a variety of malignancies. Thus the objective of this investigation is to measure the potential cytotoxicity of DNR alone and in combination with vitamins (FA, VB12 and VC) on the cancer cell line McF7. Also this study aims to assessment the mutagenic effect of DNR in mice normal cells by using different doses as well as the protective role of the previous vitamins in a trial to minimize the genotoxicity of this chemotherapy. The genotoxic potential of DNR was evaluated *in vivo* using different mutagenic end points. 1- Cytogenetic analysis (chromosomal aberrations in somatic and germ cells, SCEs in bone-marrow and sperm shape abnormalities). 2- DNA fragmentation assay in mouse spleen cells. 3- The protective role of folic acid (FA), vitamin B12 (VB12) and vitamin C (VC) on genotoxic effect of DNR. For chromosome analysis, mice were treated i.p. with 1, 3 and 5mg DNR/ kg b. wt. Samples were taken 24h, 7 and 14 days after treatments. DNR induced a statistically significant increase in the percentage of chromosomal aberrations in both somatic and germ cells with dose and time relationship, which declined with time of recovery for 14 days. The mean frequency of SCEs/ cell was three folds higher than the control indicating that DNR is a strong inducer of SCEs. DNR also induced highly significant percentage of abnormal sperms. The results indicated that DNR increased the percentage of DNA fragmentation in mouse spleen cells as measured colorimetrically with diphenylamine assay and confirmed by agarose gel electrophoresis. **The protective role of vitamins**, FA (10mg/kg b.wt.), VB12 (0.3mg/kg b.wt.) and VC (50mg/kg b.wt.) was demonstrated after oral administration of these vitamins concurrently with 3mgDNR/kg b.wt. Significant inhibitions in the percentages of chromosomal aberrations induced in somatic and germ cells were demonstrated. Also, vitamins reduced the percentages of DNA fragmentation in mouse spleen cells when administered simultaneously with 5mg DNR/kg b.wt. **In conclusion,** the results demonstrate the genotoxic effect of DNR on McF7 cell line even with vitamins indicating that vitamins do not reduce the bioactivity of DNR on cancer cell lines. Also, the study illustrates the beneficial role of vitamins against the mutagenicity of the anticancer drug on normal cells *in* vivo.
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Key words: DNR, FA, VB12, VC, McF7, cytogenetic parameters, DNA damage

1. Introduction

Daunorubicin (DNR) is an anthracycline antibiotic isolated from streptomycin products **(Blasiak et al., 2002a),** in particular the rhodomycin products. It has been used for more than 30 years for a variety of malignancies such as,. acute lymphocytic and acute myelogenous leukemias, Hodgkin's and non-Hodgkin's lymphomas, multiple myeloma, carcinomas of breast, lung, ovary, stomach, thyroid and various childhood malignancies (**Doroshow, 1986; Wiernik and Dutcher, 1992; Hortobagyi, 1997)**.

One of the main actions of the antineoplastic DNR on DNA occurs through the induction of free radicals, which in turn may produce several types of genotoxic damages is the increase in the rate of sister chromatid exchanges (**Noviello et al., 1994; Szabova, 1996).** Nowadays genotoxicity of the anticancer drugs to normal cells is one of the most serious problems of chemotherapy due to the possibility of inducing secondary malignancies. Beside, there is no doubt that DNA damage plays an important role in most mechanisms underlying the action of anticancer drugs interacting with DNA (**Gentile et al., 1998**). Recently, it is very important to search for protective substances against mutagenic-carcinogenic agents. Some vitamins may scavenge harmful species, free radicals or electrophiles, which damage DNA and other cell targets (**Ames and Gold, 1991; Odin, 1997)**. Such agents not only have the potential to diminish physiological side effects of the antitumor treatments, but also, provide the potential to limit genotoxic side effects. Our study aims to focus on natural vitamins already found

in food to see their ability to reduce the genotoxicity may be induced by DNR. These vitamins such as folic acid (FA), vitamin B12 (VB12) and vitamin C (VC) are necessary constituents of man's diet.

Folic acid (petroylglutamic acid) is converted to the active form tetrahydrofolic acid which is important in DNA biosynthesis and therefore in cell division (**Laurence et al., 1997).** Vitamin B12 (cyanocobalamine) is closely related metabolically to folic acid and taken part in methyl group transfer from N5- methyltetrahydro-FA to homocysteine (**Brody et al., 1984)**. The antigenotoxic properties of FA and/or VB12 were demonstrated in number of genetic tests (**Renner, 1990; Fenech et al., 1997)**. Vitamin C (ascorbic acid), the enolic form of 3-keto-L-glucofuranolactone takes an active part in tissue metabolism and is connected with numerous electron-transport processes, where it behaves as a strong reducing agent (**Odin, 1997).** VC decreases the genotoxicity and mutagenicity of several agents (**Sharma et al., 2000; Ghaskadbi et al., 1992; Blasiak et al., 2002b)**.

The objective of these investigations is to measurement of potential cytotoxicity of DNR and vitamins (FA, VB12 and VC) on the cell line McF7 and to study the mutagenic effect of DNR in mice at different doses by using different mutagenic end points as well as the protective role of the previous vitamins in a trial to minimize the genotoxicity of DNR.

2. Materials and Methods

2.1. Test substances:

Daunorubicin (C27H29NO10 HCL) (Fig.1) is purchased from Pharmacia and Upjiohn, Italy. The human therapeutic dose 20mg/kg was used in our study. This dose was converted to the mice dose according to **Paget and Barnes (1964)**. The vitamins were purchased as follows: Folic acid (FA) from Nile Co. for Pharm. and Chem Ind. Cairo, Egypt, Vitamin B12 (B12) from Amriya Pharm. Ind., Alexandria, Egypt and Vitamin C (VC) from S.D. Fine-Chem. Ltd., Mumbai, India.

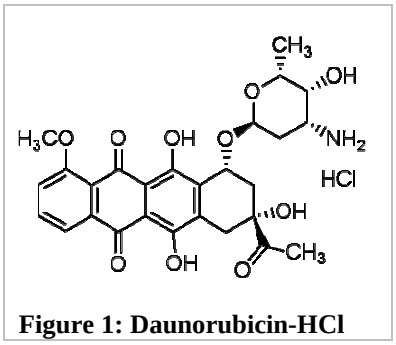

Figure 1: Daunorubicin-HCl

2.2. Measurement of potential cytotoxicity:

2.2.1. Doses of DNR and vitamins:

Tested concentrations of DNR were 10, 20, 40ug/ml. the doses of vitamins were FA (5, 10, 20ug/ml), VB12 (0.15, 0.30, 0.60ug/ml) and VC (25, 50, 100ug/ml. the study was carried out on the breast cancer cell line McF7.

2.2.2. Measurement of cytotoxicity:

Potential cytotoxicity of DNR alone and DNR with each of the three vitamins separately by Sulfo-Rhodamine-B stain (SRB) assay was carried out using the method of **Skehan and Storeng (1990).**

2.3. Cytogenetic studies:

2.3.1. Animals:

Laboratory-bred strain Swiss albino male mice of 8-10 weeks old with an average weight of 27.5+2.5g obtained from the National Research Center, Cairo, Egypt, were used .Animals were housed in groups (5animals/ group) and maintained under standard food and water *ad libitium.*

2.3.2. Doses and Treatments:

DNR doses were 1, 3, 5 mg/ kg b.wt. as single doses. Vitamins were 10mg FA/ kg b.wt. 0.3mg VB12/kg b.wt. and 50mg VC/ kg b.wt. The anticancer drug DNR and vitamins were dissolved in distilled water. Control groups of animals received distilled water and others received vitamins alone were collected continuously with the treated groups.

2.3.2.1. Chromosomal Aberrations in Somatic and Germ Cells:

Samples were harvested after 24h, 7 and 14 days of treatment with the different doses of DNR. The groups of animals received the concurrent administration of 3mg DNR/ kg b.wt. with each of the three vitamins separately were sacrificed after 24h.

2.3.2.2. Sister Chromatid Exchange (SCEs):

Samples were harvested 24h after treatment with the different doses of DNR.

2.3.2.3-Sperm-Shaped Abnormalities:

Mice were treated once i.p. with each of the three doses of DNR. Samples were collected after 35 days from the treatments.

2.3.3. Cytogenetic Paramters:

-For **chromosomal aberrations** in somatic and germ cells, bone-marrow metaphases were prepared according to **Yosida and Amano (1965)**. The diakinase-metaphase I cells collected from the spermatocytes were made following the air-drying technique of **Evans et al. (1964)**. Slides were stained with 7% Giemsa stain in phosphate buffer (pH6.8). 100 well spread metaphases per animal were analyzed for chromosome aberrations. The types of aberrations in bone-marrow cells included breaks, deletions, fragments, centric fusions, centromeric attenuations…etc. The types of aberrations in spermatocytes were XY univalents, autosomal univalents, fragments, breaks and chain IV.

-For **sister-chromatid exchanges,** the method described by **Allen (1982)** was used with some modifications. Bone-marrow cells were fixed and stained with fluorescence plus Giemsa method of **Perry and Wolff (1974)**. The frequency

of SCE's was recorded for each animal in at least 30 metaphases with second division.

-For **sperm-shape abnormalities,** the epididymides excised and minced in isotonic sodium citrate solution (2.2%). Smears were prepared and stained sperms with Eosin Y **(Wyrobek and Bruce, 1978).** At least 1000 sperm per animal (5000/group) were assessed for morphological abnormalities of the sperm shape.

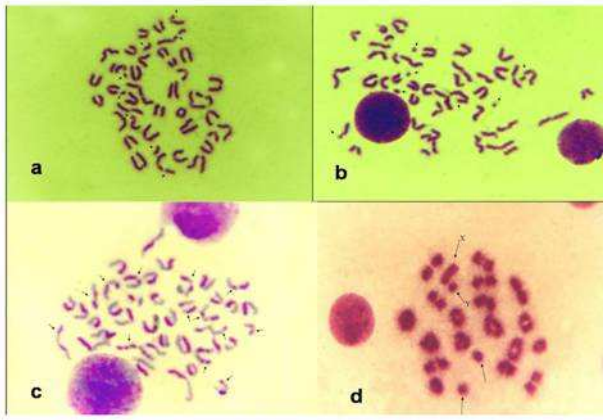

Figure (2): Metaphases with chromosomal aberrations (a&b) breaks and fragments, © sister chromatid exchanges in bone-marrow cells and (d) diaknesis-metaphase I cell with XY and autosomal univalents in mouse spermatocyte after i.p. treatment with DNR.

2.4. DNA Fragmentation Assay:

The groups of animals treated with different doses of DNR were collected 24h after treatments. The other groups of animals received concurrently 5mg DNR/kg b.wt. with each of the vitamins doses were sacrificed 24h after treatment The method of DNA fragmentation assay was carried out according to **Perandones et al. (1993)**. Mouse spleen was mechanically dissociated in hypotonic lysis buffer. The cell lysate was centrifuged at 13.000xg for 15 min. then the supernatant containing small DNA fragments was separated immediately, half the supernatant was used for gel-electrophoresis. The other half, as well as the pellet containing large pieces of DNA were used for the colorimetric determination Diphenylamine (DPA) assay.

2.5. Statistical Analysis:

The significance of the experimental from the control data was calculated using chi square statistic table (X^2-test) for chromosomal aberrations in somatic and germ cells and t-test for cytotoxicity, SCE's, sperm-shape abnormalities and DNA fragmentation assays.

3. Results

3.1. Cytotoxic effect:

The cytotoxicity of the anticancer drug DNR increased with increasing its concentrations. FA, VB12 and VC are not reducing the effect of DNR on the breast cell line (McF7). Table (1) demonstrated a highly significant percentage of bioactivity compared to control ($p<0.001$). FA, VB12 and VC statistically demonstrated non-significant percentage compared to DNR concentration treatments.

3.2. Genotoxic effect of DNR:

3.2.1. Effect of DNR on somatic cells:

3.2.1.1. Chromosomal aberrations:

A significant increase in the percentage of chromosomal aberrations was observed in mouse bone-marrow 24h, 7 and 14 days after treatment with 1, 3, and 5 mg/kg b. wt. (Table 2). The percentage of induced aberrations decreased with increasing the time.of recovery and was found to be statistically highly significant (Fig. 2a and b).

3.2.1.2. Sister chromatid exchanges:

DNR induced a highly significant and a dose dependent increase in SCE's frequencies in mouse bone-marrow cells (Table 3) and (Fig. 2c).

Table (1): Mean percentages of cell viability induced in MCF7 cell lines 48h. after treatment with DNR, FA, VB12 and VC alone or in combination.

Doses and Treatments	O.D. Mean±SE	% Cell Viability[a]
I. Control[b]	2.104±0.065	100.00
FA20 µg/ml	2.066±0.073	98.19
VB12 0.6 µg/ml	1.974±0.043	93.82
VC100 µg/ml	2.008±0.074	95.44
II. DNR 10µg/ml[c]	1.316±0.029***	62.55
DNR+5µg FA	1.377±0.014	65.45
DNR+0.15µg VB12	1.286±0.027	61.12
DNR+25µg VC	1.339±0.032	63.64
III. DNR 20µg/ml[c]	1.136±0.022***	53.99
DNR+10µg FA	1.242±0.096	57.60
DNR+0.3µg VB12	1.219±0.060	57.94
DNR+50µg VC	1.279±0.041	60.79
IV. DNR 40µg/ml[c]	1.056±0.018***	50.19
DNR+20µg FA	0.893±0.061	42.44
DNR+0.6µg VB12	0.898±0.052	42.68
DNR+100µg VC	0.978±0.031	46.48

([a]) %Cell Viability: All treatments compared to control.
[b] No significance between the O.D. of different vitamins compared to control.
[c] No significance between the O.D. of different doses of DNR plus vitamins compared to related DNR dose.
*****p< 0.001 significant of DNR compared to control. (t-test)**

Table 2: Number and mean percentage of different types of chromosomal aberrations induced in mouse bone-marrow cells 24h., 7 and 14 days after treatment with different doses of DNR.

Dose	Duration of treatments	% of cells with different types of structural aberrations												% of cells with different types of numerical aberrations				Total Chromosomal Aberrations			
		Gap		Br. or F.		Del.		C. F.		C. A.		G. + Br. and/or F.		Br. + F.		41 Ch.		Tetrap.		Including Gaps		Excluding Gaps	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
I. Control	24h.	10	2.00	5	1.00	0	0.00	1	0.20	0	0.00	0	0.00	0	0.00	0	0.00	1	0.20	17	3.40	7	1.40
	7 Days	10	2.00	4	0.80	0	0.00	1	0.20	1	0.20	0	0.00	0	0.00	0	0.00	1	0.20	17	3.40	7	1.40
	14 Days	7	1.40	4	0.80	0	0.00	0	0.00	1	0.20	1	0.20	1	0.20	0	0.00	2	0.40	16	3.20	9	1.80
II. DNR Dose																				***		***	
1mg/kg	24h.	10	2.00	23	4.60	1	0.20	7	1.40	4	0.80	2	0.40	3	0.60	0	0.00	4	0.80	54	10.8	44	8.8
																				***		***	
	7 Days	10	2.00	18	3.60	0	0.00	0	0.00	4	0.80	1	0.20	4	0.80	0	0.00	6	1.20	43	8.60	33	6.60
																				***		***	
	14 Days	8	1.60	11	2.20	0	0.00	6	1.20	3	0.60	1	0.20	3	0.60	1	0.20	9	1.80	42	8.40	34	6.80
																				***		***	
3mg/kg	24h.	45	9.00	43	8.60	2	0.40	8	1.60	1	0.20	7	1.40	8	1.60	2	0.40	7	1.40	123	24.6	78	15.6
																				***		***	
	7 Days	42	8.40	23	4.60	2	0.40	5	1.00	3	0.60	5	1.00	10	2.00	1	0.20	9	1.80	100	20.0	58	11.6
																				***		***	
	14 Days	38	7.60	26	5.20	0	0.00	2	0.40	0	0.00	2	0.40	10	2.00	2	0.40	12	2.40	92	18.4	54	10.8
																				***		***	
5mg/kg	24h.	54	10.8	66	13.2	0	0.00	2	0.40	4	0.80	14	2.80	30	6.00	1	0.20	9	1.80	180	36.0	126	25.2
																				***		***	
	7 Days	50	10.0	41	8.20	0	0.00	4	0.80	0	0.00	9	1.80	18	3.60	0	0.00	11	2.20	133	26.6	83	16.6
																				***		***	
	14 Days	46	9.20	33	6.60	1	0.20	4	0.80	5	1.00	11	2.20	3	0.60	0	0.00	16	3.20	119	23.8	73	14.6

The total number of scored cells is 500 (5 animals/ group), G.: Gaps, Br.: Breaks, F.: Fragments, Del.: Deletions, C. F.: Centric Fusions, C. A.: Centromeric AttenuationsTetrap.:Tetraploidy,*p<0.001.(X^2-test)**

3.2.2. Effect of DNR on germ cells:

3.2.2.1. Chromosomal aberrations:

Table (4) illustrates the number and percentage of diakinase metaphase I cells with chromosome aberrations induced by DNR 24h, 7 and 14 days. This percentage was increased gradually parallel to dose increasing and decreased gradually with increasing the time of recovery (Fig. 2d).

3.2.2.2-Sperm- shape abnormalities:

The changes, which may be induced in sperms by the anticancer drug, were studied. The main percentage of abnormal sperms was highly significant ($p<0.001$) with all the doses. Table (5) represents the different types of observed abnormalities (Fig. 3).

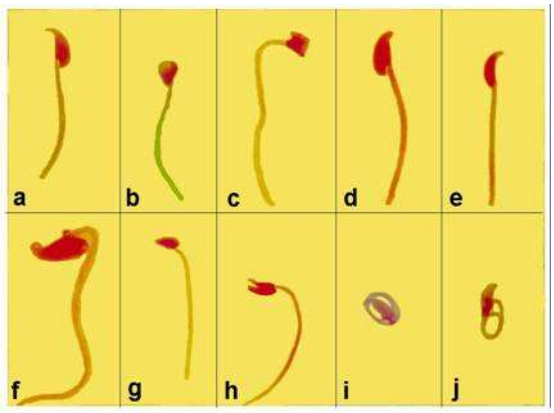

Figure (3):Types of sperm-shape abnormalities found in normal and DNR treated mice .(a) normal sperm with a definite head by a marked hook and tail , (b) amorphouse shape, (c) triangular, (d) without hook, (e) banana shape, (f) big head, (g) small head,(h) forked head, (i) coiled tail, (j) head without hook and coiled tail.

3.3. The protective effect of vitamins:

The protective effect of FA, VB12 and VC administered as a single dose on the induction of chromosome aberrations in somatic (bone-marrow cells) and germ cells (spermatocytes) is presented in tables (6 and 7) respectively. The results showed that FA, VB12 and VC exerted a significant reduction in the percentage of chromosome aberrations induced by 3mg DNR/ kg b. wt. in somatic and germ cells.

3.4. DNA fragmentation assay:

3.4.1. Effect of DNR:

3.4.1.1. DPA assay:

Table (8) demonstrates the mean percentage of DNA fragmentation induced by DNR in mouse spleen cells. The percentage of fragmentation was increased highly significantly ($p<0.001$) after treatment with the different doses. The maximum percentage of DNA fragmentation was 12.86% after treatment with 5mg DNR/kg b. wt.

3.4.1.2. Agarose gel-electrophoresis:

DNA fragmentation assessed by agarose gel-electrophoresis was increased in a dose dependent manner with increasing the dose of DNR (Fig. 4).

3.4.2. Protective effect of vitamins:

Table (8) illustrates the mean percentage of DNA fragmentation induced in mouse spleen cells after i.p. treatment with 5mg DNR /kg b. wt. and oral concurrent treatment with 10, 0.3 and 50mg /kg b. wt. FA, VB12 and VC respectively. The percentage of DNA fragmentation decreased to 5.67%, 5.13% and 8.76% after treatment with FA, VB12 and VC respectively compared with 12.86% for DNR alone. Figure (4) shows the DNA fragmentation assessed by agarose gel-electrophoresis, which was decreased after treatment with vitamins compared to that with 5mg DNR/kg b.wt.

Table3: Frequency of sister chromatid exchanges in mouse bone marrow cells induced by different doses of DNR.

Dose	No. of Scored Cells	Total No. of SCE's	Mean± SE
I. Control	150	583	3.89±0.058
II.DNRDose 1mg/kg	173	1477	8.54±0.28 ***
3mg/kg	171	2525	14.77±0.76 ***
5mg/kg	156	3214	20.60±0.73

*** p<0.001. (t-test)

4. Discussion

The majority of anticancer drugs are specifically designed to interfere with DNA synthesis, cellular metabolism, and for cell division. Due to this mode of action, these drugs are expected to cause different mutations and cytogenetic abnormalities **(Pedersen-Bjergaard et al., 1991).** Daunorubicin is one of these anticancer drugs which have the ability to intercalate with DNA, therefore blocking DNA, RNA and protein synthesis. Also, it can bind with DNA and inhibits DNA replication and DNA- dependent RNA synthesis **(Barton-Burke et al., 2001)**.

The present studies indicate that DNR has a significant ($p<0.001$) and a dose dependent genotoxic effects on mouse bone- marrow cells and spermatocytes. These results are in agreement with previously reported, induction of chromosome abnormalities by anthracyclines in patients received chemotherapy **(Pedersen-Bjergaard and Philip, 1987; Andersen et al., 1998).**

Table 4: Number and mean percentage of different types of diakinase metaphase I cells with chromosomal aberrations induced in mouse spermatocytes 24h., 7 and 14 days after treatment with different doses of DNR.

Dose	Duration of treatments	% of cells with different types of chromosomal aberrations										Total Chromosomal Aberrations	
		XY un.		Auto.un.		XY+ Auto.un.		Br. or F.		Chain (IV)			
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
I. Control	24h.	8	1.60	3	0.60	0	0.00	0	0.00	0	0.00	11	2.20
	7 Days	7	1.40	4	0.80	0	0.00	1	0.20	0	0.00	12	2.40
	14 Days	6	1.20	3	0.60	0	0.00	0	0.00	0	0.00	9	1.80
II. DNR Dose												***	
1mg/kg	24h.	22	4.40	11	2.20	2	0.40	3	0.60	0	0.00	38	7.60
												*	
	7 Days	12	2.40	11	2.20	2	0.40	2	0.40	0	0.00	27	5.40
												n.s.	
	14 Days	8	1.60	11	1.20	1	0.20	2	0.40	0	0.00	17	3.40
												***	
3mg/kg	24h.	33	6.60	17	3.40	5	1.00	4	0.80	0	0.00	59	11.8
												***	
	7 Days	19	3.80	15	3.00	2	0.40	4	0.80	0	0.00	40	8.00
												***	
	14 Days	13	2.60	14	2.80	1	0.20	4	0.80	1	0.20	33	6.60
5mg/kg												***	
	24h.	32	6.40	36	7.20	4	0.80	6	1.20	1	0.20	79	15.8
												***	
	7 Days	32	6.40	10	2.00	5	1.00	4	0.80	1	0.20	52	10.4
												***	
	14 Days	14	2.80	12	2.40	4	0.80	5	1.00	0	0.00	35	7.00

The total number of scored cells is 500 (5 animals/ group); XY un.: XY univalents, Auto.un.: Autosomal univalents, XY+ Auto.un.: XY univalents plus Autosomal univalents, Br.: Breaks, F.: Fragments, *p<0.05, **p<0.01, ***p<0.001, n.s.= not significant. (X^2-test).

In addition, many studies demonstrated the genotoxic effects of adriamycin (doxorubicin) on somatic and germ cells of mice **(William and Hsu, 1980)**, induction of chromosome damage in Ames assay **(Bhuyan et al., 1983)** and on mammalian germ cells **(Witt and Bishop, 1996). Othman (2000)** demonstrated that Epirubicin (anthracyclin analogue) induced genotoxic effect on cultured Chinese hamster cells. Our studies demonstrated that high frequencies of chromosome aberrations induced by different doses of DNR in somatic and germ cells declined with time of recovery up to 14 days. In respect to the structural aberrations, breaks and/or fragments were represented the dominant types of aberrations in bone-marrow cells. Separations of chromosomes forming univalents (XY and autosomal univalents) were the most common types of aberrations. XY univalents were much more frequency. It worthy to mention that DNR induced numerical aberrations (hyperdiploidy) which were dose dependent and increased with time recovery to reach its maximum value after 14 days. The positive correlation between tetraploid cells and long duration of treatment might lead to induction of secondary carcinoma **(Kubota et al., 1997; Silva et al., 2002)**.

The experimental data indicated that DNR was a strong inducer of SCE's. The mean frequencies were higher than three folds of the control. Other antitumor drugs induced SCE's in human cell culture such as adriamycin **(Lambert et al., 1983; Mareczewska et al., 2004).**

Sperm abnormalities are usually taken as a characteristic criterion and as an applied test for monitoring the mutagenic potential for many chemicals **(Brusick, 1980).** The results showed that DNR induced significant sperm abnormalities after treatment with different doses. Both head and tail abnormalities were recorded. The head abnormalities most probably reflect a change in DNA content **(Wyrobek and Bruce, 1978).** Coiling of sperm tail mainly involves its orientation, which gives an impression of limitation the sperm movement. Such tail deformities were reported to reduce fertility in human and animals **(Topham, 1983).**

Apoptosis is a form of programmed cell death shown to play a key role in normal development and oncogenesis. Its hallmark biochemical feature is endonuclease activation**,** giving rise to internucleosomal DNA fragmentation **(Arends et al., 1990; Perandones et al., 1993)**. DNR has the ability

Table 5: Number and mean percentage of different types of sperm shape abnormalities in mouse sperms induced by different doses of DNR.

