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Cover Page, Introduction, Contents, Call for Papers, All in one file

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An evaluation of the Antibacterial and Antifungal activity of leaf extracts of *Momordica Charantia* against *Candida albicans, Staphylococcus aureus* and *Escherichia coli.*

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ABSTRACT: The antibacterial and antifungal activities of Momordica.charantia, was investigated against S.aureus (gram+ve), E.coli (gram-ve) and C.albicans using the Stokes disc diffusion, the pour plate, well diffusion and streak plate methods. The solvent type extracts were obtained by three extractions with hexane, dichloromethane, ethyl acetate and ethanol respectively. Solvents were removed in vacuo to yield viscous oils and paste which were made up to a concentration of 0.03g in 10 mL of the respective solvents. These were tested in varying volumes of 100-600 uL/plate (i.e. concentrations of 0.03-0.18 mg/10 mL agar). The solvents were used as control whereas ampicillin and nystatin were used as references for bacteria and fungal species respectively. The solvents had no effect on the microorganisms whereas ampicillin and nystatin inhibited microbial growth. Momordica.Charantia showed antimicrobial inhibitory activity at 0.18mg/10mL plate of medium with activity most prominent with the ethanol extracts and negligible with the hexane. This study suggests that the ethanol extracts of M Momordica.Charantia,can be used in the control of E.coli and S.aureus induced diseases as herbal medicines following clinical trials. [Nature and Science. 2008;6(1):1-14]. ISSN: 1545-0740.

Key words: *Momordica Charantia*, Antimicrobial, *S.aureus, E.Coli*, *C.albicans*, Stokes Disc diffusion, Pour plate, Well diffusion, Streak plate, herbal medicines.

1.0. Introduction:

This paper discusses the microbiological properties of leaves of *Momordica Charantia, bitter melon* from the coastal plane of the Guyana flora and its possible use as an herbal medicine. Its antimicrobial properties were investigated against *S.aureus* (gram+ve), *E.coli* (gram-ve) and *C.albicans* strains using the Stokes disc diffusion sensitivity technique, Pour plate, Well diffusion and Streak plate.

Guyana has a rich flora biodiversity whose crude extracts, both organic and aqueous can be investigated for antimicrobial activity in addition to their role as global CO_2 sinks(in the context of global warming). Also, the specified plants parts of the same species be screened for natural products whose antimicrobial activity can also be correlated with the crude extracts. Following this, clinical trials can lead to the formulation of an herbal plant cream or herbal medicine. A few herbal medicine shops have now been established in Guyana. Plants extracts and fractionated plant extracts have been used for their antimicrobial properties[1-14]. Besides used as an herbal cream, following clinical trials, crude plant extracts can be subjected to chromatographic separation, leading to the isolation and purification of new and known bioactive natural products/phytochemicals, whose medicinal activity can