Dose	No. of Scored sperms	No. of Abnormal sperms	Mean (%) ± SE	No. and % of Different types of abnormal sperms															
				Amor.		Triang.		W. Hook		Ban. Shape		Big Head		Small Head		Forked Head		Coiled Tail		Head and Tail	
				No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
I. Control	5099	99	1.94±0.23	52	1.02	17	0.33	12	0.24	2	0.04	2	0.04	2	0.04	0	0.00	11	0.22	1	0.02
II. DNR Dose																					
1mg/kg	5387	387	7.18±0.25***	223	4.14	73	1.36	17	0.32	8	0.15	7	0.13	3	0.06	3	0.06	51	0.95	2	0.04
3mg/kg	5586	586	10.49±0.33***	396	7.09	46	0.82	38	0.68	16	0.29	10	0.18	15	0.27	0	0.00	64	1.15	1	0.02
5mg/kg	5719	719	12.57±0.39***	250	4.37	71	1.24	42	0.73	10	0.17	45	0.79	7	0.12	1	0.02	283	4.95	10	0.17

Amor.: Amorphous, Triang.: Triangular, W. Hook : Without Hook, Ban. Shape: Banana Shape, ***p<0.001. (t-test)

Table 6: Number and mean percentage of chromosomal aberrations in mouse bone marrow cells induced by 3mg/kg b.wt. DNR plus folic acid, vitamin B12 or vitamin C.

Dose	No. of cells with different types of structural aberrations							No. of cells with numerical aberration		Total Chromosomal Aberrations				Inhibition % Of Aberrant Cells Excluding Gaps
	G.	Br. or F.	Del.	C.F.	C.A.	G+Br. and/or F.	Br.+ F.	41 Ch.	Tetrap.	Including Gaps		Excluding Gaps		
										No.	%	No.	%	
I. Control	10	5	0	1	0	0	0	0	1	17	3.40	7	1.40	-
FA	11	9	0	0	0	2	0	0	3	25	5.00	14	2.80	-
VB12	12	8	0	0	0	1	0	0	2	23	4.60	11	2.20	-
VC	10	8	0	2	0	0	0	0	4	24	4.80	14	2.80	-
II. Treatment with the anticancer drug alone														
DNR (3mg/kg)	45	43	2	8	1	7	8	2	7	***123	24.6	***78	15.6	-
III. Treatment with the anticancer drug and vitamins														
DNR+ FA	25	17	0	1	3	2	5	0	10	•••63	12.6	•••38	7.60	51.28
DNR+ VB12	31	25	0	2	0	1	3	0	5	•••67	13.4	•••36	7.20	53.85
DNR+ VC	28	25	0	3	1	3	7	0	7	•••74	14.8	••46	9.20	41.03

The total number of scored cells is 500 (5 animals/ group); ***p<0.001: Significance compared to Control; ••p<0.01, •••p<0.001: Significance compared to treatment with DNR; G.: Gaps, Br.: Breaks, F.: Fragments, Del.: Deletions, C. F.: Centric Fusions, C.A.: Centromeric Attenuations, 41 Ch.: 41Chromosomes, Tetrap.: Tetraploidy, FA: 10mg/kg b. wt.; VB12: 0.3mg/kgb.wt.VC:50mg/kgb.wt.

Table 7: Number and mean percentage of diakinase metaphase I cells with chromosomal aberrations in mouse spermatocytes induced by 3mg/kg b.wt. DNR plus folic acid, vitamin B12 or vitamin C.

Dose	Normal Cells	XY un.	Auto.un.	XY+ Auto.un.	Br. or F.	Chain (IV)	Total Aberr. No.	Total Aberr. %	Inhibition % of Aberrant Cells
I. Control	489	8	3	0	0	0	11	2.20	-
FA	487	7	6	0	0	0	13	2.60	-
VB12	488	8	1	0	3	0	12	2.40	-
VC	490	8	2	0	0	0	10	2.00	-
II. Treatment with the anticancer drug alone									
DNR (3mg/kg)	441	33	17	5	4	0	59***	11.8	-
III. Treatment with the anticancer drug and vitamins									
DNR+ FA	475	15	7	3	0	0	25•••	5.00	57.63
DNR+ VB12	477	9	11	1	2	0	23•••	4.60	61.02
DNR+ VC	470	16	11	1	2	0	30••	6.00	49.15

The total number of scored cells is 500 (5 animals/ group), ***p<0.001: Significance compared to Control; ••p<0.01, •••p<0.00: Significance compared to treatment with DNR; Br.: Breaks, F.: Fragments, FA: 10mg/kg b.wt. VB12:0.3mg/kgb.wt.; VC: 50mg/kgb.wt. (X^2-test)

to intercalate DNA causing DNA breakage, producing oxidative stress and triggering cell apoptosis **(Aligiannis et al., 2002)**.

The present study indicates the apoptotic changes induced by DNR in mouse spleen cells (*in vivo*) by measuring the percentage of DNA fragmentation calorimetrically with diphenylamine (DPA) assay and confirming the DNA fragmentations with agarose gel-electrophoresis. The observed increase in the DNA fragmentation might be due to the induction of DNA strand breaks by this compound. These in agreement with the established mechanism underlying anthracyclines cytotoxicity- topoisomerase II mediated DNA cleavage **(Binaschi et al., 1997).**

Breaks detected by chromosome aberrations in mouse somatic and germ cells and DNA fragmentation assay may be an indication of reactive oxygen species that arise during the metabolism of DNR. These suggestions agree with **(Blasiak et al., 2002a)** who reported that idarubicin (anthracycline analogue) possesses a methyl group in its structure which by unknown way can transfer onto DNA bases, thus methylation might contribute to the DNA damaging effect of the drug. They also hypothesized that oxidative DNA damage observed upon the action of idarubicin might be evoked by free radicals generated by the drug. The genotoxic effect of DNR may be considered as the origin of its anticancer activity but that exerted by the drug on normal cells should not surpass the effect on cancer cells.

Recent studies revealed that DNR induced DNA fragmentation in Hela cells (human leukemia cells)

Table 8: Mean percentage of DNA fragmentation induced in mouse spleen cells 24h. after treatment with different doses of DNR and concurrent treatment with DNR plus folic acid, vitamin B12 and vitamin C.

Dose	DNA Fragmentation Mean%±SE	DNA Fragmentation Inhibition %
I. Control	2.95±0.621	-
FA	2.56±0.869	-
VB12	3.17±0.787	-
VC	2.87±0.716	-
II. Treatment with the anticancer drug alone		
DNR 1mg/kg	10.75±0.778***	-
3mg/kg	12.35±0.776***	-
5mg/kg	12.86±0.746	-
III. Treatment with the anticancer drug and vitamins		
DNR (5mg)+FA	5.67±0.618•••	55.89
DNR(5mg)+VB12	5.13±0.525•••	60.09
DNR (5mg)+VC	8.76±0.665••	31.84

(5 animals/group), ***p<0.001: Significance compared to Control; ••p<0.01, •••p<0.001: Significance compared to treatment with DNR, FA: 10mg/kg b. wt.; VB12: 0.3mg/kgb.wt.; VC: 50mg/kg b.wt. (t- test)

and DNA fragmentation was dose and time dependent **(Chan and Chan, 1999)**. DNR induced apoptosis chromatin condensation, nuclear fragmentation and internucleosomal DNA degradation in A-431 cells (human epidermoid carcinoma cell line **(Chen et al., 2000)**. Also, **Masquelier et al., (2004)** studied the relationship between DNR concentration and apoptosis induction in leukemia cells.

Vitamins play a beneficial role against the mutagenicity of some chemicals **(Odin, 1997).** In a trial to minimize the genotoxicity of DNR in somatic and germ cells of mice, vitamins (FA, VB12 and VC) were administered separately simultaneously with the anticancer drug DNR. The mechanism of FA action is connected with thymidylate synthetase activity and through it with DNA synthesis **(Glover, 1982)** and with modifying cellular nucleotide pools **(Kunz, 1988)**. Also, FA is involved in both methyl metabolism and in DNA repair. VB12 is required for the synthesis of methionine and S-adenosyl thionine, the common methyl donor require for the maintenance of methylation patterns in DNA that determine gene expression and DNA confirmation **(Zingg and Jones, 1997). Laurence et al. (1997)** demonstrated that VC is a powerful reducing agent (antioxidant) and play a part in intracellular oxidation/reduction system, and scavenging of free radicals produced endogenously. VC can promote the removal of oxidative DNA damage from the DNA and/or nucleotide pool, through the upregulation of repaired enzymes perhaps induced by the vitamins pro-oxidative properties **(Cooke et al., 1998)**. The used vitamins (FA, VB12 and VC) were not reducing the effective of DNR on McF7 cell line. So they must be used as antigenotoxic agents to protect the normal cells from genotoxicity of DNR.

The results indicated that the applied doses of FA, VB12 and VC inhibited significantly the percentage of chromosome aberrations in both somatic and germ cells, also the percentage of DNA fragmentation in mouse spleen cells induced by DNR. The inhibition percentage reached 51.28%, 53.85% and 41.03% in mouse bone-marrow cells after treatment with 10mg FA/ kg b.wt., 0.3mg VB12/ kg b.wt. and 50mg VC/ kg b.wt. respectively. It reached 57.63%, 61.02% and 49.15% in mouse spermatocytes after treatment with the same doses of vitamins. The results showed that VB12 has the maximum protective effect against the chromosome aberrations induced by DNR (VB12>FA>VC). A number of vitamins have been tested to determine their antimutagenic potential, among these vitamins A, E, C and B12 are known to be strong mutagenic inhibitors against the anticancer drugs **(Water et al., 1998; Blasiak et al., 2002b; Wozniak et al., 2004; Yunca et al., 2004).**

The present study indicates that the anticancer drug DNR is genotoxic in mouse somatic and germ cells. Vitamins (FA, VB12 and VC) are not reduced the effect of DNR on breast cancer cells and they play a

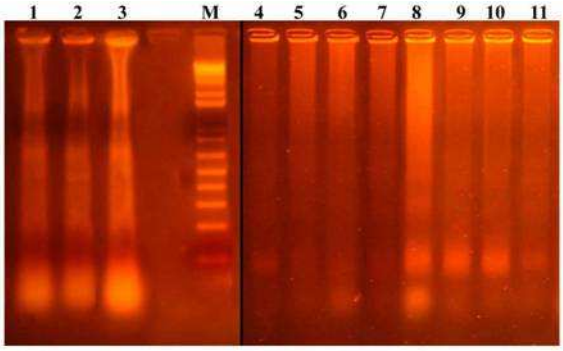

Figure (4): Effect of DNR on DNA fragmentation. Lanes 1-3: DNA of mice treated with 1,3 and 5mg DNR /kg b. wt. respectively. Lane M:1K base DNA ladder . Lane 4: control .Lanes 5-7: DNA of mice administered FA,VB12 &VC respectively. Lane 8: DNA of mice treated with 5mg DNR /kg b. wt. Lanes9-11: DNA of mice treated concurrently withDNR plus FA,VB12 &VC respectively (FA,10mg/kg b. wt.,VB12 0.3mg/kg b. wt., VC 50mg/kg b. wt.)

beneficial role against the genotoxicity of DNR on normal cells. Thus they may be beneficial with chemotherapy treatments as the active form of these agents being essential for DNA synthesis, cell proliferation and in the oxidation-reduction processes. Further investigations must be carried out to evaluate the importance of vitamins therapeutic regimens with the anticancer drugs. Suitable doses of vitamins can protect the normal cells from the genotoxic effect induced by chemotherapies.

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Co-integration and Error-Correction Modeling of Agricultural Output. A Case of Groundnut

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Abstract: Co-integration and error correction modeling tend to solve spurious regression result noticed from the analysis of macroeconomic data and also to establish an equilibrium long-run relationship which enables one to carry out a valid inferences about the explanatory variable that affect the output of such crop. First and foremost, stationarity test was carried out and it reveals that at level form output was stationary while the various variables (producer price, rainfall, hectarage and fertilizer) became stationary only at first-differencing applying the unit root test. Furthermore estimates of factor affecting the output of groundnut were derived using Johansen co-integration and error-correction representation procedures. The result indicated the existence of the one co-integrating vector at 5 percent significance's level, thus rejecting the null hypothesis of no co-integrating vector. As a result a parsimonious error-correction model was set-up. The statistical significance of the error correction model for groundnut validates the existence of an equilibrium relationship among the variables. The result therefore shows that the combine effect of producers price, hectarages, rainfall and fertilizer jointly affect the output of groundnut. [Researcher, 2009; 1(6):27-32]. (ISSN: 1553-9865).

Key words: Johansen co-integration; error-correction modeling; equilibrium relation; stationary; groundnut.

1. Introduction

In the history of Nigeria, agriculture used to be the mainstay of the economy. Nigeria is mainly an agrarian state. The emphasis on agriculture was so great that we had tremendous output in the production of groundnut in the North, cocoa in the West and palm oil in the East. As a result we had groundnut pyramids in 1970's, but this laudable measure could not be sustained due to the advert of petroleum in the early1970's, which subsequently became the major foreign earner for the country leading to the neglect of the agricultural sector. In recent times, revenue from oil hasn't been encouraging, thus, the government as a policy is diversifying the economy such that revenues can also be derived from other sectors of the economy. The agricultural sector to an extent has gained from these policies, which has witness a gradual emphasis being accorded it. This in no small way has lead to a significant but gradual increase in agricultural output of which groundnut is one them. As a result of all these, groundnut output has been fluctuating. In 11965 – 1970 it's output level was 7680 thousand tones, 1971 – 1975 it was 6004 thousand tones, 1976 – 1980 it recorded 9072 thousand tones, 1981 – 1985 it dropped to 2484 thousand tones but resumed a gradual increase in 1986 – 1990 with 3806 thousand tones and in 1991 – 1995 it's value was 7338 thousand tones. Groundnuts witness a total growth rate of 40.93 percent for the period 1965 – 1995.

This paper tends to examine whether producer's price, hectarage cultivated to groundnut, fertilizer applied and rainfall have an important effect on groundnut production. Reliable estimates of the determinations of output level are essential for policy decision to foster groundnut production. Due to the fluctuation in groundnut output, the regression of its statistical data will be spurious, which invalidate the result and interpretation. To adequately cater for this problem necessitate the use of co-integration and error-correction model in this study. During the last decade co-integration analysis has become a widely used technique for the analysis of economic time series. In recent past, co-integration analysis has been used by several authors: Ardeni, 1989; Goodwin and Schroeder, 1991; Alexander and Wyeth, 1992, to study market integration, Moss (1992) applied it to the cost-price squeeze in agriculture in U. S: Hallaam et al (1992) used it to determine the determinant of land prices. Adams (1992) applied the concept of co-integration to estimate the demand for money in Kenya. Tambi (1999) applied it to agricultural export supply in Cameroon. Tijani et al (1999) applied co-integration analysis to Nigeria Cocoa export supply.

2. Co-integration and Error-correction representation

Co-integration has assumed increased importance in analysis that purports to describe long-run or equilibrium relationships. An equilibrium relationship exists when variables in the model are co-integrated. A necessa 1 condition for integration, however, is that the data series for each variable involved exhibit similar statistical properties, that is, be integrated to the same order with evidence of some linear combination of the integrated series.

A stationary series X_t, for example, has a mean, variance and auto-correlation that are constant over time. However, most economic series tend to exhibit non-stationarity.

Stochastic process of the form

$X_t = \alpha + \beta X_{t-1} + e_t$…… (1)

Where α is a constant drift, $\beta = 1$, and e_1 is an error term. The series X_t is integrated because it is the sum of its base value X_t and the difference in X up to time t. since β is unity, X is said to have 'unit root'. If X_t is non-stationary, the variance may become infinite and any stochastic shock may not return to a proper mean level. As shown by Engle and Granger (1987), such a non-stationary series has no error-correction representation.

A non-stationary series required differencing to become stationary. X_t is integrated of order D_x or $X_t - (D_x)$ if it is differences D_x times to achieve stationarity. Engle and Granger (1987) provide appropriate tests for stationarity of individual series as the Dickey-Fuller (DF) and Augmented Dickey-Fuller (ADF) statistics. These tests are based on t-statistics on 'σ' obtained from estimates of the following respective static OLS regressions applied to each of the series:

$\Delta X_t = \alpha + \sigma X_{t-1}$ (for DF test)…….. (2)

$\Delta X_t = \alpha_0 + \sigma X_{t-1} + \Sigma\beta\Delta X_t\text{-}1 \div e_t$ (for ADF test)……..... (3)

Where the lag length K chosen for ADF ensures e_t is empirical white noise. The null hypothesis that X is 1(1) against the alternative of 1(0). The null is rejected if the t-statistic on σ is negative and statistically significant when compared to appropriate critical values established for stationarity tests. These critical values have been established by a number of studies from Monte Carlo simulations (Fuller, 1976: Dickey and Fuller, 1981: Engle and Granger, 1987: Perron, 1988: Blangiewiez and Charemza, 1990: Mackinnon, 1990).

Once the stationarity properties of the individual series are established, linear combinations of the integrated series are tested for co-integration. Should a linear combination of individual non-stationary series produce a stationary data series, then the variables are co-integrated and unless they integrates, they cannot describe equilibrium relationships. It they do not co-integrate, regressions of one 1(1) variables to another become spurious. As shown by Granger and Newbold (1974), such regressions produce high $R^{2's}$ and t-ratios that are biased towards rejecting the hypothesis of no relationship even when there is no relationship between the variables. Estimates of a linear combination of individual series tend to be reliable and constant and are fit for describing the steady-state relationships.

A number of studies have provided exposition of the co-integration methodology along with explicit tests for evaluating the co-integrating properties of a pair of non-stationary series (Hendry, 1960: Engle and Granger. 1987: Johansen, 1988: Johansen and Jusselius, 1990; Goodwin and Schroeder, 1991: Hallaam et al, 1994). The procedure consists of two steps. First, standard OLS is applied to the levels of the variables to establish the order of integration for particular combinations of co-integrating variables. Estimates of the residual error e_t are obtained as follows:

$E_t = X_t\text{-}\alpha\text{-}\beta y_t$…… (4)

The null hypothesis that e has a unit root and therefore is a random walk, is tested against the alternative that, it is stationary using the DF and ADF tests.

The Johansen procedure which is the most recent method is based on maximum likelihood estimates of all the co-integrating vectors in a given set of variables and provides two likelihood ratio tests for the number of co-integration vectors. This technique is important when testing for co-integration between more than two variables. The first test is based on the maximal eigen-value, the null hypothesis is that there are at most r co-integrating vectors against the alternatives of r + 1 co-integrating vectors. The second test, is based on the trace of the stochastic matrix, the null hypothesis is that there are at most r co-integrating vectors against the alternative hypothesis that there are r or more co -integrating vectors.

In order to achieve along-run equilibrium relationship the second step of Engle-Granger is applied by estimating an error-correcting model in which residual from the equilibrium co-integrating regression are used as an error-

correcting regressor (Ec_t lagged one period) in a dynamic model.

2.1. EMPIRICAL ESTIMATION

The general form of the equation specified in the double log form as follows:

$\text{In } \Delta Q_t = a_0 + a_1 \text{ In } \Delta A_{t-j} + a_2 \text{ In } \Delta P_{t-1} + a_3 \text{ In } f_{t-p} + a_4 \text{ In } \Delta W_{t-k} + Ecm_{t-1} + U_t$

Where;

$\text{In}\Delta Q_t$ = the quantity of groundnut output produced in thousand tonnes

$\text{In}\Delta A_{t-j}$ = the hectarage under cultivation for groundnut in hectare

$\text{In}\Delta P_{t-1}$ = the producers price for groundnut in N/tonnes

$\text{In}\Delta F_{t-p}$ = the quantity of fertilizer available in thousand MT

$\text{In}\Delta W_{t-k}$ = the weather variable

Ecm_{t-1} = error correction variable

U_t = error trem

On a prior basis all the variables are expected to have a positive effect on output of groundnut. But there could be deviation due to one reason or the other.

The data used in this study covered the period 1970 – 1998 data for the entire period were collected from of the federal office of statistics (Abstract of statistics); Central Bank of Nigeria and fertilizer yearbook of food and Agricultural organization.

3. DIAGNOSTIC RESULTS

3.1 Ordinary least square technique

The result of the static model for groundnut is shown in the table 1 below. It shows that the explanatory variable could only explain about 24 percent movement in the dependent variable. The priori sign for hectare and fertilizer are positive while that of rainfall and price are negative. The negative sign for price could be attributed to glut in the market, which pushes the price down while that for rainfall could be due to the drought nature of the northern part of the country. The D. W statistics showed no sign of serious negative auto correlations.

$R^2(R^{-2})$ =0.24 (0.11)

F-Statistics = 1.91

Durbin-Watson statistics = 2.25

Table 1: The result of static model estimate using the ordinary least square technique (1970 -1998) Dependent variable-quantity of groundnut produced

INDEPENDENT VARIABLES	CO-EFFICIENT	STANDARD ERROR	T-STATISTICS	PROBABILITY
CONSTANT ©	4.428	0.409	0.471	0.6421
LOG (G/NUT P)	-0.100	0.250	-0.401	0.6918
LOG (G/NUT HA)	0.480	0.318	1.509	0.1444
FERTILIZER	0.001	0.002	0.532	0.5999
LOG (RAINFALL)	-0.064	1.003	-0.064	0.9499

3.2. Unit root test

Table 2 below; present the ADF test-statistics for unit root for the entire variable used for groundnut. For all variables in their level form except for quantity of groundnut produced, the null hypothesis that each variable is 1(1) cannot be rejected as their ADF statistics are above the critical value of -2.98 at 5 percent significant level. Thus the variable hectare, price, fertilizer and rainfall are non-stationary at their level form. Note; critical value for level form is -2.98 at 5 percent significant level. For first differencing the critical value are -3.71,-2.98 and -2.63 at 1, 5 and 10 percent significant level respectively.

Table 2: Univariate stationary properties of the variable (ADF root test)

VARIABLES	ADF	VARIABLE	ADF	NO OF LAGS
Qty g/nut output	-3.10			1
G/nut price	-0.35	G/nut price	-3.53	1
G/nut ha	-1.94	G/nut hectare	-3.45	1
Fertilizer	-0.28	Fertilizer	-2.75	1
Rainfall	-2.36	Rainfall	-4.25	1

In the first difference form, however we can reject the null hypothesis for all the variables. At their first difference all the variables except fertilizer are stationary at 5 percent significant level while fertilizer is stationary at 10 percent significant level.

3.3. Johansen co- integration test

After stationarizing the variables, the Johansen procedure to test for the existence of more than two co-integrating vectors was applied. The result of test is shown in table 3 below. None denotes rejection of the hypothesis at 5 percent significances level. L.R test indicates 1 co-integrating equation at 5 percent significances level.

Table 3: Johansen Tests for the Number of Co-integrating Vectors for G/Nut Series in the Equation: Qty G, G/Nut P, G/Nut Ha, Fertilizer and Rainfall

HYPOTHESIS					
NULL	**ALTERNATIVE**	**LIKELIHOOD RATIO**	**5 PERCENT CRITICAL VALUE**	**EIGEN VALUE**	**HYPOTHESIS NOS OF CO-INTEGRATING EQUATION**
r = 0	r = 1	78.8085	68.52	0.7498	None
r ≤ 1	r = 2	41.4047	67.21	0.5366	At most 1
r ≤ 2	r = 3	20.6400	29.68	0.3218	At most 2
r ≤ 3	r = 4	10.1561	15.41	0.2673	At most 3
r ≤ 4	r = 5	1.7600	3.76	0.0631	At most 4

The result of the Johansen test for groundnut indicates the existence of a single co-integrating vector at 5 percent significant level. Thus rejecting the null hypothesis of no co-integrating vector, but accepting the alternative hypothesis of a single co-integrating vector. The long-run test indicates that one co-integrating equation (vector) exist at 5 percent significant level in the sets of normalized co-integrating equations. This is so because the alternative hypothesis of r = 1 is 78.8085 which is greater than the critical value at 5 percent.

3.4. Error correction model for groundnut

Since the result reveal the existence of co-integrating among the variables of the model a parsimonious error correction model (ECM) was then set-up, it is presented in table 4 below. the result indicated (going by the value of the co-efficient of multiple determinations) that the model has a good fit as the independent variable jointly explain 99 percent of the movement in the dependent variable which is a marked improvement on the 24 percent obtained with static model using OLS.

R^2= 0.999842; Prob (F-statistic) = 0.0015; R^2= 0.998263; D.W= 1.169899; F-statistic = 633.3200

Table 4: Modeling the Determinants of the Output of G/Nut by Ordinary Least Squares (A Dynamic Error Correction Model): Summary of the results of the Estimated Equations (1970 – 1998). Dependent variable = LOG OF G/NUT OUTPUT (LGNUTO)

	INDEPENDENT VARIABLE	**CO-EFFICIENT**	**STANDARD ERROR**	**t-Statistics**
1	C	-0.034793	-0.011438	-3.041833
2	D(LGNUT HA,2)	0.346290	0.048908	7.080441
3	D(LGNUT HA,(-1),2)	-0.059164	0.060049	-0.9852262
4	D(LGNUT HA, (-2),2)	0.354026	0.044498	7.956026
5	D(LGNUT HA (-3),2)	0.905166	0.101154	8.948360
6	D(LGNUT HA (-4),2)	0.747013	0.127464	5.860557
7	D(LGNUT P,2)	-0.740937	0.100189	-7.395414
8	D(LGNUT P(-1),2)	-0.225266	0.078539	-2.868216
9	D(LGNUT P(-2),2)	-0.825961	0.135119	-6.112850
10	D(LGNUT P(-3),2)	-0.757401	0.128746	-5.882915

11	D(LGNUT P(-4),2)	-0.766367	0.121703	-6.112850
12	D(LRAINFALL,2)	-0.909280	0.157490	-5.773563
13	D(LRAINFALL, (-1),2)	-0.683869	0.170690	-4.006492
14	D(LRAINFALL,(-2),2)	-1.449964	0.2502294	-5.793048
15	D(RAINFALL,(-3),2)	-0.137132	0.110911	-1.236415
16	D(FERTILIZER,2)	0.002695	0.000380	7.099002
17	D(FERTILIZER(-1),2)	0.003739	0.000646	5.787657
18	D(FERTILIZER(-2),2)	0.001294	0.000442	2.927222
19	D(FERTILIZER(-3),2)	0.004242	0.000688	6.159390
20	ECMG	0.612802	0.047291	12.95811
21	ECMG(-1)	-1.142107	0.039308	-29.05509

This means that the independent variables used in the model are the major determinant of the output of groundnut in Nigeria. The F-statistics which is significant at zero percent confirmed the goodness of fit of the model. The result shows that the coefficient of the 3rd and 15th explanatory variable is statistically significant at various levels ranging from zero to 10 percent. For instances the coefficient of the explanatory variables (LGNUTHA, 2) and its four years lagged components are significant at 1 and 5 percent respectively. Similarly the explanatory variables (LGNUTP, 2) and its four years lagged component are significant at 1, 5 and 10 percent respectively. While the variable (LRainfall, 2) and its two years lagged component are significant at 5 percent. Furthermore, the variable (Fertilizer, 2) and its various three years lagged component are significant at 1, 5 and 10 percent respectively. The ECM coefficient and that of its one year lag are both significant at zero percent, which is an indication of its high feed back mechanism, thereby ensuring non-less of information and a confirmation of the validity of an equilibrium relationship among the variable in the co-integrating equation. The result therefore reveals that the combine effect of producer's price, hectarage cultivated, fertilizer and rainfall jointly affects the output of groundnut.

4. Conclusion

Estimation of Nigeria's groundnut production was approached through Johansen co-integration and correction model. The unit-root reveals that groundnut output was stationary at level first differencing. The Johansen co-integration reveals the existence of one co-integrating vector, thus a parsimonious error-correction model was set-up. Statistical significance of the error-correction terms validates the existence of an equilibrium relationship among the variables in the co-integrating vector.

The conclusion from this is that the combine effect of producer's price, the hecterage, fertilizer and rainfall jointly affects the production of groundnut in Nigeria. In order to boost production a positive price policy should be put in place, a well defined land use policy should be pursued and an efficient management of available surface and underground water resources should be emphasized.

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Model for Prediction of the Quantity of Absorbed Water In Clay Materials Exposed To Hot-Humid Environment

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Abstract: Model for prediction of the quantity of absorbed water in clay materials exposed to hot-humid environment has been derived. These clay materials were prepared using different grain sizes; <100μm, 100-300μm, 300-1000 μm and their respective mixtures. The derived model;

$$\beta = \left(\frac{\gamma}{\left(\alpha\,(S)^{0.995} \right)} \right)$$

was found to be dependent on the bulk density, apparent porosity and the shrinkage sustained on the clay body at any point in time under the hot-humid condition. The validity of the model is rooted on the expression; $S = (\gamma/\alpha\beta)^{1.005}$ where both sides of the expression are correspondingly almost equal. The maximum deviation of the model-predicted quantity of absorbed water from the corresponding experimental values is 8% which is within the acceptable range of deviation limit for experimental results.[Researcher. 2009;x(x):xx-xx]. (ISSN: 1553-9865). [Researcher, 2009; 1(6):33-37]. (ISSN: 1553-9865).

Keywords: Model, Water Absorption, Clay Materials, Hot-Humid Environment.

1. Introduction

Following studies by Reed (1988), firing of clay was found to proceed in three stages; preliminary reactions which include binder burnout, elimination of gaseous product of decomposition and oxidation, sintering as well as cooling which may include thermal and chemical annealing. Barsoum (1997),Viewey and Larrly (1978) and Keey (1978) have studied the shrinkage of clay during drying. In all these works, porosity has been shown to influence the swelling and shrinkage behaviour of clay products of different geometry. Reed (1988) reported that drying occurs in three stages; increasing rate, constant and decreasing rate. He pointed out that during the increasing rate; evaporation rate is higher than evaporating surface hence more water is lost. At constant rate, the evaporation rate and evaporation surface are constant. The researcher posited that shrinkage occurs at this stage. In a similar study, Keey (1978) suggested that at this stage, free water is removed between the particles and the inter-particle separation decreases, resulting in shrinkage. During the decreasing rate, particles make contacts as water is removed, which causes shrinkage to cease.

Model for calculating the volume shrinkage resulting from the initial air-drying of wet clay has been derived by Nwoye (2008). The model;

$$\theta = \gamma^3 - 3\gamma^2 + 3\gamma \qquad (1)$$

calculates the volume shrinkage when the value of dried shrinkage γ, experienced during air-drying of wet clays is known. The model was found to be third-order polynomial in nature. Olokoro clay was found to have the highest shrinkage during the air drying condition, followed by Ukpor clay while Otamiri clay has the lowest shrinkage. Volume shrinkage was discovered to increase with increase in dried shrinkage until maximum volume shrinkage was reached, hence a direct relationship.

Nwoye et al.,(2008) derived a model for the evaluation of overall volume shrinkage in molded clay products (from initial air-drying stage to completion of firing at a temperature of 1200^0C). It was observed that the overall volume shrinkage values predicted by the model were in agreement with those calculated using conventional equations. The model;

$$S_T = \alpha^3+\gamma^3-3(\alpha^2+\gamma^2)+3(\alpha+\gamma) \qquad (2)$$

depends on direct values of the dried γ and fired shrinkage α for its precision. Overall volume shrinkage was found to increase with increase in dried and fired shrinkages until overall volume shrinkage reaches maximum.

Successful derivation of a model for calculating the quantity of water lost by evaporation during oven drying of clay at 90^0C has been carried out (Nwoye,2009). The model;

$$\gamma = \exp[(\ln t)^{1.0638} - 2.9206] \quad (3)$$

indicated that the quantity of evaporated water, γ during the drying process is dependent on the drying time t, the evaporating surface being constant. The validity of the model was found to be rooted in the expression $(\text{Log}\beta + \ln\gamma)^N = \ln t$.

Model for predictive analysis of the quantity of water evaporated during the primary-stage processing of a bioceramic material sourced from kaolin has been successfully derived (Nwoye et al.,2009a) .The model;

$$\alpha = e^{(\ln t/2.1992)} \quad (4)$$

indicates that the quantity of water α, evaporated at 110^0C, during the drying process is also dependent on the drying time t, where the evaporating surface is constant. It was found that the validity of the model is rooted on the expression $(\ln t/\ln\alpha)^N = \text{Log}\beta$ where both sides of the expression are correspondingly approximately equal to 3. The respective deviation of the model-predicted quantity of evaporated water from the corresponding experimental value was found to be less than 22% which is quite within the acceptable deviation range of experimental results.

Nwoye et al. (2009b) derived a model for quantifying the extent and magnitude of water evaporated during time dependent drying of clay. The model;

$$\gamma = \exp((\ln t/2.9206)^{1.4}) \quad (5)$$

indicates that the quantity of evaporated water γ during the drying process (at 90^0C) is dependent on the drying time, t the evaporating surface being constant. It was found that the validity of the model is rooted in the expression $\ln\gamma = (\ln t/\text{Log}\beta)^N$ where both sides of the expression are correspondingly almost equal.

The present work is to derive a model for prediction of the quantity of absorbed water by Otamiri clay materials exposed to hot-humid environment.

2. Materials and Methods

2.1. Model Formulation

Results of the experiment previously carried out (Nwoye,2006) were used for the model derivation. These results as shown in Table 1 indicate that;

$$S = \left(\frac{\gamma}{\alpha\beta}\right)^N \quad \text{(approximately)} \quad (6)$$

Introduction of the value of N to equation (6) reduced it to;

$$S = \left(\frac{\gamma}{\alpha\beta}\right)^{1.005} \quad (7)$$

Dividing the indices of both sides of equation (7) by 1.005 reduces it to;

$$S^{1/1.005} = \left(\frac{\gamma}{\alpha\beta}\right) \quad (8)$$

$$(S)^{0.995} = \left(\frac{\gamma}{\alpha\beta}\right) \quad (9)$$

$$\beta = \left(\frac{\gamma}{\alpha\left(S\right)^{0.995}}\right) \quad (10)$$

Where

N = 1.005; Coefficient of shrinkage Otamiri clay at 1200^0C (determined in the experiment (Nwoye,2006)

(γ) = Bulk density of the clay body in the hot-humid environment (g/cm^2)

(α) = Fractional value of apparent porosity of the clay body in the hot-humid environment

(β)= Fractional value of water absorbed by the clay body under the hot-humid environment

Equation (10) is the derived model

3. Boundary and Initial Conditions

Consider a rectangular shaped clay product of length 70mm, width 17mm, and breadth 9mm exposed to drying in the furnace while it was in wet condition. Initially, atmospheric levels of oxygen are assumed. Atmospheric pressure was assumed to be acting on the clay samples during the drying process (since the furnace is not air-tight). The grain sizes for the clay materials used are, <100μm, 100-300μm, 300-1000μm and their respective mixtures. The hot-humid environment was at a temperature; 1200^0C, and the resident time of clay bodies under the environment; 18hrs. The boundary conditions are: atmospheric levels of oxygen at the top and bottom of the clay samples since they are dried under the atmospheric condition. No external force due to compression or tension was applied to the drying clays. The sides of the particles and the rectangular shaped clay products are taken to be symmetries.

4. Model Validation

The formulated model was validated by direct analysis and comparison of the model-predicted β values and those from the experiment (Nwoye, 2006) for equality or near equality.

Analysis and comparison between these β values reveal deviations of model-predicted β from those of the experimental values. This is believed to be due to the fact that the surface properties of the clay and the physiochemical interactions between the clay and binder, which were expected to have played vital role

during the evaporation of water were not considered during the model formulation. This necessitated the introduction of correction factor, to bring the model-predicted β value to that of the corresponding experimental value.

Deviation (Dv) (%) of model-predicted values of β from the experimental values is given by

$$Dv = \left(\frac{\beta_M - \beta_{exp}}{\beta_{exp}} \right) \times 100 \quad (11)$$

Correction factor (Cf) is the negative of the deviation i.e

$$Cf = -Dv \quad (12)$$

Therefore

$$Cf = -100 \left(\frac{\beta_M - \beta_{exp}}{\beta_{exp}} \right) \quad (13)$$

Introduction of the value of Cf from equation (13) into the model gives exactly the corresponding experimental value β_{exp}.

5. Results and discussions

The model is equation (10). It was found that the model is dependent on the bulk density, apparent porosity and the shrinkage sustained on the clay body at any point in time under the hot-humid condition. The validity of the model was found to be rooted on the expression; $S = (\gamma/\alpha\beta)^{1.005}$ where both sides of the expression are correspondingly almost equal. Table 2 also agrees with equation (6) following comparison of the value S and that of $(\gamma/\alpha\beta)^{1.005}$ evaluated from Table 1 as a result of corresponding computational analysis. Fig. 1 shows appreciable close alignment of the curves from model-predicted values of absorbed water (βmod) and that from the corresponding experimental values (βexp). It is strongly believed that the degree of alignment of these curves is indicative of the proximate agreement between both experimental and model-predicted quantities of absorbed water by the clay material. Table 3 shows that the maximum deviation of the model-predicted quantity of absorbed water from the corresponding experimental values is less than 8% which is within the acceptable range of deviation limit for experimental results.

The model can be useful to engineers for carrying out failure or survival analysis of clay materials (exposed to hot-humid environment while in service) relative to the varied clay porosity, water absorption, bulk density and shrinkage sustained in the clay bodies under this service environment. This is because swelling of clay materials is likely when water absorbed by the materials becomes excessive. Nwoye (in press) found that swelling process weakens the grain boundaries and also loosen the clay-binder interface leading to collapse of the microstructure of the clay material. This implies failure.

Conclusion

The model computes the quantity of absorbed water in clay materials exposed to hot-humid environment. The model is dependent on the bulk density, apparent porosity and the shrinkage sustained on the clay body at any point in time under the hot-humid condition. The validity of the model is rooted on the expression; $S = (\gamma/\alpha\beta)^{1.005}$ where both sides of the expression are correspondingly almost equal. The maximum deviation of the model-predicted quantity of absorbed water from the corresponding experimental values is less than 8% which is within the acceptable range of deviation limit for experimental results.

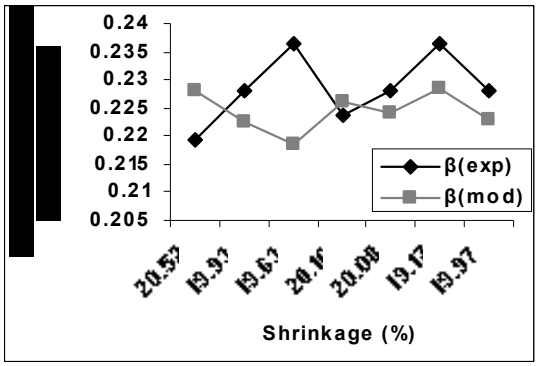

Fig.1: Comparison of the quantities of water absorbed by the clay body under the hot-humid environment as obtained from experiment (Nwoye,2006) and derived model.

Table1: Variation of apparent porosity, water absorption, bulk density and volume shrinkage with grain size of Otamiri clay body exposed to hot-humid environment.(Nwoye,2006)

$(\gamma/\alpha\beta)$	$(\gamma/\alpha\beta)^{1.005}$	S (%)
21.0364	21.3593	20.52
19.1634	19.4484	19.93
17.8518	18.1109	19.63
20.0678	20.3710	20.16
19.4604	19.7514	20.08
18.2583	18.5254	19.17
19.2456	19.5323	19.97

Table 2: Variation of $(\gamma/\alpha\beta)^{1.005}$ with S

Dv (%)	Cf (%)
+4.06	-4.06
-2.41	+2.41
-7.69	+7.69
+1.03	-1.03
-1.62	+1.62
-3.34	+3.34
-2.19	+2.19

Table 3: Deviations (from experimental values) of model-predicted volume shrinkage and the associated correction factors

Grain size (μm)	α	β	γ	S (%)
(A) <100	0.2559	0.2192	1.18	20.52
(B) 100-300	0.2608	0.2281	1.14	19.93
(C) 300-1000	0.2628	0.2366	1.11	19.63
A + B	0.2584	0.2237	1.16	20.16
A + C	0.2593	0.2279	1.15	20.08
B +C	0.2618	0.2364	1.13	19.17
A + B + C	0.2598	0.2280	1.14	19.97

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Distribution and Indigenous Uses of Some Medicinal Plants in District Uttarkashi, Uttarakhand, India

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Abstract: The present study was based on a field survey in district Uttarkashi of Uttarakhand, to find the plants of medicinal values. [Researcher. 2009;1(6):38-40]. (ISSN: 1553-9865).

Keywords: Distribution; Indigenous Uses; Medicinal Plants; Uttarakhand; India

Introduction

The plants have been used as a source of medicines by man from ancient time to the present day. Initially these were the main parts of folk or ethanomedicine, practiced in India and other parts of the world like China, Middle East Africa and South America. Later a considerable part of this indigenous knowledge was formulated, documented and eventually past into the organize systems of medicines such as Ayurveda, Yunani, Sidha or other systems. Subsequently, with the advanced in the techniques of phytochemistry and pharmacology, a number of active principles of medicinal plants were isolated and introduce as valuable drugs in modern system of medicine (Ved Prakash, 2001). The Himalaya represents the largest mountain chain covering approximately 8 million km^2 in surface area and occupying a length of approximately 3000 km. Owing to enormous size and elevation, the Himalaya represents a complete transaction from tropical to temperate conditions despite its location near the tropics. 1748 species of medicinal plant have been reported from the Indian Himalayan region (IHR), of these 701 species occur in Uttarakhand state (West Himalaya). In the region most medicinal plants are being extracted for drug and pharmaceutical industries from the wild (Mehta, 2001). In India earliest references of the curative properties of plants appear in *Rigveda*, which is said to be written between 3,500-1,600 B.C. *Ayurveda* describes use of large number of drugs their properties and uses in some details. Works of charka and susruta namely '*Charak Sanhita*' deals with about 700 drugs, few of which were not indigenous to India (Jain, 1968).

Lying in the upper Himalayas, Uttarkashi contains within itself varying geographic environment ranging from snow free valley and outer hills to the peaks with perpetual snow and glaciers. The widely varying climate and topography produce a wide range of vegetation and serve as habitats to diverse species of wild life. Forest occupies a place of pride in the environment of the district not only for sheer bulk of the area they occupy but also for the richness of variety of vegetation. The descriptions of forest of district Uttarkashi are as follows:

Pine forest (900-2000 meters)
Deodar forest (2000-3000 meters)
Fix and spruce forest (over 3000 meters)
Kharshu, Birch and Junipers forests (up to 4000 meters)
Alpine zone (3500-4877 meters)

Material and Methods

The present study was based on a field survey in district Uttarkashi, to find the plants of medicinal values. The work was conducted among local people, rural persons, farmers and vaidyas to know the local names and medicinal importance of mentioned plants. The plants with medicinal values, as known from local people and rural persons were collected and studies were made to know their medicinal uses.

Result and Discussion

A large number of medicinal plants of great commercial value grow spontaneously in the forests. Some of these grown in the valleys, some in sub-mountain tracts while some other in high altitudes. Forestry plays an important role in the economy of the district. In the present study some important medicinal plants of Uttarkashi are described as follows:

Mitha (*Aconitum balfourii*):
Family: Ranunculaceae
Altitude: 2800-4000 m.
Aconitum balfourii found in sub-alpine to alpine zone of district Uttarkashi. It is an extremely poisonous herb grows on shady moist slopes usually along the edges of brich, rhododendron forest. Root pest, in small quantity after frying in butter, is applied and massaged on joints for the treatment of rheumatism. Best populations are seen at Dayara and Gidara (Rawat, 2005).

Atis (*Aconitum heterophyllum*):
Family: Ranunculaceae
Altitude: 2200-4000 m.
Atish is an herb found in sub-alpine to alpine zone of district Uttarkashi. Fruits are follicles with 16-18 mm large seeds. Root (tuber) is used part of the plant for medicine. Roots are tuberous, white in color and useful in dysentery, diarrhea, stomach disorders, fever, malaria fever and helminthiasis etc. local people store the dry roots as emergency medicine. Best populations of Atis are seen at Gidara (Rawat, 2005).

Gokhru (*Tribulus terrestris*):
Family: Oxalidaceae
Altitude: 3000-4000 m.
A prostate-spreading herb densely covered with minuet heirs found in sub-alpine to alpine region of Uttarkashi forests. The fruit of the plant are useful in urinary compliances and sexual weakness. Rarely leafs used as pot herb. Seed powder of the plant with *Swertia chirayita* given in cough and asthma; seed pest applied on skin eruptions (Gaur, 1999).

Cacrasinghi (*Pistacia integerrima*):
Family: Anacardiaceae
Altitude: 2000-3000 m.
A moderate sized deciduous tree up to 18 mt. high with dark gray or blackish bark found in deodar forests. Galls produced on leaves are used commercially as 'karkkatasrgi'. Galls on leaves are the used part of the plant as medicine, as anti-inflammatory, depurative, digestive and expectorant with terebinthine odor. They are useful in asthma, cough, dysentery and fever, consumption irritability of stomach, skin diseases and useful at the time of teething of children.

Barberry (*Berberis aristata*):
Family: Berberidaceae
Altitude: 2000-3000 m.
A large thorny shrub found in deodar forests of Uttarkashi. Fruits are ovule bluish purple with few seeds. Although whole plant is useful as medicine but particularly roots and seeds of the plant is important part of the plant for drugs. The main use of drug maid by barbery is a diuretic i.e. promote urination in dropsy and in jaundice and gonorrhea, it is also recommended for asthma.

Guggul (*Tanacetum dolichophyllum*):
Family: Asteraceae
Altitude: 2500-4000 m.
A small armed free with spine scent branches and colored rough bark found in deodar forests and in alpine zone also, having small flowers and fruits avoid red when ripe. The resinous gum obtained from the bark, yellowish to brown in color, is a part use as medicine. The gum is bitter acid, astringent, thermogenic, aromatic, digestive, anti-inflammatory, antiseptic, liver tonic, antispasmodic and also useful in vitiated condition of vatic, gout, facial paralysis, heiminthiasis, dyspepsia cough, asthma, fever and anemia.

Gubankh (*Angelica archangelica*):
Family: Apiaceae
Altitude: 2700-3400 m.
Distributed on the Western Himalayas, in alpine shrub and found on moist and shady slopes. Seeds are used as spice for seasoning vegetables in some parts of Uttarakhand. Essential oils distilled from the seeds and roots used in perfumes and flavoring various liquors (Vashishta, 2006).

Bel (*Aegle marmelos*):
Family: Rutaceae
Altitude: 800-2500 m.
A medium size deciduous tree bearing strong axillary throne found commonly through out the district. Fruit (8-20 cm. diameters) is a part use as medicine. Globosely green, color is changed to grayish when fruit mature. The bel fruits are valuable chiefly for its mucilage and pectin; it is very useful in chronic diarrhea and dysentery. Sweet drinks (sharbat) prepared from the pulp of the fruit are useful as soothing agents for in testing patient who have just recovered from bacillary dysentery (Jain, 1968)

Brahm Kamal (*Saussurea obvallata*):
Family: Asteraceae
Altitude: 3100-4000 m.
A small and middle size 15 cm. long annual, aromatic herb grow wild, found in alpine region of Uttarkashi. Whole plant is use as medicine; the drug is used in treatment of headache and other pains (Rawat, 2005).

Datura (*Datura starmonium*):
Family: Solanaceae
Altitude: 2500 - 3500m.
A bushy plant up to 1 mt. height found in Uttarkashi city and in sub-alpine to alpine region of Uttarkashi. Leaves tops of flowers and seeds of the plant are the parts use as medicine. Leaves are large ovate and toothed, flowers are white and large. The chief active principle in the leaves is hvoscvamine, the drug is therefore useful in the same manner as belladonna or hvosvamus. The drug is useful in bronchitis or asthma and controls saiivation in mouth. The seeds also contain hvoscvamine and have similar properties as the leaves.

Chirayata (*Swertia chirayata*):
Family: Gentianaceae
Altitude: 1500-2000 m.
Chirayata is an annual herb of deodar forests of Uttarkashi. The whole plant is use as a medicine. Leaves of the plant are opposite pairs about 10 cm. long without stalks, the flowers are pale green, tinged of green glands. Chirayata is well known far its bitter, stomach ache, and

febrifuge, anathematic properties. It is given in fever, diarrhea and weakness (Nautiyal *et al*., 2004).

Ashvagandha (*Withania somnifera*):
Family: Solanaceae
Altitude: 1000-1800 m.
A small or middle size under shrub, found through out the district in drier regions. Roots are the uses part of the plant in drugs. The drug consists of the dried roots of the plant. Ashvagandha is useful in consumerism, sexual and general weakness and rheumatism. It is diuretic i.e. it promotes urination and removes functional obstructions of body. The root powder is applied locally on ulcers and inflammations. Crystalline principle 'Withaferin- A' has been obtained from leaves and is reported to possess significant antibiotic properties (Jain, 1968).

Uttarakhand, on account of its unique setting within the Himalaya region, possesses luxuriant and varied vegetation. Almost every plant has economic value from either a nutritional, esthetics or medicinal viewpoint. In fact a large percentage of crude drugs in the Indian market come from this Himalayan area (Badoni and Badoni, 2001). Nearly 30 species of Garhwal Himalaya have been listed in various categories under threat in the Indian Red Data Books (Nayar and Shastri, 1987-90) of which 24 species are from high altitute alpine regions. Recently, Rawat *et.al*. (2001) listed 45 more species (excluding Red Data Book) which need special attention for conservation, and this list also contains as many as 30 species from high altitudes (Nautiyal et. al., 2004). There has been a considerable decrease in the number of families in sub alpine and alpine zones of district Uttarkashi dependent on grazing. Most of the medicinal plant exploited indiscriminately from the wild. Due to non-availability in abundance, different populations are completely wiped off for economizing the collection activity.

Medicinal plants and their uses in the indigenous medicine are well known to many Indian communities. The recent trend has been to blend the traditional knowledge with modern heath care practices to provide effective health care services to a wider population. The basic ingredients in the traditional medicine are the medicinal plants, which are depleting at a faster rate due to increase in consumption and indiscriminate drawl of resources from the wild. With the changing scenario, there is a need to enhance and promote the conservation and cultivation of these natural resources especially medicinal plants. In addition to the requirement for conservation of medicinal plants it has also become essential to protect and patent the traditional knowledge (Raghupathy, 2001).

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Land utilization farming with reference to Uttarkashi, the Hilly District of Uttarakhand

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ABSTRACT: Traditional knowledge system has been a key to the survival of the hill society, be it in cropping, forestry or health. It has not only ensured continuous livelihood of farm households but also ecological sustainability. Farming in the hills is highly interdependent with forestry and animal husbandry. The present study deals with the major environmental problem related to deforestation in Uttarkashi district of Uttarakhand, their remedial measures, socioeconomic status and the links of forestry, animal husbandry and agents of change**.** [Researcher. 2009;1(6):41-47]. (ISSN: 1553-9865).

Key Words: Uttarkashi, Development Strategy, Traditional system

1. INTRODUCTION

Large-scale indiscriminate cutting of trees, uncontrolled overgrazing overexploitation of communities, unscientific exploitation of natural resources reflects their effect on vegetation and environment. At present there is hardly 46 million hectare area with reasonable forest cover but according to national forest policy, there should be at least 110 million hectare area under forest (Pant et.al. 1999). The national target is to reclaim annually 3-5 million hectare of waste land and to plant trees and grasses on vacant government land, community land, marginal agricultural land and the Agro forestry will be vital for bridging the gap between demand and availability of various forms of wood. Traditional Agroforestry system and fixed farming are well established in the Ganga and Yamuna valley, planting and harvesting of trees for wood products, fruit, fodder, leaves etc since ancient time, the type of agroforestry system found in a particular area is determined to same extent by agro-ecological and socio-economic factors if these agro forestry system are modified properly play an important role in reclamation of waste land and soil conservation. Rearing of livestock is an integral part of the economy of the people of the district, due to over-grazing, desirable nutritive grasses and medicinally important species have been depleted considerably, during past times the grazing incidence has decreased due to bringing more and more area under agriculture, horticulture and closing of existing grazing areas by state forest department as a measure of soil conservation and also under different afforestation programmes. High density of human and livestock population over exploitation of community, unscientific exploitation of natural resources, reflect their effect on the vegetation and indirectly on environment in various ways like soil erosion, global warming, irregular rain fall extinction of various species these are caused mainly by cleaning forest for agriculture, horticulture, illicit lopping and cutting of forest vegetation for fuel, food, fodder, charcoal, removal of litter from forest floor for manures, grazing and commercial exploitation of important forests species. The study describes how environmental legislation has slowly taken away the traditional livelihoods of vast numbers of people. Wood carvers, whose handworkers be seen in the traditional houses, have disappeared over years, nomadic sheep's and goats herders are slowly dying and agriculture is Back-breaking work that does not yield enough for subsistence.

2. MATERIALS AND METHODS

The present study was conducted in the Uttarkashi district of Uttarakhand which is basically divisible as Ganga and Yamuna Vallies located between 31º 02´ north latitude and 78º 44´ and 78º 43.4´ east longitude of western Himalaya covering about an area of 8016 sq. km. Uttarkashi is the north most district of the Uttarakhand bordering Himachal Pradesh to northwest, Chamoli district on eastern side Dehradun district on western side, Tibbet on northern side and Tehri district on southern side. The district bears unique cultural, heritage, significant forest and water resources. The detailed information about the study materials was collected with the co-operation of Statistical department, forestry department, and horticulture department. The information regarding the problem is based on following parameters:

1 Population and its growth rate
2 Live stock population
3 Forest composition
4 Land use statistics
5 Area and production of fruits and vegetables
6 Area and yield of principal crops

3. RESULTS AND DISCUSSION

The total population of Uttarkashi district during year 2001 is 294179 in the comparison of Uttarakhand population (8479562) (Mittal, et.al. 2008). The growth rate of the population is high as 22.72 beside this the population density of this hilly district is lowest in Uttarakhand (Table- 1).

Table 1- Population and its growth rate

Site	Male population	Female population	Total population	Rural population	Urban population	Sex ratio	Population density	Growth rate
Uttarakhand	4316401	8479562	6309317	2170245	2170245	963	159	19.20
Uttarkashi	151599	142580	294179	271255	22924	941	37	22.72

The sheep and goats are migratory taken for grazing to alpine pastures during summer and lower hills during winter while the cow and buffaloes grazed in an area near the villages, free grazing are practiced for these livestock**.** The live stock population increased from 394466 to 438086 from 1998 onward, which is the maximum value in Uttarakhand (Table 2).

Table 2- Live stock population

Year	Cow	Buffalo	Sheep	Goat	Total
1993	210632	38280	89329	95613	433854
1998	199263	38594	72367	84242	394466
2003	202535	38690	101268	95593	438086

Milk availability in the district is low and the milk societies require capital to develop infrastructure and markets. There is also no fodder department. Cattle bought from outside are less adaptable to the cold weather of Uttarkashi and thus cross-breeding is needed within the district, but vaccine is a constraint. Since Uttarkashi is rich in livestock, wool-rearing is a viable option. The total forest area of the district is 88.86%. On the basis composition the forest of the region are broadly classified as coniferous forest and broad leaved forest includes undisturbed forest, *Pinus roxburghii Cedrus deodara, Pinus wallichiana, Picea smithiana, Abies pindrow* are important conifers while Oak (*Quercus leucotrichophora, Quercus semicarpifolia, Quercus floribunda)* are important broad leaved species with a number of other temperate and tropical hardwoods growing in this region. *Quercus leucotrichophora* has maximum area 33724.04 (ha) while *Pinus roxburghii* have least area of 1284.06 (ha) (Table 3).

Table 3- Forest composition and growing stock

Species	**Area (ha)**
Quercus leucotrichophora	33724.04
Quercus semicarpifolia	24308.30
Quercus floribunda	14471.75
Pinus roxburghii	1284.06
Cedrus deodara	3346.54
Abies pindrow	1619.06
Picea smithiana	3288.94

The destruction and degradation of forests are taking a heavy toll on soil and water resources, making the land less productive and leading to impoverishment of the rural population. It is essential to regenerate degraded forest and wasteland (Malhotra, 2005). All land, which is used wholly or partly for agricultural production, are operated as one technical unit by one person alone or with others without regard to the title, legal form, size or location. Already, an area about 3.10% is under agriculture including fallow land beside this Barren and unculturable wasteland is 4.65%, current fallow and other fallow land is 0.57% (Table 4).

Table 4- Land use statistics

Characteristics of Uttarkashi	**Area (ha)**	**Percentage of total land area**
Total area	812415	100
Forest	721661	88.83
Agriculture land/Cultivable land	2278	0.29
Current fallow land	1539	0.16
Other fallow land	3099	0.38
Land put to non-agricultural uses	5381	0.65
Culturable waste land	40694	5.00
Barren and uncultivable waste land	37763	4.65

The area under agriculture is about 3.97% of the total land area, due to large agricultural population and limited arable area the size of land at present is about 23.23%, about 86.21% of the farmers are small and marginal owing about 49.40% of the land holdings area. The numbers of holdings bigger than 10 hectare area are negligible (Table 5).

Table 5- Distribution of land holdings by size classes

Size class (ha)	Number of land Holdings	Percentage (%)	Area (ha)	Percentage (%)	Average size of Holdings
Less than 0.5	20182	52.41	3212	9.42	0.16
0.5- 1.0	6346	16.48	4132	12.12	0.65
Marginal farmer	26528	68.88	7344	21.54	0.28
1-2	6670	17.32	9500	27.86	1.42
Small and marginal farmer	33198	86.20	16844	49.40	0.50
2-4	4282	11.12	11673	34.24	2.73
4-10	1014	2.63	5326	15.61	5.25
10 and above	21	0.05	257	0.75	12.24
Total	**38515**	**100.00**	**34100**	**100.00**	**23.23**

The important fruits are *Pyrus malus, Pyrus communis, Prunus persica*, *Juglans regia*. Among these the *Pyrus malus* occupies larger area of about 6928 ha and lowest, 170 ha for *Prunus persica* (Table-6).

Table 6- Fruit production during year 2006-07

Sl. No	Name of Blocks	*Pyrus malus*		*Pyrus communis*		*Prunus persica*		*Pyrus persica*		*Prunnus armeniaca*	
		Area (ha)	Production (M tones)	Area (ha)	Production (M tones)	Area (ha)	Production (M tones)	Area (ha)	Production (M tones)	Area (ha)	Production (M tones)
1	Bhatwari	277	4941	225	1647	30	4941	225	1647	20	205
2	Dunda	235	1955	159	1179	32	1955	159	1179	18	180
3	Chinyalisaur	235	1534	169	1161	21	1534	169	1161	15	145
4	Naugaon	2380	20314	290	2588	60	20314	290	2588	25	233
5	Purola	709	3416	250	1217	13	3416	250	1217	20	214
6	Mori	**3092**	10312	270	1904	14	164	67	490	24	92
	Total-	**6928**	**42472**	**1363**	**9696**	**170.**	**1126**	**682**	**3887**	**122**	**1069**

Continued….

Sl. No	Name of Blocks	*Juglans regia*		*Citrus species*		*Mangifera indica*		Other Fruits		Total	
		Area	Production (M tones)	Area	Production	Area	Production (M tones)	Area	Production (M tones)	Area	production

		(ha)		(ha)	(M tones)	(ha)		(ha)		(ha)	(M tones)
1	Bhatwari	182	151	47	153	2	8	200	320	1098	8273.00
2	Dunda	184	126	62	200	37	65	180	310	1027	4860.00
3	Chinyalisaur	210	142	42	150	25	55	220	315	1062	4369.00
4		288	202	28	125	87	290	290	332	3583	25028.00
5	Purola	164	132	16	62	25	-	180	303	1479	6199.00
6	Mori	252	113	10	63	18	-	168	280	3915	13418.00
	Total	**1220**	**866**	**205**	**753**	**194**	**418**	**1238**	**1860**	**12182**	**62147.00**

The production of the *Pisum sativum* is highest 1600 M tonnes among all vegetables and all blocks also the total area and production of the vegetables of the district are 2745 ha and 41506 M tones respectively (Table7).

Table 7- Vegetable production during year 2006-07

Sl. No	Name of Blocks	*Pisum sativum*		*Brassica oleracea*		*Solanum melongena*		*Allium cepa*	
		Area (ha)	Production (M tones)	Area (ha)	Production (M tones)	Area (ha)	Production (M tones)	Area (ha)	Production (M tones)
1	Bhatwari	44	210	17.37	248.55	3.50	20.60	0.50	12
2	Dunda	65	550	35.68	640.45	7.50	210.40	18.10	375
3	Chinyalisaur	27	240	8.40	117	8.50	10.50	10.50	150.50
4	Naugaon	260	1600	10.75	228	3.50	40.50	40.40	800.50
5	Purola	155	1250	5.00	50	0.50	7.50	1.50	18.50
6	Mori	55	130	8.80	142	1.50	27.50	1.00	15.50
	Total	**606**	**3980**	**86**	**1177.45**	**25**	**317**	**72**	**1372**

Continued…

Sl. No	Name of Blocks	*Capsicum annum*		*Lycopersicon esculentum*		*Solenum tuberosum*		Other vegetables		Total	
		Area (ha)	Production (M tones)	Area (ha	Area (ha)	Area (Ha)	Production (M tones)	Production (M tones)	Production (M tones)	Area (Ha)	Production (M tones)
1	Bhatwari	0.50	35.00	35.00	480.79	298.35	7045.60	5572.4	6257.55	298.35	7045.60
2	Dunda	2.50	60.00	60.00	165.5	150.65	3528.40	4371.1	6231.95	150.65	3528.40
3	Chinyalisaur	4.75	10.50	10.50	117.75	160.65	3725.80	2240.4	2869.60	160.65	3725.80
4	Naugaon	4.25	125.50	125.50	596.95	550.35	15205.20	885.5	21681.70	550.35	15205.20
5	Purola	1.00	70.00	70.00	76.00	370.00	8745.00	479.5	2369.50	370.00	8745.00
6	Mori	1.00	8.00	8.00	17.80	400.00	9045.00	1712.7	2095.70	400.00	9045.00
	Total-	**14**	**309**	**309.00**	**1454.79**	**1930**	**47295.00**	**15261.6**	**41506**	**1930**	**47295**

Total cereal area and production is 40589 ha and 53599 M tones respectively while total food grain area and production are 46811 ha and 59032 M tones respectively (Table-8).

Table-8- Area and Production of principal agriculture crops

Sl.No.	**Name of the crops**	**Area (ha)**	**Production (M tones)**
1	*Oryza sativa*	9884	16476
2	*Triticum aestivum*	15643	18393
3	*Zea mays*	5982	7969
4	*Barley*	175	203
5	*Glycine max*	48	37
6	*Macrotyloma uniflorum*	604	438
7	*Eleusine corocana*	5640	7308
8	*Echinochloa frumentacea*	2613	2775
	Total cereal	**40589**	**53599**
1	*Cicer arietinum*	4	2
2	*Lens culinaris*	40	100
3	*Phaseolus mungo*	593	213
4	*Cajanus cajan*	180	90
5	*Pisum sativum*	342	212
6	*Phaseolus vulgaris*	2195	2469
7	*Other pulses*	2868	2347
	Total food grains	**46811**	**59032**

The extension of cultivation to this area will be expensive, since it requires extensive work for soil and water conservation, irrigation and reclamation **(**Dewan, and Bahadur. 2005). On the basis of diagnostic survey and appraisal of existing traditional farming system for satisfying farmer needs which are ecologically and economically feasible, the following aspects should need immediate care and attention:

1- Preservation of genetic resources of the local species mostly exploited by the farmers

2- Identification of multipurpose woody species

3- Identifying crop associations which can be fitted in to different intensities of shed

4- Qualitative and quantitative interaction between plants and soil in different type of associates

5- Awareness among the rural people through trainings, workshops and seminars

6- Involvement and encouragement of rural women in awareness programmes by organizing site and need specific training, workshops and seminars.

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Constraints of Resource Poor Farmers and Causes of Low Crop Productivity in a Changing Environment

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Abstract: This paper reviewed the constraints of resource poor farmers and courses of low productivity in a changing environment with a particular focus on sub-Saharan Africa. In doing these books, journals, newspaper interaction with farmers and observation including the internet were used in the production of this paper. In most sub-Saharan African countries, there is serious low crop yield as a result of use of poor planting materials, soil infertility, erosion problem and climate change. Also, the farmers attitude through improper use of agro-chemicals or excessive use of high external inputs causing damage and posing danger to the farmer, his crops, livestock, man and his environment. Government intervention is required to improve extension services, train farmers on proper use of agro chemicals, proper soil management and good methods of pest control techniques through monitoring. Adequate funding of research institutes and universities is required to help them find solutions to these mounting problems including climate change and to avert immediate occurrence of famine now and in the future. Organic agriculture is advocated for as it is sustainable and environment friendly. [Researcher. 2009;1(6):48-57]. (ISSN: 1553-9865).

Keywords: Resource poor farmers changing environment and crop productivity.

Introduction

Famine occurrence has always been the lot of man and his livestock including wildlife, and has not been restricted to the remote and distant parts of the earth. When it occurs, all are affected and the ecosystem is stressed up to overcome and sustain human and animal life. However, in order to survive, man has often strive to secure his harvest but the variations and changes that occur locally or globally, on and off season had restricted him to the control by environmental factors such as temperatures, rainfall, wind, pressure relative humidity etc including pests and diseases.

The activities of man in the pursuit of food, fiber and energy production are creating changes in our ecosystem. The carbon dioxide emission and other green house gases are on the increase and people are complaining of global warning or climate change. This is evident as state of the art equipments such as computer models using as much theoretical understanding of the earths weather behaviour and data as possible to confirm that global warning is occurring along with shifting patterns of rainfall and incidences of extreme weather events.

If this scenario continues unabated, the global community will witness serious changes in our environment otherwise called climate change. Thus, the global environment will have serious effects and consequences for natural and agricultural ecosystems and for the society as a whole. The objective of this

paper therefore is to x-ray some of the constraints of resource poor farmers and causes of Low crop productivity in a changing environment including those caused by the farmers themselves.

Changes in our Agro-Ecology

The changes in the ecosystems could alter the location of the major crops production regions on the earth. Hence, agricultural production and productivity is particularly vulnerable to disruption by weather (IFPRI, 2004). To contend with these happening induced by weather and climate changes, we have to produce more food, fibre and energy including other commodities and to secure them thereafter, to cope with increasing population under diminishing per capita arable land, water, degrading soil resources and expanding biotic stresses (Paarlberg, 2002).

In addition to all these stringent constraints, shifting from normal weather with its associated extreme events, zones of crop adaptation and cultural practices required for good crop production will surely change and problems will escalate. Also, plant responses to climate changes are not uniform and thus there will be winners and losers within a given agro-ecosystem and this marks variations and diversity within species and cultivars (Spore, 2008)

All these have implications for crop production and productivity within a given agro-ecology. Therefore, the induced instability by climate and weather in food, fibre and energy supplies will alter social and economic stability and regional competitiveness and global consciousness. Thus the developing and poor nations of the world will suffer more. For example, the global food crises recently witness in 2008 saw dramatic rise in world food prices, creating global crises and causing political and economic instability and social unrest in both poor and rich countries.

The singular item "food" shows its strength in world politics, peace or war since one must eat to sustain life on earth. Therefore, mechanisms, ways and means to produce food fibre and energy and too sustain it, would be an arbiter to world peace because "a hungry man is an angry man". Food production is therefore central to human development and world peace. In charting the future, scientists agree that more extreme weather pattern is on the horizon. A range of forecasts predict increased drought in some parts of Africa and flooding in others while rising sea levels and tropical cyclones threaten small island states. Thus nothing can stop the march of climate change, but there is still time to temper its effects (Spore, 2008).

The Resource Poor Farmers and Farms

In Nigeria, about 70-75% of the populations are farmers. Members of the family participate in cultivating family lands with the wealthy ones engaging in outright purchase from others or on lease to produce food and fibre. Generally, the people are poor and most of them are small scale farmers who produce majority of the food in Nigeria. They are said to be resource poor and practice small scale farming (0.1-2 ha). As there are many poor and developing countries in the world today so also is the number of resource poor farmers who produce in small portions and hectarages of land and the excess after family requirements and needs are met are pulled together for the markets to feed other families who cannot farm or have limited access to land resources. The small scale farmer is central in food and fibre production in the world. They play significant role in economy stabilization and in hunger mitigation. However, recent production trends in Africa indicate a serious farming lag (IFPRI, 2004). According to Paarlberg (2002); in developing countries as a whole between 1970 and 2000, per capita food production increased by 51 percent and this is reasonable if it were secured.

The only way out of the woods of hunger is through strongholds in family farms to produce food through confirmed experiments (adapted on farm research) which can sustain and drive small holder productivity forward. These family farms have lower labour related transaction cost and have more family workers per hectare, each motivated to work and each able to find, screen, and supervise hired workers (Lipton, 2005).

Soil Quality and Fertility as a Constraint in Food Production.

Soil is a natural body upon the earth on which crops grow, and its quality varies widely, ranging from very old, weathered and leached rocks to soils inherently low in nutrient because of their clay and organic matter content (DFID, 2002).

In sub-Saharan Africa, soil quality is classified as degraded on about 72 percent of arable land and 31 percent of pasture land (Scott, *et al* 2000). In addition to the natural nutrient deficiencies in the soil, soil fertility is declaring by the year through nutrient mining "whereby nutrients are removed over the harvest period and lost through leaching erosion or other means (Mark *et al* 2005).

According to UNU-INRA/World Bank report, (1999), nutrient levels have declined over the past 30 years, resulting in low levels of minerals like nitrogen (N), phosphorus (P) and potassium (K) because for an estimated 1 million square kilometers of cultivated land, the rates were 660 $kgha^{-1}$ for N, 75

kgha^{-1} for P and 450 kgha^{-1} for K. they explained that in contrast farms in North America have actually increased the average nutrient level per hectare up to 2000kg for N, 700kg for P and 1000kg for K over the same period. The contrast is found in Nigeria where the tropical lush growth of the rainy season may give an illusion of a Garden of Eden where food production could never be a problem (Babalola 2002). The basic physical, chemical and biological limitations of both soil and its environment are not always realized by the ordinary man. Accordingly, Babalola (2002) and Eshett (1993) pointed out that our soils in Nigeria and indeed any other tropical soils are inherently infertile, highly weathered, leached and contain low activity clay minerals which make them behave like "sieves" retaining little water during rainfall and irrigation and little nutrients or plant food. Also, organic matter content of the soil, the seat of plant food is very low and this confers a weak structure on the soil. Thus, the soils are fragile and their aggregates collapse readily under the impact of raindrops making them highly susceptible to soil erosion (Babalola, 2002). Further to these problems, the too low soil moisture retention and the erratic and poor distribution of rainfall cause severe water stress in plants which reduces yield. The situation calls for cheap ways and means of ameliorating the degraded tropical soils of Nigeria and others especially, areas with high demographic pressure. Hence the use of dehydrated pig manure as recommended by Onweremadu *et al* (2003) and use of poultry manure as recommended by Ibeawuchi *et al* (2006) will help rebuild the water holding capacity of these soils by building up organic matter content and increase crop yields of the tropical soils.

Many of the soils in the semi-arid parts of Nigeria have a strong liability to surface crusting or sealing which reduces rainfall penetration into the soil encourages run-off and subsequent soil erosion (Babalola 2002). FAO (1991) has classified the Nigerian landmass as high productivity 0%, good productivity 5.0%, medium productivity 46.5% and low productivity 48.5%. Hence only about 50% of soils in Nigeria are considered to have medium to good productivity potentials for food, fibre and energy production and this is mainly on the account of their depth, and quality of clay content. It is obvious that majority of our low crop productivity can be traced to a large extent to soil infertility.

Table 1: National average yield t ha^{-1} of some crops in Nigeria and the USA for 1993

Crops	Nigeria	USA	Percentage of USA yield
Maize	1.25	8.60	14.5
Rice	2.28	6.71	34.00
Soybean	1.00	2.81	35.60
Sorghum	1.00	4.58	21.80
Groundnut	1.14	2.99	38.10
Sweet potato	8.00	18.14	44.10
Yam	11.00	-	-
Tomato	10.00	63.66	15.70

Source: FAO Year Book Vol. 48 1994.

The Table 1, showed some selected crops in Nigeria compared with those of the USA. The differences are largely due to differences in the nature of the soil clay fraction (Babalola, 2002) and too high technology farming with improved hybrid seeds and planting materials. Yields of maize, rice, sorghum and sweet potatoes per hectare in Nigeria were less than 40% of the average yields per hectare of crop yield in the USA (FAO, 1994). Several studies point to the possible causes of soil fertility depletion in Africa and elsewhere in the tropical and subtropical environment (Barret *et al*, 2002).

The major factors commonly identified include:

- The limited adoption of inorganic fertilizer.
- Organic fertilizer replenishment strategies
- Limited adoption of soil and water conservation measures.
- Use of heavy machinery on soils with weak soil structure.
- The declining use and length of fallow periods
- The expansion of agricultural production into marginal and fragile areas such as

cultivation on steep slopes or an arid area without proper anti-erosion measures.

- The use of animal dung and crop residues as fuel and feed rather than as soil amendments and
- The removal of vegetation through overgrazing, logging, development (urban and industrial) and domestic use.

Soil fertility depletion can also be related to many socio-economic, institutional and policy related factors. Rapid population growth as witnessed in Nigeria in the last 30 years can help give an insight on land resources management and use. Limited access to agriculture related technical assistance and lack of knowledge about profitable soil fertility. Management practices can lead to expansion into less-favoured lands according to (Pender, *et al* 1999).

Access to Fertilizer can also be Constrained by:

- Market liberalization and trade policies that increase fertilizer prices relative to commodity prices.
- Limited access to market and infrastructure
- Limited to development of output, input and credit markets and
- Poverty and cash constraints that limit farmers' ability to purchase fertilizer and other inputs and cause them focus on the short term.

However, a number of approaches have been adopted to deal with soil infertility in sub-Saharan Africa. These include approaches such as organic farming, High external input agriculture, low external input sustainable agriculture, and integrated soil fertility management (Pender and Mertz 2004, Mokokha *et al* 2001)

Excessive Use of High External Inputs

Most farmers (the small scale or resources poor farmers) apart from being poor are mostly illiterates who do what pleases them to the detriment of the environment and high yield of crops. The improper use of chemicals such as pesticides and herbicides is a major environmental concern in sub-Saharan Africa, although majority of them have limited access to these chemicals and often cannot justify their need and use. Those of them who come in contact with these chemicals over use them to the detriment of their lives, and crops including animals and man. According to Hijmans *et al.* (1999), farmers in some sub-Saharan countries spray their potato fields up to 15 times during a single growing season of 4-6 months in order to combat late blight potato disease caused by *Phytophora infestans.* The problem with pesticides is that as one uses them on these organisms, they emerge to be renewed emerging more virulent to the fact that the chemicals cannot do them any harm again. This problem of misuse of agro-chemicals is a serious constraint to farmers in Nigeria and elsewhere in sub-Saharan Africa.

Furthermore, on the other hand, excessive or incorrectly proportioned doses of chemical fertilizer represent various forms of environmental risk (Scott *et al*, 2000). For instance, too much fertilizer application to the soil may result in residues, contaminating local water supply including ponds otherwise available for fish farming. Conversely, too little fertilizer application can result in low yields, declining soil fertility and eventually soil exhaustion. At any point or anywhere, inefficient use of fertilizer causes problems to the environment and contributes to environmental pollution. In fact, the spread of fertilizers or pesticides residue into water supplies through irrigation systems (***fertigation***) or field run-off attract a growing problem to human habitation especially in the southeast agro ecological zone of Nigeria where there is high rainfall, erosion problems and destruction of the ecosystem in search of the liquid gold i.e. petroleum resources. These chemicals that escape into our environment damages plants, insects (non-target organisms) and livestock and poses great threat to the source of human drinking water in many farming communities. However, looking at it from different angles, water pollution is not restricted to production only but also includes post harvest activities (Scott *et al* 2000).

Pest and Diseases

The aspect of pest and disease in reducing crop productivity cannot be over emphasized. Pests are all organisms causing significant economic damage to crops while diseases are disorders or physiological disturbances of the normal functions of plants caused by physical, chemical or biological factors. These pests and disease reduce the income of the farmer, crop yield, market prices and value of the affected crop. Food and cash crops constitute the bulk of agriculture production and the productivity is generally known to be low in most parts of Nigeria. This is partly due to losses from pests and diseases which nearly all crops suffer. These losses in most cases begin with the planting of the seed and continue throughout the filed phases of production, storage and processing. They range from hidden losses due to soil inhabiting nematodes, insects, fungi, bacteria, viruses and other organisms. According to Ume *et al* (2000), diseases are estimated to cause about 20-30 percent loss of annual agricultural production in developing countries.

Conclusion

This paper looked at some of the constraints our farmers have in producing healthy food in a changing agro-ecosystem. The farmer is central to food production and causes some damages to the soil which is the base of production through improper use of farm machines, farm inputs and improper soil management. The farmer also is implicated in the use of excessive or incorrect does of chemical inputs which affect the targeted and non-targeted organisms and pollute the environment. All these problems reduce the soil fertility and cause low productivity. Hence, farmers should be taught proper external input management, proper soil management and good methods of post harvest activities. By these shortcomings, organic agricultural production is advocated for at this point which has been proved to be more sustainable and environment friendly. Farmers have to be on alert as better observations at the local levels will help produce more accurate forecasts of climate and weather to enable them target planting and harvesting dates of crops to escape pests and disease and the effect of climate change.

There should be improved extension services to farmers through government support. Also, government should support the farmers for improved planting materials and environmental friendly high external inputs for increased yield of crops. This should be done through increased funding of research institutes and the universities to help them find solutions on the way out of impending flood, drought and possible future famine at the global scale as climate related disasters, are already taking heavy toll, and causing massive damage to crops, livestock and infrastructure.

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Diversity of Aquatic Fungi in Relation to Environmental Conditions in Tunga River (South India)

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Abstract: The present investigation was carried out on studying the impact of environmental conditions on diversity of aquatic fungi. Tunga river water was assessed for the physico-chemical factors of the water with respect to seasonal variations with reference to aquatic and aero-aquatic fungal diversity. Tunga river water was assessed at 6 different study stations with study period from April, 2007 to March, 2009. During the study, 12 aquatic fungal species and 14 aero-aquatic species, a total of 26 fungal species belonging to class *chytridiomycetes*, *oomycetes*, *phycomycetes Ascomycetes*, *Zygomycetes* and *Deuteromycetes* were isolated and identified. Maximum fungal distribution was recorded during the monsoon period followed by pre-monsoon periods in all the four stations. But comparatively less percentage of fungal incidences was recorded during post monsoon period. The present investigation revealed that the difference in the percent of occurrence, distribution of aquatic fungi and aero aquatic in periodically proved to depend on the physico-chemical factors of the water and also on seasonal variations. [Researcher. 2009;1(6):54-62]. (ISSN: 1553-9865)

Key words: Tunga river, aquatic fungi, physiochemical factors, aero aquatic fungi.

1. Introduction

Ecology of aquatic fungi has not attained the degree of prominence as the ecology of soil fungi. The qualitative composition of the fungal population in water is now becoming fairly well known. Heterotrophic organisms are usually present in natural water in direct proportion to the physico-chemical nature of aquatic environment. A wide variety of aquatic fungi such as *chytridiomycetes*, *Saprolegniales* and *Peronosporales* are found in fresh water. Till 1942, available information was mainly concerned to "oosporic phycomycetes" or "water molds" which till then were regarded as true water fungal flora. In 1942, Ingold reported a distinctive and abundant flora of conidial fungi as aquatic hypomycetes. Another assemblage of aquatic hypomycetes was recognized as aero aquatic fungi with the mycelium in submerged decaying leaves (Glen-Bott, 1951). The fungi encountered in fresh water are divided into two principal groups, The Hydro fungi which require the presence of water to complete their life cycle and geo-fungi or typical soil fungi which were not specially adopted to aquatic existence, but they might be found in water because of adequate supply of nutrients, these were regarded as "Facultative aquatic fungi" (Cooke 1963).

The aquatic fungi play a key role in the decomposition of leaf litter in aquatic environments (Barlocher, 1992; Gessner, Chauvet & Dobson, 1999). Fungal activity on leaves is affected by several environmental factors, such as dissolved nutrients in water (Suberkropp & Chauvet, 1995; Gulis & Suberkropp, 2003), temperature (Chauvet & Suberkropp, 1998), turbulence (Webster, 1975) and pH (Dangles et al., 2004). Generally, low to moderate nutrient concentrations stimulate fungal activity (Gulis et al., 2006). Number of species of aquatic hyphomycetes was lower in a side arm of the Rhone River than in the main channel, and this difference was attributed to lower water velocity and dissolved O_2 in the side arm (Chergui,1988).

Fresh water bodies receives various category of waste materials, many of them are organic in nature. These organic wastes are easily degraded by microbes like fungi and bacteria, naturally present in river water. Hunter (1975) studied the water molds and their role in the degradation of wastes in the river great use and its tributaries. The various wastes accumulated in the water bodies creates several problems and are responsible to kill aquatic fungi, which are useful for the bio-degradation process. Thompstone and Dix (1985) identified 25 isolates of *Achlya* and *Saprolegnia sp*. Singh (1982) studied the

distribution, occurrence and cellulose decomposition of the five species of aquatic hypomycetes. The study of aquatic fungi have been carried out in all over the world by Coker (1923), Dick (1990), Johnson (1956), Scott (1961), Middleton (1943), Seymour (1970), and Robertson (1980). The studies of aquatic fungi in Indian was carried out by Sati (1997), Paliwal and Sati (2009), Bhargava (1946), Dayal (1968), Khulbe (1977), Mer et al. (1980), Manoharachary (1991), and Mishra and Dwivedi (1987). The present investigation was carried out on diversity, distribution and periodical variation of aquatic fungi and aero aquatic fungi in Tunga River, Shimoga district, Karnataka, South India.

2. Materials and Methods

Study site Shimoga - a South west region of India, is located between 13° 27' and 14° 39' North latitude and 74° 38' and 76° 4' East longitude. Shimoga is located almost at the central part of Karnataka state in the Malnad region bounded by Sahyadri ghats on the east direction. The eastern part of the district comes under the semi-Malnad zone with plain topography and occasional chains of hills covered with semi-deciduous vegetation. Shimoga is a true picture of nature's bounty-landscapes dotted with waterfalls. Hence, climatically major parts of this study area represent a temperate zone and monsoon pattern of rain fall with dry summer and winter. The Tunga river of South India selected for the present study originates in the Western Ghats on a hill known as *Varaha Parvata* at a place called *Gangamoola*. From here, the river flows through two districts in Karnataka -Chikmagalur District and Shimoga District. It is 147 km long and merges with the Bhadra River at Koodli, a small town near Shimoga City, Karnataka.

Initially, physico-chemical characteristics of Tunga river water was studied, water samples were collected from each of the identified sampling stations at monthly intervals for a period of one year during April 2007 to March 2009 from Tunga river in sterilized plastic bottles periodically. The detailed information of the sampling stations selected for the study purpose are given in Table 1.

To study the distribution and occurrence of aquatic fungi of Tunga river water and organic waste material's like twigs, decaying aquatic plant parts were collected from lake. Water was collected in 2 litres plastic cans and decaying plant materials were collected in polythene bags of 1kg capacity from the identified stations at monthly intervals.

Isolation of fungi was carried out by following isolation techniques *viz.*, Incubation and baiting techniques in the laboratory. In incubation method, decaying leaf litter, aquatic plant parts, woody materials were collected from the river. The materials were broken into small pieces and incubated on wet blotters in petriplates, The materials along with petri-plates were kept in the incubator under laboratory condition (22$\pm$ 2ºC temperature) for about 8 days. In baiting method, sterilized broken pulses and pieces of blotter papers were used as fungal baits. The known quantity of water was taken in the sterilized petri-plates, and then broken pulses and paper pieces of blotter paper were added. The plated materials were kept for incubation under laboratory conditions (22$\pm$2ºC temperature) for about 7 to 8 days, at the end of the incubation period the colonized fungi were found on the incubated materials.

The isolates were purified by single hypha culture method. Culturing of few aquatic and Extra-aquatic was done in the laboratory on cornmeal and potato dextrose agar media for pure culturing. Identification and characterization of fungi were made with the help of aquatic fungi manual by Khulbe (2001) with support of various standard monographs (Coker, 1923; Johnson, 1956; Scott, 1961; Dick, 1990 and Barnett.H.L, 1962).The physico-chemical properties (pH, water temperature, and total organic matter) of water analyzed by following standard methods of APHA (1989). Calculations of total and individual fungal occurrence were made at the end. All calculations were made in terms of percentage by following simple formula.

$$\frac{\text{Number of samples in which fungi appeared}}{\text{Total number of samples plated}} \times 100$$

The individual fungal occurrence of each fungal species was calculated by using following simple formula.

$$\frac{\text{Individual fungal species appeared in the samples}}{\text{Total number of colonies of fungi that grew from sample}} \times 100$$

3. Results

The six different sample collection stations (station-I, station-II, station-III, station-IV, station-V, station-VI) were selected in the river based on the extent of pollution and Anthropogenic activities. From all the six sampling station, a total of 26 aquatic and aero-aquatic fungal species were isolated and identified, about 12 species of aquatic fungi belonging to 7 genera of class *chytridiomycetes and oomycetes* were encountered, which includes: *Achlya debaryana, A. Orion, A. prolifera, A. recurva, Allomyces arbuscular, A. anamalus, Aphonomyces laevis, Pythium elongatum, P. debarianum*

,Saprolegnia ferax, S. parasitica and S. terrestries. The two yearly average percentage of aquatic fungi distribution of individual fungi has summarized in the table 2. And about 14 species of aero-aquatic fungi belonging to 8 genera of aquatic *phycomycetes, Ascomycetes, Zygomycetes and Deuteromycetes* were isolated. Which includes, *Alternaria alternata, Aspergillus flavus, A. fumigatus, A. niger, Chaetomium globosum, Cladosporium cladosporides, Fusarium oxysporum, F. solani, F.equisiti, F. semitectum, P. citrinum, Rhizopus nigricans, Trichoderma viride and T. harzianum.* The two yearly average percentage of aero aquatic fungi distribution can be depicted from Table 4.

Table 1. Sampling stations selected for the present study

Sl. No	Sampling Stations	Location
01	S_1	Thirthahalli, Near new Bridge (Shimoga District), South India.
02	S_2	Mandagadde, Near Bird wild life Sanctuary (Shimoga District), South India.
03	S_3	Sakarebile, (Shimoga District), South India.
04	S_4	Gajanur,Near dam site upstream stream (Shimoga District) , South India.
05	S_5	Shimoga,Near old Bridge (Shimoga city) , South India.
06	S_6	Koodli, Near Nandi temple(Shimoga District)), South India. (Before confluence point)

Table 2. Seasonal average values of occurrence of Aquatic fungi in different stations of Tunga River from Apr 2007 to March 2009.

Sl No	**Species of Fungi**	**Station-I**			**Station-II**			**Station-III**		
		PM	**M**	**PO-M**	**PM**	**M**	**PO-M**	**PM**	**M**	**PO-M**
1.	***Achlya debaryana***	31.5	43.0	30.4	30.2	28.5	20.6	29.5	29.2	26.2
2.	***Achlya orion***	27.3	31.6	19.8	13.4	19.0	11.2	16.5	16.9	15.0
3.	***Achlya prolifera***	7.9	11.5	8.9	6.0	8.9	3.5	6.2	7.3	4.5
4.	***Achlya recurva***	4.6	7.8	5.0	3.7	6.0	2.5	3.7	5.9	2.0
5.	***Allomyces arbuscula***	16.5	18.9	17.5	11.6	12.5	13.5	12.5	10.9	13.8
6.	***Allomyces anomalus***	13.7	19.3	12.0	8.5	11.5	14.5	9.2	10.0	9.5
7.	***Aphanomyces laevis***	13.2	20.5	12.5	11.5	13.5	11.0	12.3	14.0	10.5
8.	***Pythium elongatum***	27.6	30.0	23.2	24.2	26.5	14.5	22.6	25.3	13.5
9.	***Pythium debarianum***	32.0	40.0	31.5	19.4	20.5	19.0	17.0	16.6	18.5
10	***Saprolegnia ferax***	24.0	28.3	22.5	15.5	19.0	13.4	13.0	16.2	11.6
11	***Saprolegnia parasitica***	16.2	20.5	18.6	11.5	12.8	10.0	11.5	14.6	8.9
12	***Saprolegnia terristries***	12.5	14.8	9.7	11.5	13.0	8.8	10.8	12.0	8.5
Sl. No	**Species of Fungi**	**Station-IV**			**Station-V**			**Station-VI**		
		PM	**M**	**PO-M**	**PM**	**M**	**PO-M**	**PM**	**M**	**PO-M**
1.	***Achlya debaryana***	19.5	24.0	19.5	18.2	23	17.5	24.5	28.0	19.5
2.	***Achlya orion***	14.0	16.5	12.3	13.5	15.0	12.3	16.3	19.2	16.3
3.	***Achlya prolifera***	5.0	6.0	3.5	4.9	4.5	3.0	4.0	4.9	3.2
4.	***Achlya recurva***	3.0	4.5	1.5	2.5	3.5	2.0	3.0	3.5	2.9
5.	***Allomyces arbuscula***	10.5	10.7	11.2	12.0	10.0	11.1	13.0	16.8	12.0
6.	***Allomyces anomalus***	14.3	14.5	11.5	13.8	14.0	12.2	13.5	13.8	12.0

7.	***Aphanomyces laevis***	13.3	13.8	10.0	12.2	12.6	9.3	11.0	12.0	9.0
8.	***Pythium elongatum***	21.0	24.3	12.0	20.0	21.0	11.0	23.5	25.6	14.5
9.	***Pythium debarianum***	15.5	16.0	17.5	14.8	15.7	15.0	14.0	15.2	14.6
10	***Saprolegnia ferax***	12.5	14.5	10.6	11.5	13.5	9.8	11.0	12.5	8.0
11	***Saprolegnia parasitica***	10.5	13.6	7.0	9.2	12.0	7.3	11.5	11.0	9.5
12	***Saprolegnia terristries***	8.0	8.5	7.0	7.0	7.5	6.0	8.6	9.1	6.5

PM-Pre Monsoon; M- Monsoon; PO-M-Post Monsoon.

Table 3. Percent occurrence of different of Aquatic fungi in different stations of Tunga river from Apr 2007 to March 2009.

Sl. No	**Species of Fungi**	**Station-I (%)**			**Station-II (%)**			**Station-III (%)**		
Study period		**07-08**	**08-09**	**Avg**	**07-08**	**08-09**	**Avg**	**07-08**	**08-09**	**Avg**
1.	***Achlya debaryana***	35.0	45.0	40	20.0	27.2	23.6	20.2	24.0	22.1
2.	***Achlya orion***	29.3	30.6	29.9	20.9	29.2	25.0	16.5	20.2	18.35
3.	***Achlya prolifera***	7.9	11.5	9.7	14.0	22.0	18	7.2	8.3	7.75
4.	***Achlya recurva***	6.5	8.6	7.55	5.8	10.2	8.0	3.0	6.9	4.95
5.	***Allomyces arbuscula***	18.5	20.4	19.4	3.5	6.8	5.15	12.5	14.8	13.65
6.	***Allomyces anomalus***	14.7	20.4	17.5	10.9	12.5	11.7	10.2	13.5	11.8
7.	***Aphanomyces laevis***	14.4	21.5	17.9	7.4	11.5	9.4	13.3	17.0	15.15
8.	***Pythium elongatum***	27.5	31.5	22.5	12.2	14.4	13.3	22.6	19.3	20.9
9.	***Pythium debarianum***	32.0	40.0	36.0	23.0	24.5	23.75	20.6	21.05	18.0
10	***Saprolegnia ferax***	24.0	28.3	26.15	14.9	20.2	17.5	12.0	15.2	13.6
11	***Saprolegnia parasitica***	17.2	20.5	18.85	12.4	16.4	14.4	93.8	12.6	11.2
12	***Saprolegnia terristries***	11.5	14.3	12.9	17.4	20.2	18.8	8.0	10.5	10.25
Sl. No	**Species of Fungi**	**Station-IV(%)**			**Station-V (%)**			**Station-VI (%)**		
Study period		**07-08**	**08-09**	**Avg**	**07-08**	**08-09**	**Avg**	**07-08**	**08-09**	**Avg**
1.	***Achlya debaryana***	22.2	25.0	23.6	21.1	24.0	22.5	26.3	27.6	26.9
2.	***Achlya orion***	14.5	18.2	16.3	12.1	16.1	14.1	18.3	20.0	19.1
3.	***Achlya prolifera***	7.0	7.5	7.2	7.2	6.3	6.75	7.25	9.1	8.15
4.	***Achlya recurva***	5.0	3.8	4.4	4.5	3.2	3.8	8.0	8.5	8.25
5.	***Allomyces arbuscula***	11.5	12.8	12.1	10.5	11.5	11.0	13.0	16.0	14.5
6.	***Allomyces anomalus***	9.2	12.1	10.6	7.2	11.1	9.16	14.3	14.7	14.5
7.	***Aphanomyces laevis***	12.9	16.0	14.4	9.7	15.0	12.3	10.8	12.5	11.6
8.	***Pythium elongatum***	21.6	20.0	20.8	18.3	19.0	18.65	25.8	27.2	26.5
9.	***Pythium debarianum***	21.0	19.5	17.3	20.0	18.65	22.0	26.8	26.8	24.4
10	***Saprolegnia ferax***	11.0	14.2	12.6	9.1	13.2	11.15	13.6	16.8	15.2
11	***Saprolegnia parasitica***	8.5	11.5	10.0	6.5	9.0	7.75	13.5	14.5	14.0
12	***Saprolegnia terristries***	8.0	9.5	8.5	6.0	5.5	5.7	10.6	11.2	10.9

Table 4. Percent occurrence of different of Aero- Aquatic fungi in different stations of Tunga river from Apr 2007 to March 2009.

Sl. No	**Species of Fungi**	**Station-I (%)**			**Station-II (%)**			**Station-III (%)**		
Study period		**07-08**	**08-09**	**Avg**	**07-08**	**08-09**	**Avg**	**07-08**	**08-09**	**Avg**
1.	***Alternaria alternata***	18.0	16.5	17.2	10.0	9.2	9.6	10.0	8.6	9.3
2.	***Aspergillus flavus***	39.5	36.	37.9	19.6	2.6	21.1	20.5	23.3	21.9
3.	***Aspergillus fumigatus***	34.0	32.5	33.2	20.3	21.4	20.8	17.0	16.7	16.7
4.	***Aspergillus niger***	38.6	39.5	39.0	25.0	22.5	23.5	21.5	22.4	21.9
5.	***Chaetomium globosum***	13.0	12.6	11.8	8.5	5.7	7.1	7.6	7.0	7.3
6.	***Cladosporium cladosporides***	17.8	13.5	15.6	5.7	5.0	5.3	4.5	4.9	4.7
7.	***Fusarium eqiusiti***	26.6	23.7	21.1	20.3	18.4	19.3	18.6	16.5	17.5
8.	***Fusarium oxysporum***	30.6	31.4	31.0	22.3	21.5	21.9	19.3	18.8	19.5
9.	***Fusarium semitctum***	32.3	30.5	31.4	27.3	22.6	24.9	19.0	20.3	19.6
10	***Fusarium solani***	28.6	26.5	27.5	19.0	16.2	17.6	18.0	15.4	16.7
11	***Penicillium citrinum***	16.8	15.0	15.9	10.6	12.0	11.0	10.0	11.5	10.7
12	***Rhizopus nigricans***	17.5	14.6	16.5	10.8	7.5	9.5	11.0	9.5	8.0
13	***Trichoderma viride***	15.5	14.0	14.7	8.16	8.5	8.3	7.3	6.5	6.9
14	***Trichoderma harzianum***	15.5	18.6	17.0	8.5	6.8	7.6	8.5	6.2	7.3

Sl. No	Species of Fungi	Station-IV(%)			Station-V (%)			Station-VI (%)		
Study period		07-08	08-09	Avg	07-08	08-09	Avg	07-08	08-09	Avg
1.	*Alternaria alternata*	9.6	8.2	8.9	7.3	8.5	7.7	13.0	14.7	13.8
2.	*Aspergillus flavus*	19.3	22.0	20.6	18.9	21.0	19.9	23.5	22.7	23.1
3.	*Aspergillus fumigatus*	16.2	17.3	16.7	15.2	17.0	16.1	18.3	20.8	19.5
4.	*Aspergillus niger*	20.5	21.3	20.9	19.2	20.3	19.8	19.4	26.0	22.7
5.	*Chaetomium globosum*	6.2	7.1	6.6	5.2	7.3	6.2	10.1	9.8	9.9
6.	*Cladosporium cladosporides*	4.3	5.0	4.6	4.1	4.5	4.3	6.8	7.1	6.9
7.	*Fusarium eqiusiti*	16.6	17.0	16.8	15.0	14.5	14.5	19.1	16.5	17.8
8.	*Fusarium oxysporum*	18.2	18.5	18.3	17.2	19.3	18.2	24.0	22.6	23.3
9.	*Fusarium semitctum*	17.0	19.5	18.2	16.0	20.5	18.2	22.3	18.0	20.1
10	*Fusarium solani*	17.0	14.4	15.7	16.0	19.0	17.5	19.5	17.5	18.5
11	*Penicillium citrinum*	9.8	10	9.9	8.9	10.1	8.5	13.8	14.5	14.1
12	*Rhizopus nigricans*	10.1	11.2	10.6	9.4	10.3	9.8	13.5	11.6	12.5
13	*Trichoderma viride*	6.2	8.5	7.3	6.0	7.1	6.5	9.3	10.5	9.7
14	*Trichoderma harzianum*	7.3	5.2	6.3	6.8	8.1	7.4	11.0	8.5	9.7

Table 5. Seasonal average values of occurrence of Aero-Aquatic fungi in different stations of Tunga River from Apr 2007 to March 2009.

Sl. No	Species of Fungi	Station-I			Station-II			Station-III		
		PM	M	PO-M	PM	M	PO-M	PM	M	PO-M
1.	*Alternaria alternata*	35.4	39.2	18.6	26.3	29.2	12.6	21.4	23.5	14.6
2.	*Aspergillus flavus*	36.2	38.3	21.0	20.5	21.2	15.4	19.6	20.3	13.3
3.	*Aspergillus fumigatus*	37.8	39.3	28.2	24.4	26.5	14.3	20.9	22.3	13.2
4.	*Aspergillus niger*	39.9	43.2	30.0	21.2	27.6	18.0	19.2	22.3	15.0
5.	*Chaetomium globosum*	14.5	18.0	11.5	9.5	14.0	8.0	8.5	13.0	7.3
6.	*Cladosporium cladosporides*	20.1	22.4	14.0	12.5	14.5	9.0	11.4	15.3	6.5
7.	*Fusarium eqiusiti*	27.6	33.8	22.5	23.5	25.6	18.6	21.6	22.5	16.8
8.	*Fusarium oxysporum*	38.0	40.0	22.7	22.6	28.4	20.2	23.8	25.6	18.0
9.	*Fusarium semitctum*	31.6	37.4	22.5	20.6	23.1	18.9	21.5	23.8	10.7
10	*Fusarium solani*	29.8	33.5	18.6	10.5	14.6	10.5	12.5	13.0	7.5
11	*Penicillium citrinum*	20.0	23.0	15.5	13.4	15.5	6.8	11.8	13.4	12.0
12	*Rhizopus nigricans*	19.5	23.0	16.2	12.6	15.5	9.5	11.6	14.7	8.8
13	*Trichoderma viride*	14.5	18.5	13.5	7.5	10.0	6.5	6.0	12.5	4.6
14	*Trichoderma harzianum*	18.6	20.2	14.5	11.0	13.5	7.5	8.5	12.0	9.13
Sl. No	Species of Fungi	Station-IV			Station-V			Station-VI		
		PM	M	PO-M	PM	M	PO-M	PM	M	PO-M
1.	*Alternaria alternata*	20.4	22.0	13.0	19.0	21.0	12.0	26.3	27.4	19.6
2.	*Aspergillus flavus*	18.0	19.5	12.0	17.2	18.0	11.0	22.5	31.4	19.5
3.	*Aspergillus fumigatus*	19.0	21.8	12.5	18.3	22.0	11.3	20.7	22.0	13.6
4.	*Aspergillus niger*	18.5	21.4	13.2	16.5	20.0	10.2	23.2	26.4	20.5
5.	*Chaetomium globosum*	7.2	9.3	6.0	6.5	8.5	5.0	9.5	14.5	6.5
6.	*Cladosporium cladosporides*	10.5	14.0	5.8	9.3	12.0	4.0	12.0	15.5	8.0
7.	*Fusarium eqiusiti*	20.8	19.0	15.8	19.5	18.0	16.0	18.4	23.5	117
8.	*Fusarium oxysporum*	22.5	24.3	17.0	20.5	22.0	16.9	20.5	25.5	18.4
9.	*Fusarium semitctum*	19.2	19.8	8.2	17.0	18.5	6.7	20.2	23.0	15.8
10	*Fusarium solani*	9.8	11.6	10.0	7.0	10.0	6.8	13.4	18.6	10.6
11	*Penicillium citrinum*	10.5	12.2	11.1	9.8	10.7	7.9	14.5	15.0	12.7
12	*Rhizopus nigricans*	10.3	13.0	7.0	9.1	12.3	6.8	13.4	14.5	13.2
13	*Trichoderma viride*	5.3	9.8	3.0	4.7	6.8	3.4	6.2	5.8	4.0
14	*Trichoderma harzianum*	12.2	12.6	9.9	10.1	11.3	7.2	9.8	10.3	8.9

PM-Pre Monsoon; M- Monsoon; PO-M-Post Monsoon.

Table 6. Average values physico-chemical parameters of Tunga river for the year Apr 2007 to March 2009.

Sl. No	Physico-Chemical Parameters	Station-I			Station-II			Station-III		
Study period		**07-08**	**08-09**	**Avg**	**07-08**	**08-09**	**Avg**	**07-08**	**08-09**	**Avg**
1.	Air Temp. (^{0}C)	30.5	29.8	30.15	32.3	31.2	31.7	31.5	30.5	31
2.	Water Temp. (^{0}C)	25.0	23.0	24.0	24.6	24.8	24.7	25.0	26.0	25.5
3.	pH	7.6	7.1	703	7.59	7.7	7.6	7.6	7.8	7.7
4.	Electrical Conductivity	123	121	122.0	122.7	124.0	123.3	123.0	122.3	122.6
5.	Total Hardness (mg/l)	35.6	32.3	33.95	30.0	31.5	30.75	39.6	37.2	38.4
6.	Carbonates (mg/l)	0.10	0.12	0.11	0.12	0.11	0.11	0..16	0.15	0.155
7.	Bio-carbonates (mg/l)	1.8	1.6	1.7	2.0	1.5	1.7	1.8	1.6	1.7
8.	B.O.D (mg/l)	2.90	2.50	2.7	2.69	2.74	2.7	2.79	2.63	2.7
9.	D.O. (mg/l)	7.81	8.01	7.9	7.74	8.0	7.8	7.72	7.68	7.7
10	C.O.D (mg/l)	9.3	9.4	9.3	9.48	10.1	9.79	10.02	9.59	9.7
11	Sulphate (mg/l)	19.2	17.2	18.2	16.3	15.2	15.75	21.3	18.2	19.7
12	Chloride (mg/l)	18.7	17.5	18.1	18.0	18.3	18.1	24.2	22.8	23.5
13	Phosphate (mg/l)	0.13	0.11	0.12	0.08	0.10	0.09	0.16	0.14	0.15
14	Sodium (mg/l)	2.65	2.33	2.49	2.08	2.05	2.06	2.58	2.56	2.5
15	Potassium (mg/l)	1.13	1.10	1.1	0.8	1.10	0.95	1.35	1.33	1.34
16	Calcium (mg/l)	16.5	15.8	16.15	13.7	14.3	14	18.32	18.6	18.46
17	Magnesium (mg/l)	3.8	2.7	3.25	3.9	3.5	3.7	4.52	4.59	4.55
18	Total Dissolved solids	113.7	110.2	111.9	112.0	111.0	111.5	118.23	120.20	119.2
Sl. No	**Physico-Chemical Parameters**	**Station-IV**			**Station-V**			**Station-VI**		
Study period		**07-08**	**08-09**	**Avg**	**07-08**	**08-09**	**Avg**	**07-08**	**08-09**	**Avg**
1.	Air Temp. (^{0}C)	32.0	32.5	32.3	34.0	34.2	34.1	34.5	33.2	33.8
2.	Water Temp. (^{0}C)	26.12	26.7	26.4	26.61	26.8	26.7	26.4	26.5	26.45
3.	pH	7.98	7.8	7.8	8.0	8.2	8.1	7.9	7.8	7.85
4.	Electrical Conductivity	124.12	125.0	124.5	127.0	128.0	127.5	126.89	127.0	126.94
5.	Total Hardness (mg/l)	36.8	37.0	36.9	41.2	42.0	41.6	39.45	40.0	39.72
6.	Carbonates (mg/l)	0.17	0.18	0.17	0.19	0.20	0.195	0.18	0.17	0.175
7.	Bio-carbonates (mg/l)	1.9	2.0	1.9	2.4	2.3	2.35	2.39	2.35	2.37
8.	B.O.D (mg/l)	3.19	3.3	3.2	4.64	4.7	4.67	3.80	3.83	3.81
9.	D.O. (mg/l)	7.74	7.62	7.6	5.25	5.5	5.35	7.5	7.7	7.6
10	C.O.D (mg/l)	10.60	9.2	9.9	13.45	13.62	13.53	13.26	13.30	13.28
11	Sulphate (mg/l)	22.0	22.8	22.4	24.85	23.89	24.3	23.75	24.0	23.87
12	Chloride (mg/l)	24.31	24.8	24.5	26.91	26.70	26.8	24.0	24.5	24.3
13	Phosphate (mg/l)	0.17	0.18	0.17	0.21	0.22	0.215	0.14	0.15	0.145
14	Sodium (mg/l)	2.89	2.96	2.92	3.0	3.15	3.7	2.30	2.32	2.31
15	Potassium (mg/l)	0.9	0.11	0.10	1.42	1.47	1.44	1.80	1.82	1.81
16	Calcium (mg/l)	19.7	21.3	20.5	23.0	23.6	23.3	21.0	22.14	21.5
17	Magnesium (mg/l)	5.0	5.2	5.1	5.46	5.47	5.465	4.62	4.7	4.66
18	Total Dissolved solids	122.5	123.0	122.7	125.47	126.0	125.8	123.4	124..0	123.7

Table 7. Seasonal average values physico-chemical parameters of Tunga river for the year Apr 2007 to March 2009.

Sl. No	Physico-Chemical Parameters	Station-I			Station-II			Station-III		
		PM	M	PO-M	PM	M	PO-M	PM	M	PO-M
1.	Air Temp. (^{0}C)	34.02	27.69	29.22	35.0	26.50	29.0	34.72	27.53	30.0
2.	Water Temp. (^{0}C)	26.22	22.92	25.12	26.12	24.62	25.0	26.39	23.13	25.08
3.	pH	7.89	7.42	7.81	7.94	7.35	7.81	8.1	7.56	7.82
4.	Electrical Conductivity	124.57	118.0	119.18	126.12	118.8	122.6	127.7	130.59	124.5
5.	Total Hardness (mg/l)	44.12	22.23	28.0	46.12	22.1	23.5	45.18	24.59	33.5
6.	Carbonates (mg/l)	0.11	0.06	0.009	0.18	0.08	0.12	0.195	0.008	0.15
7.	Bio-carbonates (mg/l)	1.9	1.3	1.7	2.45	1.4	1.7	2.65	1.6	1.92
8.	B.O.D (mg/l)	2.88	2.37	2.6	3.58	2.39	2.84	3.68	2.59	3.15
9.	D.O. (mg/l)	7.18	8.82	7.8	7.09	870	7.79	7.19	10.0	7.80
10	C.O.D (mg/l)	9.71	8.65	9.1	11.48	9.52	9.82	12.84	10.12	10.1
11	Sulphate (mg/l)	20.22	13.32	17.3	24.09	14.54	18.0	24.9	18.26	21.6
12	Chloride (mg/l)	23.78	17.47	18.4	23.05	19.60	18.75	25.12	18.59	22.68
13	Phosphate (mg/l)	0.15	0.09	0.07	0.17	0.15	0.13	0.23	0.10	0.16
14	Sodium (mg/l)	2.69	1.56	1.9	2.95	1.86	2.52	3.47	2.12	2.71
15	Potassium (mg/l)	1.14	0.6	0.8	1.35	0.8	1.01	1.49	0.9	1.35
16	Calcium (mg/l)	15.41	10.25	13.2	15.5	12.85	15.4	19.41	17.85	18.74
17	Magnesium (mg/l)	3.57	2.50	2.7	4.14	3.58	3.82	5.11	4.0	4.71
18	Total Dissolved solids	117.3	109.3	113.0	118.12	112.1	114.5	120.8	113.56	120.50
Sl. No	**Physico-Chemical Parameters**	**Station-IV**			**Station-V**			**Station-VI**		
		PM	M	PO-M	PM	M	PO-M	PM	M	PO-M
1.	Air Temp. (^{0}C)	35.17	26.50	30.50	36.42	26.95	31.33	36.4	24.6	31.23
2.	Water Temp. (^{0}C)	25.45	24.0	25.2	26.8	23.56	26.34	26.90	23.0	26.0
3.	pH	8.35	7.6	7.96	8.3	7.7	7.90	8.7	7.2	8.0
4.	Electrical Conductivity	127.8	121.6	125.6	128.8	122	126.71	130.0	123.56	125.63
5.	Total Hardness (mg/l)	45.8	28.0	37.85	46.0	27.0	35.42	48.0	29.8	36.0
6.	Carbonates (mg/l)	0.19	0.007	0.18	0.23	0.009	0.16	0.25	0.1	0.16
7.	Bio-carbonates (mg/l)	2.73	1.7	2.0	2.8	1.8	2.3	3.0	1.9	2.39
8.	B.O.D (mg/l)	3.8	2.64	2.85	5.1	3.52	4.12	5.18	3.0	3.85
9.	D.O. (mg/l)	6.03	9.8	7.89	4.23	8.04	6.0	4.0	8.56	7.8
10	C.O.D (mg/l)	14.0	10.2	9.94	14.38	11.8	13.0	15.7	11.22	13.67
11	Sulphate (mg/l)	26.5	18.33	22.32	27.0	22.3	25.0	29.9	21.34	24.0
12	Chloride (mg/l)	27.3	20.18	24.38	28.7	24.5	26.4	29.6	22.22	26.0
13	Phosphate (mg/l)	0.23	0.11	0.14	0.24	0.14	0.2	0.28	0.13	0.2
14	Sodium (mg/l)	3.58	2.0	2.81	4.0	2.63	2.89	4.12	2.20	2.3
15	Potassium (mg/l)	0.26	0.11	0.16	1.8	1.45	1.64	2.8	1.0	2.1
16	Calcium (mg/l)	22.0	17.9	19.67	23.5	21.4	22.56	27.6	21.56	24.18
17	Magnesium (mg/l)	5.25	4.12	4.80	5.8	4.5	5.64	6.0	4.3	5.68
18	Total Dissolved solids	123.05	117.86	120.5	124.50	120.36	123.0	130.45	120.0	123.45

PM-Pre Monsoon; M- Monsoon; PO-M-Post Monsoon.

4. Discussion

The data obtained from the analysis regarding distribution of aquatic fungi and aero aquatic fungi of all the six stations reveals that, maximum percentage of fungal distribution was recorded in the station –I, when compared to other five stations (station-II, station III, station –IV, station V, station VI).

The higher fungal incidence that occurred in station-I was observed, may be due to unpolluted nature of water and less anthropogenic activities was encountered at this station. Whereas, the lowest percent distribution of aquatic and aero aquatic fungi was recorded in the station-V. This was may be due to the polluted nature of the water and increased anthropogenic activities at this station (Table 2 and 4). Seasonal variation in relation with aquatic and aero aquatic fungi distribution in all the six stations was studied and recorded in the Table 3 and 5. The data obtained during the present investigation reveals the maximum fungal distribution was recorded during the monsoon period followed by pre-monsoon periods in all the six stations. But, comparatively less

percentage of fungal incidences was recorded during post monsoon period (Table 3 and 5). The occurrence of maximum percentage of aquatic and extra aquatic fungi during monsoon period is probably due to low temperature, high organic load and low pH of the water (Table 6 and 7).

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Predicting Surface Water Contamination from the Kaduna, Yola and Maiduguri Landfill Sites

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Abstract: Surface water contamination from effluents from solid waste dump site is a phenomenon in Nigeria and the government planned to construct Central Integrated Waste Management Facilities in most of the State Capitals of Nigeria in order to control the menace of flooding arising from blockage of drainages and littering of the environment by solid wastes. This paper modeled the impact of the proposed Sanitary Landfills sites contained in the facilities on their immediate basin's surface water resources using the US Soil Conservation Service Model and the Streeter-Phelps dissolved oxygen balance equation. The results show that the critical dissolved oxygen concentration could be as bad as 730 to 786 mg/l and could occur within 1.98 to 2.17 days of first contact with the stream which will begin to show sign of recovery as early as the 25th day of first contact. [Researcher. 2009;1(6):63-68]. (ISSN: 1553-9865)

Keywords: Contaminants, Landfill, Waste management, rainfall-runoff, dissolved oxygen.

Introduction

Indiscriminate dumping of solid and liquid wastes has constituted a major obstruction to the flow of runoff water in drainages in urban settlements of Nigeria. The flood events of Ogunpa 1986, Kaduna 2003, Lagos 2007, and Abeokuta 2007 are typical solid waste induced flooding. Overrun of solid waste dump sites by runoff waters and subsequent discharge into river systems has also led to source contamination of the urban water supply systems.

Kaduna, Yola and Maiduguri are the capital cities of Kaduna, Adamawa and Borno States of Nigeria respectively, and are among the major cities where Integrated Waste Management Facilities (IWMF) are planned for construction in Nigeria. These cities have large concentration of industries such as agricultural, food and beverages, automobiles, textiles, oil mills, paper manufacturing and conversion, hospitality businesses, plastic, tanneries, flour mills, breweries, poultry feed mills, pharmaceuticals, industrial chemical, fertilizer, markets, printing and publishing, refinery/petrochemical plants and academic institutions. Large quantities of wastes are generated from these establishments and their nature and characteristics are so diverse and coupled with poor management practices, they are currently an eye sore in our cities thereby constituting environmental health hazard. The construction of integrated waste management facilities in these cities will not only improve environmental health but also reduces associated problems.

The integrated waste management system planned for some cities in Nigeria is focused on the establishment of sanitary landfills. Sanitary landfills are engineered disposal systems that are operated in accordance with environmental protection standards (USEPA, 1994). Several studies on waste management in Nigeria have been published, but most of them are limited in scope and extent, and they rarely include prediction of leachate migration and pollution. They include Fulani and Abumere (1983), Massey (1992), Beecroft et al (1983), Ademoroti (1988) Egboka et al, (1989) and Bichi (2000) among others.

The sites for these Integrated Waste Management Facilities (IWMF) were located on the outskirt and upstream of these cities. Between the sites and the cities, there are smaller communities within the same river basin that depend on the river water for their domestic and agricultural water supply. These facilities would therefore constitute potential danger to human and animal health if the leachate inflow into the surface water bodies is not properly managed.

This paper therefore examines the impacts of the proposed sanitary landfills at Kaduna, Yola and Maiduguri on their immediate surface water sources. The paper modeled the concentrations of the leachate from these landfills over a period of time to predict their impacts on the surface water quality.

Method of Investigation

The capacity of the surface water environment to assimilate contaminants and pollution is a function of both the source quality, the nature of the physical, chemical and biological properties of the contaminants and the re-aeration capacity of the system. In order to ensure that the loading of pollutants from the landfill sites in the receiving waters does not exceed its capacity, and that the quality of water in the river systems remain satisfactory to the downstream users, it therefore became important to examine:

- Whether the volume of available water running between the landfill site and the stream as well as the flow rate of the stream is sufficient for effective dilution; and
- Whether the degree of dilution and dispersion to be achieved while in transport will be sufficient to prevent adverse effects to both the aquatic ecosystem of the receiving water and the human population downstream and around the landfill sites.

It was also critical to examine runoff flows and volume under storm events. Direct precipitation, especially high energy raindrops, can penetrate the landfills and promote the reaction of storm water with various constituents of the landfill.

The method of investigation was divided into the following stages at the three locations:

- Solid waste sampling and categorization from fifteen sites strategically located within each of the cities where the wastes would be moved to the sanitary landfill sites.
- The chemical characteristics of the waste were determined by chemical digestion of representative soil samples taken at 0-30cm beneath the heap of waste at each of the fifteen solid waste dump sites considered for each city.
- Permeability tests were also carried out on soil samples taken from six pits dug to 0.5 – 1.0m deep at each of the sanitary landfill sites.
- The effect of contaminants in surface water is measured by the dissolved oxygen deficit in the water, therefore the Biochemical oxygen Demand (BOD) is the most critical parameter considered in this model. To this effect, the surface water contamination was investigated by modeling the dissolved oxygen deficits consequent to:
  - ✓ leachate spring discharge of effluents from the Landfill sites and
  - ✓ contaminants dissolved by overland flow over the landfill sites arriving the nearby stream channel,

to determine the natural stream water recovery time using the Streeter-Phelps equation (1):

$$D_t = \frac{K_D L}{K_R - K_D}(10^{-K_D t} - 10^{-K_R t}) + D_O 10^{-K_R t} \quad (1)$$

Where:

Dt = dissolved oxygen deficit at time, t (mg/l)

L = ultimate first stage BOD at point of waste discharge (mg/l)

D_o = initial oxygen deficit, (mg/l)

K_D = deoxygenation coefficient

K_R = reoxygenation coefficient.

The time t_m at which the minimum dissolved oxygen occurs can be obtained from equation (2) given as follows

$$t_m = \frac{1}{K_R - K_D}\log\left[\left(\frac{K_D L - K_R D_O + K_D D_O}{K_D L}\right)\frac{K_R}{K_D}\right] \quad (2)$$

and the corresponding critical oxygen deficit, D_c determined by equation (3)

$$D_C = \frac{K_D L}{K_R} 10^{-K_D t_m} \quad (3)$$

Estimation of Volume of Runoff

The United States soil conservation services (SCS) model is one of the rainfall-runoff models popularly used to estimate the runoff contribution form a unit rainfall. The SCS model used in this study is based on a hydrological soil parameter known as runoff curve number, type of soil and infiltration characteristics, and antecedent moisture condition. The SCS model (Schwab et al, 1981) is given by equation (4).

$$Q = \frac{(P - 0.2S)^2}{(P + 0.85S)} \quad (4)$$

Q = total direct surface runoff in depth, in mm

P = Rainfall intensity, in mm

S = Maximum potential abstraction of water by soil, mm, given by equation (5)

$$S = \frac{25400}{N} - 254 \quad (5)$$

N = runoff curve number for hydrological soil – cover complexes.

When storm water runs through landfills, many of its constituents will move into solution, some bio-accumulate in the water while others remain in suspension as it flows towards the receiving surface water downstream. On arrival at the recipient stream, it combines with existing contaminants in flow and moves through various channel physical conditions to ensure proper dilution. The contaminated water is further diluted at confluences or where effluent flow from the groundwater recharges the stream flow.

Considering the contaminants individually as tracer of concentration Cs at each landfill site flowing overland at the rate of Qs and discharging into the adjoining river with existing tracer concentration C_R flowing at the rate of Q_R, the concentration C of the resulting mixture in the streamflow is given by equation (6)

$$C = \frac{C_S Q_S + C_R Q_R}{Q_S + Q_R} \quad (6)$$

The processes of re-oxygenation and deoxygenation progress simultaneously as the stream flows downstream towards the cities, and the Streeter-Phelps equation was used at five days interval to simulate the variation in the dissolved oxygen deficit concentration.

Results and Discussion

The solid waste sampling and categorization results as presented in Table 1 indicated that food wastes constitute a major part of the wastes generated in all the cities resulting in high concentration of biogas emissions from the dump sites. As presented in the table, metallic waste is still very significant in the wastes generated despite the activities of the scavengers who collect them for re-use by the steel mills in the country. Plastic films wastes, especially polyethylene, are another significant element in the waste constituting environmental nuisance and clog drainage channels.

Comparative analysis of chemical composition of the digested soil samples collected from beneath solid waste dump sites with the standard upper limits and World Health Organization (WHO) International Standards for drinking water are presented in Table 2.

Table 2 shows that the parameters whose maximum values exceeded the W.H.O. international Drinking Water standard are BOD, COD, TDS, Total hardness, Potassium, Iron, Chromium, bicarbonate, sulphate and chloride. However the values obtained for calcium, magnesium, nitrate and carbonate are within permissible limits for drinking water. Consequently with soil permeability coefficients ranging between 1 .44 x 10^{-4} and 3.6 x 10^{-2} mm/s in Kaduna, and between 2.81 x 10^{-3} and 4.54 x 10^{-1}mm/s at Yola and Maiduguri, the hydraulic resistance to the flow of these contaminants to underlying aquifers is higher in Kaduna and lower in Yola and Maiduguri.

Modeled dissolved oxygen deficit concentrations are presented in Table 3 while the critical dissolved oxygen and time of occurrences are presented in Table 4. The results presented in Tables 3 & 4 shows that the critical dissolved oxygen concentration could be very bad, ranging between 730 and 786 mg/l at the location 1 in each City due to the washing of the landfill constituents into the adjoining river/stream. This situation occur within 1.98 to 2.17 days of contact with the water body at the stream will begin to show sign of recovery as early as the 25th day of first contact at location 1. The recovery time is expected to be much more shorter due to more dilution from the tributaries, but could be more critical during the dry season.

Table 1: Comparison of some solid waste Generation (%) of study Areas

Composition	Maiduguri 2002	Yola 2002	Kaduna 2002
Total Paper	5.73	6.5	7.2
Food Waste	46.04	44.12	49.73
Textiles	3.97	4.21	4.5
Ashes / Dust	12.72	13.05	9.64
Metal (Ferrous & Non Frerrous	10.68	9.41	9.32
Plastic & Plastic films	12.71	13.50	9.94
Glass	5.75	6.84	6.43
Biodegradable Total	68.46	67.88	71.07
Non Biodegradable Total	29.14	29.75	25.69
Miscellaneous	2.4	2.37	3.24

Table 2: Maximum Leachate Concentration compared with Upper Leachate Limits and W.H.O. Standard (mg/l)

Substance	Maximum Leachate Conc. Obtained	*Standard Leachate Upper Limits	+WHO Maximum Guideline Value for Drinking Water
BOD_5	500.5	54610	6.0
COD	750.7	8950	10.0
PH	8.8	8.5	6.5-8.5
T.D.S	2000	-	500
E.C. (µs/cm)	2400	-	-
Total Hardness	1100	-	100
Acidity	280.5	-	500
Alkalinity	460.3	-	500
Sulphate	268.1	1826	250
Chloride	2241	2800	250
Nitrate	2.0	1416	10 as N; 45 a $N0^-_3$
Bicarbonate	800.4	20850	500
Carbonate	120.0	22800	500
Calcium	45	4080	200
Magnesium	13.0	15600	150
Iron (as Fe2+)	1.80	5500	0.3
Manganese	0.0	1400	0.1
Chromium	0.15	-	0.05
Sodium	181	7700	200
Potassium	650	3770	15
Lead	-	5.0	0.05
Copper	-	9.9	1.0
Zinc	-	1000	5.0

+ Source: World Health Organization, 1971

* Source: After Bower, 1978

Table 3: Modeled Dissolved Oxygen Deficit Concentration

Time (days)	KADUNA		YOLA		MAIDUGURI	
	Sample Location 1	Sample Location 2	Sample Location1	Sample Location 2	Sample Location 1	Sample Location 2
5	436.85	289.91	447.16	268.91	No immediate Surface Water Source	
10	73.56	77.41	87.93	89.81		
15	9.63	19.52	13.31	28.48		
20	1.16	4.90	1.83	9.0		
25	0.13	1.23	0.24	2.85		
30	0.015	0.31	0.03	0.9		
35	0.0017	0.078	0.004	0.28		
40	0.00020	0.020	0.0005	0.09		
45	0.000022	0.0049	0.0000641	0.28		
50	0.0000025	0.0012	0.000008	0.09		
55	0.0000003	0.00031	0.000001	0.003		
60	0	0.00008	-	-		

Table 4: Critical Dissolved Oxygen and Time of Occurrence

Parameter	KADUNA		YOLA		MAIDUGURI	
	Sample Location 1	Sample Location 2	Sample Location1	Sample Location 2	Sample Location 1	Sample Location 2
Dc (mg/l)	786.133	474.614	730.58	390.29	No immediate Surface Water Source	
Tm (days)	1.984	1.984	2.13	2.17		

Conclusion and Recommendations

From this study, it can be inferred that surface water pollution is expected to be active for about 25days at each location, after which the stream is able to recover naturally. Minimization of leachate generation through the control of surface infiltration water into the landfill is recommended as an effective management strategy. Containment of leachate within the landfill through the use of double or composite liner systems, perimeter and base blinding with low permeability bund walls are recommended as effective construction control techniques. In order to prevent outbreak of disease epidemic, alternative sources of water supply should be provided for the communities adjoining the sites because low flow Biochemical Oxygen Demand conditions would be critical. The results presented herein are predictive, based on existing limited data and expected waste composition. It is highly recommended that parameter measurement indices be initiated immediately at the commencement of the project for future model study and analysis.

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Genomics in Traditional African Healing and Strategies to Integrate Traditional Healers into Western-Type Health Care Services- A Retrospective Study

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Abstract: This study explores genomic insights into the process of traditional African healing; the use of medicinal plants, their therapeutic benefits and the application of biotechnology to enhance the exploitation of the active principles of these herbs and the removal of alkaloids that are most likely toxics to unsuspecting patients. This study also examines the attitude of health care consumers towards the integration of traditional healers into primary health care delivery system in Nigeria. Additionally, the attitude of medical and nonmedical students towards the utilization of traditional healers at primary health care centers was compared. Cluster sample of 1000 respondents participated in the study from the urban, rural, and isolated villages in kwara State, Nigeria. A total of 960 questionnaires were mailed to all the university students; 480 to the medical and 480 to the nonmedical students. Established in this study, was the shortage of health manpower. In Nigeria, over 30% of the respondents from the three communities indicated positive attitude toward the utilization of traditional healing service for orthopedic and mental health care. Over 60% of the respondent stated that they will never patronize traditional healer for oracular consultation. Although statistically significant relationships existed between professional background and attitudes toward the acceptance of traditional healer into primary health care system, the fact that the magical component of traditional medicine will constitute a barrier for the cooperation of physicians was highly significant (X^2=101.5, 4df; p<.01). To enable planners to salvage the beneficial aspect of traditional medicine, it was recommended that the mode of healing adopted in traditional healing should be empirically evaluated. [Researcher. 2009;1(6):69-79]. (ISSN: 1553-9865)

Key Words: Genomics, Traditional African Healing, Western-Type Medicine, Primary Health Care Services, Retrospective Study

1. Introduction

In Genomics and the Public's health in the 21st Century, the Institute of Medicine (2005) defined Genomics as "the study of the entire human genome". The expert committee at IOM also echoed the potential benefits of genomics in improving the health of the public and by differentiating genomics from genetics. The latter deals with the study of functions and effects of single genes while the former explores not only the actions of single genes, but also the interactions of multiple genes with each other and with the environment.

In the same vein, genomics is the study of the whole genome of living organisms. This branch of biological science focuses on the development and application of more effective mapping, sequencing, bio-informatics and computational tools. Scientists with profound background in genomics apply large scale molecular techniques for linkage analysis, physical mapping, and the sequencing of genomes to generate detailed data which are subjected to analysis using high-speed computer facility. The new international tools of genomics include the high-throughput DNA sequences, and the large scale DNA arrays (DNA chips). These scientific tools have the capacity to analyze thousands of genes promptly and accurately. These devices can be used to study cells of virtually all living organisms. A typical genome is the entire collection of chromosomes which are present in the nucleus of each cell of an individual organism (CDC, 2004; Ebomoyi and Srinivasan, 2008). We must underscore how the Human Genome project has spurred a revolution in biotechnology innovation worldwide and continue to play significant role in making the United States and China leaders in biotechnology field. Genomic technology continues to unveil the therapeutic benefits of many plants and their lethal components which must be discarded. Hitherto, both traditional healers and western-type physicians had adopted trial and error modality to expunge many pharmaceutical products which routinely poison patients. But today through pharmacogenomics and human genome sequencing, efficient titer and adequate applications of many drugs have been compiled (Kayser and Quax, 2007).

The utilization of herbal products in traditional African medicine is widespread in virtually many African communities most especially in the rural areas and many medically isolated

villages in Africa. To illustrate, the plant Combretum mucronatum was identified as a warm expeller at a calculated dose of 0.8gm/kilogram. To alleviate the symptoms of bronchial asthma, various plants were identified which include: Desmodium adscendens, Papilionacea, Thonningia sanguine—Balanophoraceace and Deinbolia pinnata sapindaceae. Collaboration between Western-trained physicians, botanists, and African traditional healers has yielded so much benefits and the development of clinical drug trials in Nigeria, Ghana and many West African nations (Ebomoyi and Akwawua,2006).

The leaves of Eleophorbia drupifera and Itilleria latifolia taken with palm oil soup preparation acts as a filariacide and aginst guinea-worm (Dracunculus mendinences). The local application of guava leaves, ground into paste with kaolin or white clay and piper guineense, and twice a day heals the infection in about ten days. The late President of Ghana, Dr. Kwame Nkruman, emphasized some of the philosophical tenets of the Western African model as(1) to uphold, protect and promote the best in psychic healing, (2) to encourage the establishment of advanced training programs in traditional healing,(3) to introduce and train medical students to appreciate the work of traditional healing, (4) to encourage research work into traditional healing (5)to establish clinics in remote places and to educate traditional healers in the rural areas in order to improve upon their practices.

In Nigeria, 70% of the inhabitants live in rural areas and in such places traditional healers are generally the only available health care providers. Since the proficient healers could be rendering beneficial services to rural dwellers, it is common to encounter quacks among them who poison their patients and migrate further into the hinterland (Ebomoyi, 1982). Although traditional healers have not been accorded full official recognition, they render health care services to about 75% of Nigerians living in urban, suburban and medically-isolated villages (Ademuwagun, 1969). With regards to the current health manpower situation, conventionally accepted health indicators such as infant mortality rate, life expectancy and physician population ratio reveal an unsatisfactory picture. The data from The Nigerian federal ministry of health and the World Health Organization estimates (2008), and Time Almanac (2008) reveal: The health status of Nigerians is still relatively poor considering these indicators:

Infant Mortality Rate:	71.3 per 1000 live births
Crude death rate:	13.8 per 1000 live births
Crude birth rate:	38.80 per 1000 live births
Maternal death rate:	15.20 per 1000 live births

Life expectancy at birth 51.1 years for female
Life expectancy at birth 50.9 years for male
Adults (Ages 15-49) Living with HIV: 5.4%
Physicians 25,914 (1per 4,722 persons)
Hospital beds 54,872(1per 2,230 persons)

Objectives of the Study

The primary objective of this research was to explore the attitudes of primary Health care (PHC.) consumers towards the utilization of traditional healing services at the primary health care centre. A secondary objective was to compare the attitude of respondents in the urban, rural and medically-isolated villages about the utilization of traditional medical services. A third objective was to compare the attitudes and belief of medical and nonmedical undergraduates towards the utilization of traditional medicine services at the primary health care center. The underlying assumption is that Nigerian students enrolled in American universities and those enrolled in Nigerian medical school constitute an important source of future influence and leadership in Nigeria society. Given the findings from the survey, it should then be possible to evolve a suitable scientific strategy for the integration of traditional healers into primary health care delivery system in this country. The guiding theory for this approach comes from a body of research in communication known as diffusion of innovation discussed elsewhere (Ebomoyi, 1984).

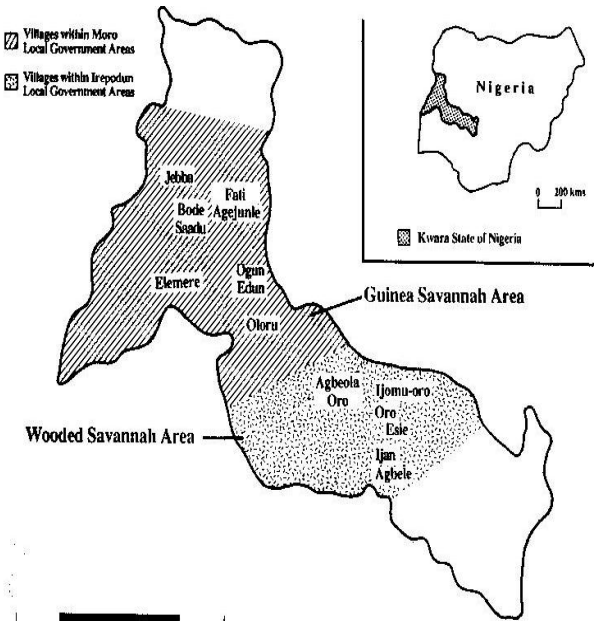
The Study Area

The three communities where PHC services were studied are located in Kwara State, Nigeria. The urban area is situated in the heart of Ilorin township. This area is rectangular- bounded by the Emir's palace, the central mosque, the market and residential houses. Demographically, about 80% of the inhabitant are Islamic and an average income is less than 2,400 (two thousand four hundred naira). There is a district health centre which provides PHC services to a catchment area of 10,000 inhabitants who live in 660 houses. The rural community is Shao where there were 434 houses. Shao is a village of 3,756 females and 3,510 males in Kwara state. It is

about 16 kilometer north of Ilorin. Shao is a farming community in which most of the women are small scale traders. About 80% of the inhabitants are Yoruba's, there are Fulani's, Ibos, Hausas, and Agatus. In the community, about 48% of the inhabitants are Muslims, 50% Christians while about 2% of them are Animists (Ebomoyi, 1985).

The area studied in the Olowu district consists of 34 small farm hamlets (Fig. 1). Geographically, these remote villages are in the Guinea savanna belt of West Africa with a biome of evenly dispersed grasses and scattered trees. The temperature ranges from 22 ^{O}C to 34 ^{O}C with an annual rainfall between 40 to 45 inches. The Olowu district farm hamlets have a dejure census of 6,586 inhabitants living in 403 houses (Ebomoyi, 1987).

Figure 1- Map of Nigeria showing Kwara State



With regards to the two groups of Nigeria students; medical students who were enrolled in four medical schools and non-medical Nigeria students enrolled in six universities in the United States participated in this study. The universities involved were University of Ibadan, University of Benin, University of Ife, and University of Lagos. In the United States, the universities included: University of California, Los Angeles, University of Illinois, University Iowa, State University of New York, University Michigan Ann Arbor, and University of Austin Texas.

Material and Method

The field workers recruited for this study were given one month induction training on the general nature of the Nigeria Primary Health Care (PHC) programs particularly its component of traditional medical services, mental health care, orthopedic, immunization, traditional birth attendance, oracular consultation, psychosomatic healing, dental services and the management of eye diseases. This study was conducted using cluster sampling method. At the urban, rural and isolated villages, 1000 respondents were interviewed in each community.

To assess the validity and reliability of the two instrument employed for this study, the Mermac Test Analysis was performed. For pilot field assessment, reliability coefficient of 0.951 for the Kuder-Richardson 20 formula and 0.921 for the Kuder-Richardson 21 formula were found for the community PHC questionnaires. The questionnaires designed for the student's group had coefficients of .881 for the Kuder-Richardson 20 formula and 0.899 for the Kuder-Richardson 21 formula. These results revealed that the questionnaires had a good degree of internal consistency and reliability. Every house was visited, and adults of child-bearing ages were interviewed.

Instruments

Eight questions requiring one mutually exclusive response, tapped attitude towards utilization of traditional healer at the PHC centers. Each respondent's position on a questionnaires item was reflected by his or her respond in one of five possible mutually exclusive responses. The continuums include 5 – strongly agree, 4 – agree, 3 – neither agree nor disagree, 2 – disagree, 1 – strongly disagree. The university students were asked whether federal Government should build hospitals to accommodate traditional healers at the local, state and Federal levels. Other questions on traditional healers were the extent of their acceptance by the people, efficacy, their role in identification of plants of therapeutic value and whether western-trained physician will co-operate with traditional healers.

With regards to the three communities studied, questions on the utilization of traditional healing were designed on a continuum of: 1 – I will never patronize, 2 – I may patronize, 3 – I will patronize, 4 – I will strongly patronize. Respondents were asked whether they will ever patronize traditional healers in specific medical services such as mental illness, psychosomatic disease, oracular consultation, gastroenteritis, immunization, family planning, dental services and eye care services. Questions on the socio-demographic characteristic of respondents were the closed-ended types and they were in numerical order.

Results

Observed in the data of PHC recipients from urban, rural and the hinterland is the preponderance of females. They made up over 50% of the respondents while the males were generally less than 40%. Among the respondents, 54.4% of the urban dwellers were illiterate, at the rural area, (Shao) 79.6% were illiterate, whereas in the isolated villages 98.8% were illiterate (Illiterate have not attained even 8th grade education).

Of the students groups, 369 were in medical field while 343 were in nonmedical profession. There were 532 males and 148 females. Among the nonmedical students, class rank was well distributed with 13.3% first-second year, 19.5% third-fourth year, 12.2% M.S. and M.A. degree candidates. The composition in class rank among medical students group was 13.9% first-second year, 17.8% third-fourth year, 16.9% fifth-sixth year. A striking observation among respondents by education pertained to the consistency of highest response rate by the third-fourth year class among both medical and nonmedical students. The mean age of respondents was 24.8% years with the range of 15 and 40. The highest percentage of respondents was found in the 20 to 24 category.

Shown in tables 1 – 3 are the attitudes of PHC recipients towards the utilization of traditional medical services. In the three district communities, over 60% of the respondents in each area stated they will never patronize traditional medical practitioners in oracular consultation. In each of the communities, well over 35% of the respondents stated that they will patronize traditional healer for either mental illness or orthopedic disease. Table 4 reflects the mean response values on the attitude-believe items based on eight variable of traditional medicine. The highest attitude score was that traditional healers are needed to identify our local plants which have healing properties. The least recorded attitude score was that traditional medical practices are more effective in the management of orthopedic disease than western-type medicine.

Since the healing of bone fractures is a physiological process by virtue of the respondents' education, they were probably quite aware of the fact this fact. Table 5 is chi-square comparison of medical and nonmedical student attitudes toward traditional medical practices. Although statistically significant relationships exist between professional background and attitudes toward utilization of traditional medicine; the fact that magical component of traditional medicine will constitute a barrier for the co-operation of western-type physician was highly significant (X^2 = 101.5; 4df, p< .01). Presented in Table 6 are the inter-correlation coefficient matrix of attitude-believe statement based on eight items of traditional medical practices. The correlations between the variables 1 and 4 (Federal Government of Nigeria should build hospitals to accommodate traditional medical practices at Local, State, and Federal levels; and respondents opinion about the integration of Western-type medicine into traditional medicine) were significantly correlated (.55 p< .01). Table 6 indicates all the significantly correlated variables at the p < .01 and p < .05) levels. Shown in Table 7 is the correlation between each independent variable and their discriminant functions. The table reveals that variable 1 is negatively correlated with variable 8. However, these negative correlations are associated with the discriminant scores by values of 23 percent each.

Table 1 – Attitude of PHC Recipients (at Ojaoba) Toward The Utilization Of Traditional Healers For Specific Clinical Problems

Traditional medical services	I will never patronize Trad. Healers	I may Patronize Trad. Healers	I will Patronize Trad. Healers	I will Strongly Patronize Trad. Healers
N = 1000	(%)	(%)	(%)	(%)
Mental Illness	60.3	-	4.7	35.0
Psychosomatic Disease	50.8	2.2	1.0	47
Oracular Consultation	90.8	1.2	-	8.0
Orthopaedic Consultation	60.4	1.6	2.0	36
Gynaecological Related Disease	68	-	12	20

Gastroenteritis & Related diseases	70	3.8	1.2	25
Immunization	88	1.2	5.8	5
Family Planning	92	-	3.4	4.6
Dental Services	80.8	6.2	10.5	2.5
Eye Care Services	84.4	-	4.4	11.2

Table 2 – Attitude of PHC Recipients (at Shao) Toward the Utilization of Traditional Healers for Specific Clinical Problems

Traditional medical services	I will never patronize Trad. Healers	I may Patronize Trad. Healers	I will Patronize Trad. Healers	I will Strongly Patronize Trad. Healers
N = 1000	(%)	(%)	(%)	(%)
Mental Illness	48.1	0.1	1.1	50.7
Psychosomatic Disease	81.9	-	0.1	18.0
Oracular Consultation	82.1	-	-	17.9
Orthopaedic Consultation	45.0	.43	2.9	51.7
Gynaecological Related Disease	81.9	-	0.1	18.0
Gastroenteritis & Related diseases	72.3	-	0.4	27.6
Immunization	99.6	-	0.1	0.3
Family Planning	82.8	0.1	16.7	0.3
Dental Services	99.4	0.1	0.1	0.4
Eye Care Services	99.4	0.1	-	0.5

Table 3 – Attitude Of PHC Recipients (at Remote Villages) Toward the Utilization of Traditional Healers for Specific Clinical Problems

Traditional medical services	I will never patronize Trad. Healers	I may Patronize Trad. Healers	I will Patronize Trad. Healers	I will Strongly Patronize Trad. Healers
N = 1000	(%)	(%)	(%)	(%)
Mental Illness	24.2	8.5	7.3	60.0
Psychosomatic Disease	40.7	6.10	6.1	47.1
Oracular Consultation	66.8	3.7	6.6	22.9
Orthopaedic Consultation	31.9	4.4	8.8	54.9
Gynaecological Related Disease	45.6	2.7	6.1	45.6
Gastroenteritis & Related diseases	71.2	5.1	6.6	17.1
Immunization	48.8	4.6	5.4	41.2
Family Planning	78.0	3.9	4.9	13.2
Dental Services	81.3	3.2	5.0	10.5
Eye Care Services	78.8	6.1	4.2	10.9

Table 4 – Item Pool for Traditional Medicine Attitude Scale

Traditional Variables	Statement	Means	Standard Deviation
1	Federal government should build hospitals to accommodate traditional medicine practitioner at local, state and federal level	4.14	1.16
2	The indigenous Nigerian people living in rural parts of the country prefer traditional medicine to western-type medical practices	3.58	1.16
3	Traditional medical practices are effective (produce results) in the treatment of mental illness	3.85	1.05
4	Traditional medical practices are effective (produce results) in the treatment of orthopedic (muscular skeletal) class than		

	western-type services.	3.55	1.29
5	If you have used the services of traditional medical practitioners before, you were satisfied.	4.04	0.90
6	In implementing the PHC the services of traditional healers are needed to identify our local plants which have healing properties.	4.43	0.69
7	What do you think of the integration of western-type medicine services into traditional medical practice in Nigeria?	4.22	1.1
8	Aspect of traditional medical practices which include magical thinking will make it very difficult for western-trained physician to cooperate with traditional services.	3.600	1.1

Table 5 – Chi-squared comparison of medical and nonmedical students' attitude toward traditional medicine

	Medical students			Nonmedical students			Chi-square with	
Traditional medicine variable	Percent DA	M	A	Percent DA	N	A	df= 4	Sign.
Federal government to build hospital accommodation for traditional healers	20	12.9	66	5.1	8.5	86.4	77.6••	.01
Satisfied with traditional medical practice in Nigeria	15.1	21.1	63.8	4.9	10.8	84.4	39.0••	.01
Traditional medicine is effective in mental illness	24.3	28	47.6	15.2	17.6	82.4	58.7•	.01
Traditional medicine is effective in orthopedic illness	44.1	21.5	34.4	17.6	19.6	62.7	76.5•••	.001
We required traditional healers to identify local medicine plants	8.4	4.8	86.8	5.6	5.9	88.5	12.2•	.05
The magical component of traditional medicine will make it difficult for W.T. (western-trained) physicians to cooperate with T.M. (traditional medicine	8.4	8.6	83.0	26.6	24.8	48.7	101.5•	.01

practitioners								
Nigerians in rural areas prefer T.M. to W.T. medicine	35.9	16.5	47.6	28.8	21.2	57.4	53.0•	.01

●Results are significant at .05
●●Results are significant at 0.1
●●●Results are significant at .001

DA = disagree
N = neutral
A = agree

Table 6 – Pearson Correlation Co-Efficient Matrix of Attitude-Belief Components of the PHC Based on Eight Items (Acceptance Of Traditional Medical Practice)

Attitude-Belief components	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8
Q1	1.0							
Q2	.33●●	1.0						
Q3	.46●●	.45●●	1.0					
Q4	.41●●	.31●●	.48●●	1.0				
Q5	.43●●	.29●●	.40●●	.44●●	1.0			
Q6	.45●●	.22●●	.33●●	.23●	.26●●	1.0		
Q7	.55●●	.31●●	.37●●	.29●●	.42●●	.35●●	1.0	
Q8	-.22●	-.18●	-.16	-.21	-.16	-.09	-.23	1.0

●Results are significant at 0.05
●●Results are significant at .01

Table 7 – Correlation between each independent variable and discriminant function (TMP)

VARIABLE (FACTOR)	CORRELATION (r)	Square multiple correlation r^2
1	.61	.46
2	.43	.25
3	.60	.40
4	.48	.33
5	.53	.32
6	.43	.23
7	.51	.37
8	-.27	.08

Discussion

In view of the current health workforce shortage in Nigeria, efforts should be harnessed to salvage pertinent technological resources from traditional healers. The results of the study suggest that overall, both medical and nonmedical students favor the integration of Western-type physicians with traditional healers. The accomplishment of such integration scheme was sufficiently supported by the Chinese political ideology (Horn, 1969).

Mahler (1969) has has cautioned that there is a need to integrate traditional healers into Western-type medicine. The most suitable strategy is to emphasize the just distribution of PHC resources and to utilize traditional medicine and its practitioner imaginatively. This will involve a collaborative research with international health experts to enable us have an objective assessment of the useful aspect of traditional medicine practice.

Viewed from the provider perspectives, both medical and nonmedical students favor the utilization of traditional medical services at the PHC centers. A statistically significant relationship exist between educational status of sttudents and the utilization of various traditional medical service ($X^2 = 29$, 12df, $p < .01$). The implications of these findings could be that the higher the education acquired, the more tendency to perceive the neccesity of integrating traditional medical practice into the official health care delivery system. Additionally current advocates of integration of both health care system such as Lambo (1956) and WHO (1978) experts committee on traditional medicine are highly enlighten health professionals. Igbinosa (1981) points out education as the most instrument by which individuals in rural areas can improve their well-being, understand and participate in health care services and develop the environment. Education definitely dispels human ignorance and enables him or her to compare and constrast relationships between events.

In most urban areas of Nigeria, there is the proliferation of western-type health care services. It is not suprising that over 50% of the respondents in urban area stated that they will never patronize traditional healers. Only 35% of them agreed to utilize the services of traditional healers for mental illness. The effectiveness of traditional healers for treatment of mental illness has been confirmed by many investigators (Prince, 1960; Torrey, 1959). At Shao rural community, not only were PHC recipients willing to patronize traditional healers in mental health care but also 51% of them stated that they will strongly patronize traditional healers in clinical problems involving bone setting. At the isolated villages, over 40% of the respondents were willing to patronize traditional healer in mental health care, psychocomatic diseases, orthopaedic, gyneacology and imminization services. Paxima et al. (1979) has confirmed the supportive role which traditional healers can play in the health sector as traditional birth attendants, bone setters and native pyschiatrists.

In spite of the apparent benefit of integrating traditional healers with western-trained physicians, even the urban and rural PHC recipients have their reservations about such a scheme. Also, among university students, in spite of the fact that 81.9% of both medical and nonmedical students preffered the combination of traditional medicine and western-type medicine, the medical student groups were more conservative on this question. The clinical assessment of the effectiveness of traditional healing practice can be evaluated by western-trained researchers; therefore, the Federal Government has an important role to play by encouraging the medical students to express and identify their specific concerns about traditional medicine. In essence, being in favor of a combined approach is one thing, but designing the

PHC program is another. Pertinent questions which government and medicalofficers need to address are:

a) What are the specific essential plants drugs, or techniques employed in traditional medicine for use at grassroot levels or for primary health care?
b) What specific role can and should traditional medicine person play?
c) Is it possible to get their suggestions in order to develop the cooperative approach?
d) In order to be able to develop a cooperative approach between traditional medicine and western-type medicine, it seems axiomatic that a special effort be made to learn more about the beliefs and practices of the traditional leaders.
e) Are there areas of health care in which cooperation could be more easily established: for example, in areas where there is a lack of trained personnel, could the traditional healer be trained to take over certain services?
f) Who are the legitimate authorities in traditional medical practice?
g) In those areas of health care, where western trained physicians have the most serious concerns, such reservations, need to be recognised before undertaking a cooperative approach.
h) Are there certain area of health problems in which traditional practitioners are more effective and therefore would be more accepted to the western trained physicians?

In addition to resolving the above questions, integration of both medical system should not be initiated until government researchers conduct preliminary pilot studies. Such projects can be carried out by: selecting pilot or experimental sites wherein the program would be initiated for a limited period of time. Based upon the experience gained from trying out new program approach, the attitude toward PHC can again be studied and the general effectiveness of the new procedure can be evaluated.

In some instances more formal experimental field trials may be conducted in order to have confidence in the results that are reported from these "tryout" experiences. Also the involvement of local residents and traditional medical practitioneers to secure their view, their most important health needs and to detremnine whether the PHC is helping them solve their health problems.

Program planners should visit herbal or traditional medicine stores in local areas and talk with consumers of traditional medicine in order to achieve a better understanding of their perceived needs. It would seem expedient to explore written materials on traditional medicine and to investigate theories on the source of care. For example, what type of people use the services of western-type medicine? What are the general attitudes of community members towards various western trained physicians and the dervices they offer?

A thorough understanding of these issues may enable planners to recognize factors likely to inhibit or promote implementation of the PHC. Although religious affliation appears to be an important factors in the type of healthcare required for the implementation of the PHC, the respondents studied were largely Muslims and Christians. A special study of the Nigeria animists can furnish more data about the relationship between various religions and the acceptance of health care practitioners for primary health care delivery.

In this study, efforts were made to assess the attititudes of PHC recipients toward the integration of traditional healers into the Nigerian PHC delivery system. The attitudes of the medical and nonmedical students were explored because they are potential physicians and health care administrators. Cognizance was taken of their not being completely comparable groups. However, their opinions, beliefs and concerns are quite pertinent in the development and implementation of primary health care in Nigeria.

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Assessment of Granite-derived Residual Soil as Mineral Seal in Sanitary Landfills

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Abstract: This study investigates the geotechnical properties of granite - derived soil from southwestern Nigeria for its potential use as mineral seal in sanitary landfill. The required parameters for soils to be considered as mineral seal such as grain size distribution, Atterberg consistency limits, maximum dry density and coefficient of permeability were determined using the BSI 1377 1990 standard. Results obtained show that the hydraulic conductivity is lower than the suggested limit (1 x 10^{-7}cm/s) of the various waste regulatory agencies. In addition, it has adequate basic geotechnical properties and strength characteristics which suggest the potential suitability of the soil as mineral seal in containment facility for disposal of solid waste material. [Researcher 2009; 1(6): 80-86]. (ISSN:1553-9865).

Keywords: Mineral seal, Hydraulic conductivity, Residual soil, Unconfined Shear Strength, Nigeria.

1.0 Introduction

Waste material in waste containment facilities are made isolated from the surrounding environment by providing mineral seals. The mineral seal is to control or restrict the migration of pollutant into the environment (Ogunsanwo, 1996). Commonly use mineral seals are composed of natural inorganic clays or clayey soils. The low hydraulic conductivity of the compacted clayey soils combined with their availability and relatively low cost make them potential materials to use as mineral seals in sanitary landfills for environmental protection. Since it is desirable for containment system to achieve its purpose at minimum cost; careful consideration should therefore be given to the choice of materials for the construction of the mineral seal. The environmental and health hazards associated with "unengineered" landfills are well known (Asiwaju-Bello and Akande, 2004; Onipede and Bolaji, 2004, and Fred and Anne, 2005). In the U.S.A, Lee and Jones (2005) asserted that 75% of unengineered landfills pollute adjacent water body with leachate. This is because deposited waste undergoes degradation through chemical reaction thereby contaminating usable surface and subsurface water supplies. In addition, the produced leachate forms complexes with the sesquioxides of lateritic soil (Orlov and Yeroschicheva, 1967) thereby weakening their in-situ geotechnical properties (Ogunsanwo and Mands, 1999).

Granite-derived residual soils, like other soils of basement complex origin, are widely distributed over the country. Its traditional geotechnical properties have been studied (Alao, 1983; Ogunsanwo, 1988, 1996). The potential use of the soil, if found suitable, will reduce cost of construction of sanitary landfills and encourage friendly environment. However, for soil usefulness as mineral seal, certain recommendations have been proposed by several previous investigators (e.g ÖNORM S 2074, 1990; Daniel, 1993; Bagchi; 1994, Benson *et al*, 1994, Benson and Trust, 1995 and Ogunsanwo, 1996). The list of some of the required geotechnical parameters with the recommendations was presented in Ige (2009). Also minimum unconfined pressure of 200kPa was recommended by Tay *et al* (2001), Daniel and Wu (1993).

This study aims at assessing the geotechnical properties of a granite-derived residual soil for potential usage as mineral seals in landfills. The typical tests that are generally used to investigate soils proposed as mineral seals in landfill such as the grain size distribution, Atterberg limits, compaction, unconfined compressive strength and hydraulic conductivity were conducted on sample of the compacted granite-derived residual soil. If on the basis of these tests, the soil proves to have properties desirable for a mineral seal material, then it should be considered as a potentially suitable material for the isolation of waste material in sanitary landfill.

2.0 Materials and Methods

The material used for this study was granite-derived residual soil. The soil was taken at 1.83m depth of gully erosion – exposed soil profile, 2.7km, along Oke-Oyi/Oloru road in Ilorin, Nigeria. The sample was collected into a plastic bag and transported to the soil laboratory of the Yaba college of Technology, Yaba, Lagos. The basic test such as specific gravity, particle size distribution and Atterberg limits of the soil were performed. All analyses were carried out in accordance to the BSI 1377 (1990) version. The data of the index properties were used to classify the soil following the

Unified Soil Classification System (USCS) classification.

3.0 Results and Discussion

Several limits have been proposed by various researchers with respect to the geotechnical properties of soil to be useful as mineral seal. Such limits are presented here along with the results obtained from this study.

3.1 Grain Size Distribution and Atterberg limits tests

The specific gravity of the granite residual soil is 2.60. The particle size analysis shows that the soil contains 70% clay, 81% fines,15% sand and 3% gravel(Fig. 1) Moreover, the results of Atterberg limits reveal the liquid limit (LL) is 68.4%, the plastic limit (PL) is 24.0% and the plasticity index (PI= LL- PL) is 44.4%. On the basis of these data, the granite residual soil is classified as CH (Inorganic clay with high plasticity) according to the USCS (Fig. 2). Inorganic clay with high plasticity (CH) is recommended for landfill liner (Oweis and Khera, 1998).

Hydraulic conductivity behaviour of mineral seal is greatly influenced by the particle size distribution because the relative proportions of large and small particle sizes affect the size of voids conducting flow (Kabir and Taha, 2006). Mineral seal should have at least 30% fines (Daniel 1993b; Benson *et al*; 1994) and 15% clay (Benson *et al,* 1994) to achieve hydraulic conductivity $\leq 1\times10^{-7}$ cm/s. Thus, the granite-derived residual soil can be used as mineral seal to achieve a hydraulic conductivity $\leq 1\times10^{-7}$ cm/s, as it possesses suitable amount of clay and fine fractions. Moreover, the soil contains adequate amount of sand, which may offer notable protection from volumetric shrinkage and impart adequate strength as well.

Liquid limit is an important index property since it is correlated with various engineering properties. Soils with high liquid limit generally have low hydraulic conductivity. Benson *et al* (1994) recommended that the liquid limit of mineral seal material be at least 20%. Most of the specifications for mineral seal proposed by various researchers or waste regulatory agencies do not generally prescribe any limit (maximum value) for their liquid limit. As long as it does not create any working problem, soils with high liquid limit are generally preferred because of their low hydraulic conductivity. Thus, the granite-derived residual soil with liquid limit of about 68% appears to be promising for use as mineral seal.

The plasticity index is one of the most important criteria for the selection of soils as mineral seal in sanitary landfill construction. It is the key property in achieving low hydraulic conductivity. Literatures suggest that the plasticity index must be more than 7% (Daniel 1993; Benson *et al*; 1994; Rowe *et al,* 1995). Thus, the granite-derived residual soil has suitable plasticity property (PI is about 44.4%) to minimize hydraulic conductivity.

The activity (PI/% clay fraction) of granite residual soil is 0.63. Thus, according to Skempton's classification it is inactive clay. Inactive clayey soils are the most desirable materials for compacted soil mineral seal (Rowe *et al,* 1995). In order to achieve a hydraulic conductivity $\leq 1\times10^{-7}$ cm/s for the soil mineral seal, soil with an activity of > 0.3 has been recommended (Benson *et al,* 1994, Rowe *et al,* 1995). An activity is an index of the surface activity of the clay fraction. Soils with higher activity are likely to consist of smaller particles having larger specific surface area and thicker electrical double layers (Taha and Kabir, 2006). Therefore, hydraulic conductivity should decrease with increasing activity.

Thus, the comparison between the index properties of granite-derived residual soil and the recommendations of various researchers for a good mineral seal material shows that the investigated granite residual soil has suitable properties to be use as mineral seal.

3.2 Compaction Properties.

The compaction curves (at two different energies of compaction) for the granite-derived residual soil are shown in Fig 3. The compaction curves clearly illustrate that the dry density is a function of compaction water content and compactive effort. For each compactive effort, at the dry side of optimum water content, the dry density increases with the increasing water content. This is due to the development of large water film around the particles, which tends to lubricate the particles and makes them easier to be moved about and reoriented into a denser configuration (Holtz and Kovacs, 1981). Whereas, at the wet side of optimum water content, water starts to replace soil particles in the compaction mould and since the unit weight of water is much less than the unit weight of soil, the dry density decreases with the increasing water content.

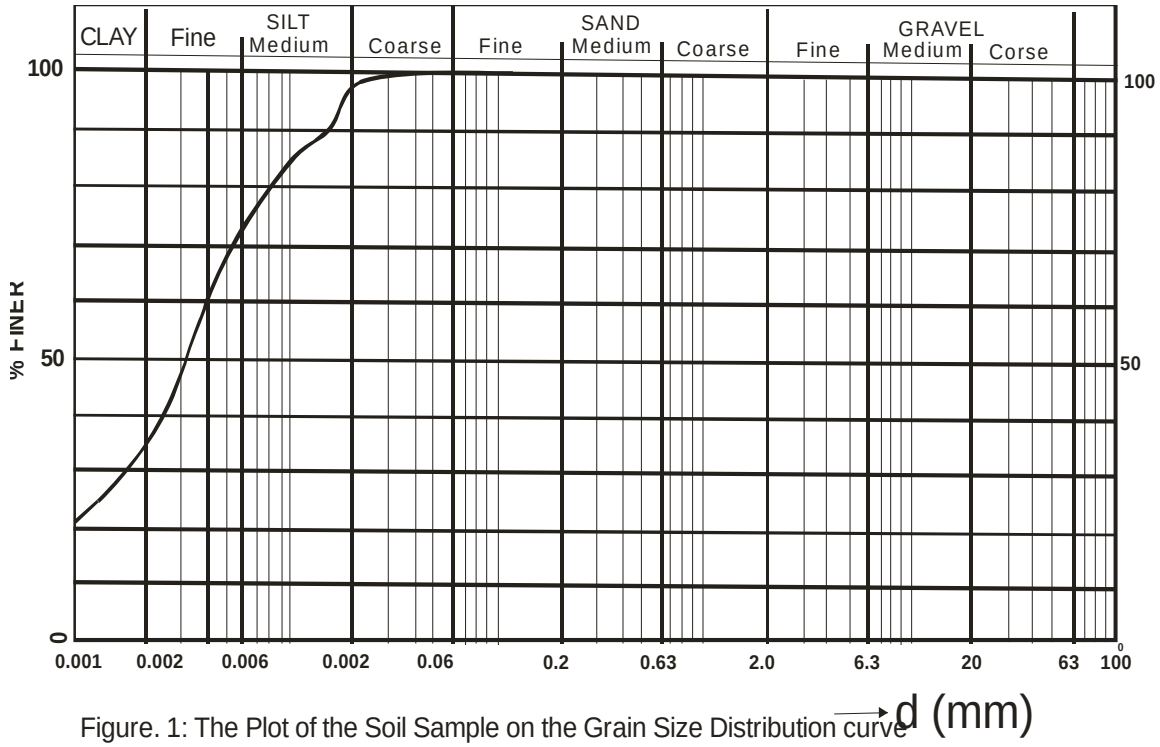

Figure. 1: The Plot of the Soil Sample on the Grain Size Distribution curve

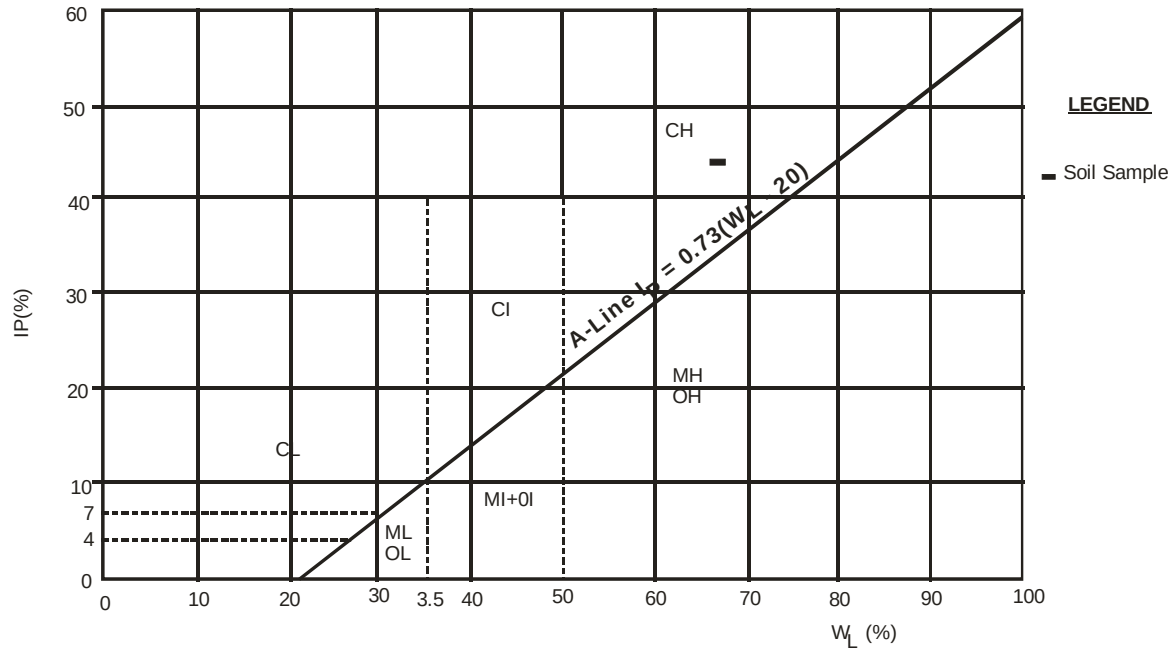

Figure 2 : Plot of the Soil sample on the Casagrande's Plasticity Chart.

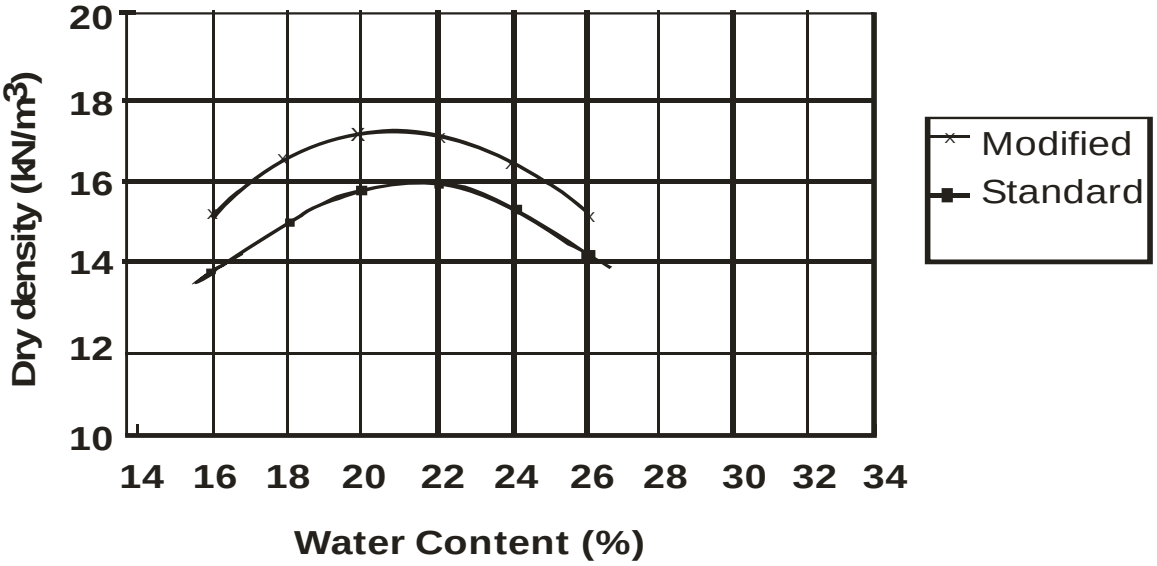

Figure 3. The Dry density versus Water content

The peaks of the curves (Fig. 3) represent the maximum dry density and corresponding optimum water content for a given compactive effort. The maximum dry density and the optimum water content obtained from these tests are given in Table 1. An increase in compactive effort increases the maximum dry density but decreases the optimum water content. This is because higher compactive effort yields more parallel orientation of the clay particles, the particles become closer and a higher unit weight of compaction results (Das, 1998). Hence, high compaction energy is preferred.

Table 1. Maximum Dry Density and corresponding Optimum Water Content.

Compactive Efforts	Optimum Water Content (w_{opt}%)	Max.Dry Density, γ(KN/m^3)
Modified Proctor	21.00	17.30
Standard Proctor	21.80	16.11

3.3 Hydraulic Conductivity

The relationship between hydraulic conductivity, water content and compactive effort is shown in Fig. 4. The hydraulic conductivity decreases with the increasing compactive effort because increasing compactive effort decreases the frequency of large pores and can eliminate the large pore mode (Acar and Oliveri, 1989). The reduction in pore size yields lower hydraulic conductivity. Figure 4 also show that the hydraulic conductivity of the soil changes with the change of compaction water content. Soils compacted at dry of optimum water content tend to have relatively high hydraulic conductivity whereas soils compacted at wet of optimum water content tend to have lower hydraulic conductivity. Increasing water content generally results in an increased ability to breakdown clay aggregate and to eliminate inter - aggregate pores (Mitchell *et al.*, 1965; Garcia-Bengochea *et al.*, 1979 Benson and Daniel, 1990). Moreover, increasing water content results in reorientation of clay particles and reduction in the size of inter- particle pores (Lambe, 1954; Acar and Oliveri, 1989 and Benson and Trust, 1995). Mineral seals should have a hydraulic conductivity of at least 1×10^{-7} cm/s. Figure 4 shows that the two different compaction efforts caused hydraulic conductivity less than 1×10^{-7} cm/s. The minimum hydraulic conductivity and corresponding water content at various compactive efforts is presented in Table.2.

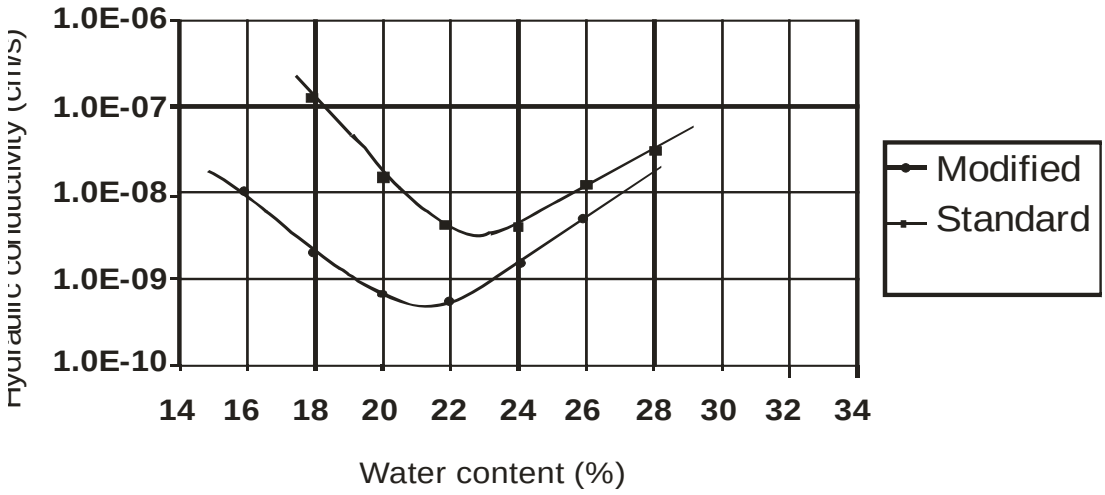

Figure 4: Hydraulic conductivity versus compaction water content

Table 2: Minimum Hydraulic Conductivity and Corresponding Water Content at various Compactive Efforts.

Compactive Efforts	Minimum hydraulic conductivity (cm/s)	Water Content (%) at Minimum Hydraulic Conductivity	Optimum Water Content (%)
Modified Proctor	3.2×10^{-9}	21.7	21.00
Standard Proctor	2.8×10^{-8}	22.5	21.80

3.4 Unconfined Compressive Strength.

The result of unconfined compression test against compaction water content is shown in Figure 5. The strength of compacted soil decreases with increase of compaction water content. Since increase in water content also increases the electrolyte concentration is reduced, leading to an increase in diffused double layer. Compactive effort also has a great influence on soil strength. For instance, at low compaction water content, unconfined compressive stress increases with increasing compactive effort. But at higher water content no clear trend is noticed.

Mineral seal in waste containment system is supposed to sustain certain amount of static load exerted by the overlying waste materials. In this regard, the mineral seal material must have adequate strength for stability. The bearing stress act on the mineral seal system depends on the height of landfill and unit weight of waste. Thus, the minimum required strength of soil used for compacted soil mineral seal is not specified but Daniel and Wu (1993) recommended that soils should have minimum unconfined compression strength of 200KPa. Test result shows (Fig.5) that the soil possesses higher strength than the recommended minimum strength of 200KPa for all the two compactive efforts.

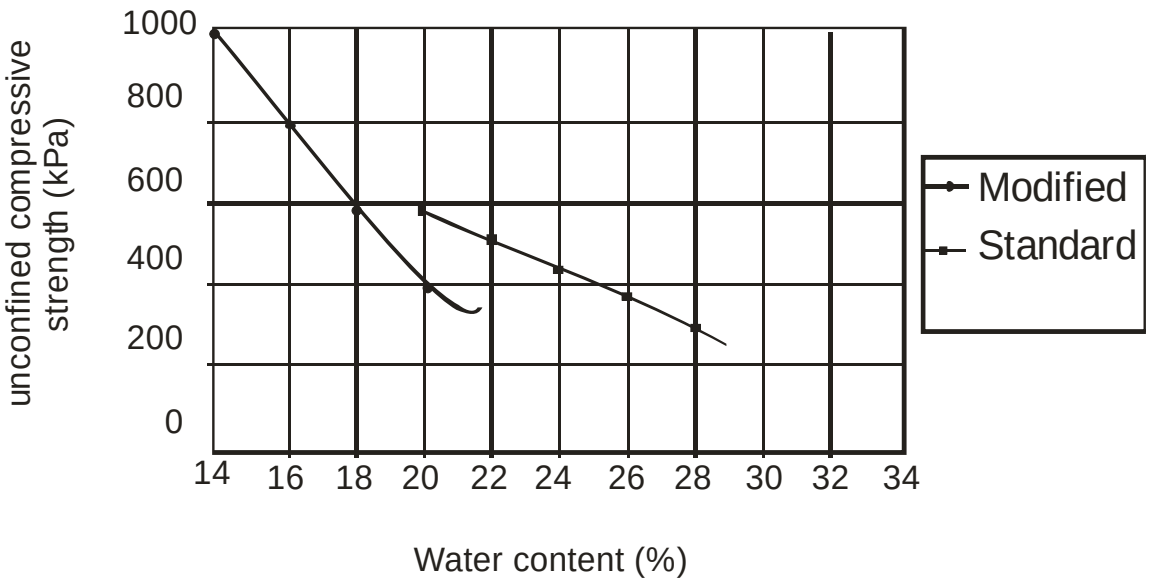

Figure 5: Unconfined compression strength versus compaction water content

4.0 CONCLUSIONS

The following conclusions can be drawn form the investigation of granite-derived residual soil:

The residual soil is inorganic clay with high plasticity. Generally, this type of soil possesses desirable characteristics to minimize hydraulic conductivity, and is frequently used for the construction of compacted soil mineral seals. The index properties (liquid limit, plastic limit, % clay content, % fines, activity etc.) of the soil satisfy the basic requirements as a mineral seal.

It is inactive clayey soil. Thus, the soil will be less affected by waste leachate. The soil has hydraulic conductivity of less than 1×10^{-7} cm/s, when it is compacted with both modified and standard Proctor compaction efforts.

Moreso, the soil has average strength higher than 200kPa

Thus, it is concluded that the granite-derived residual soil can be used as a suitable mineral seal material in sanitary landfills. Its potential use as isolation mineral seal will enhance the waste management programs in Nigeria since granite-derived soils are locally readily available

Although the soil meets all the basic requirements as a good mineral seal material, it may pose little difficulties during filed application due to its high plasticity. Therefore, during construction, great attention should be focused on soil preparation. The soil should be properly blended and homogenized to achieve a mixture of relatively small clods.

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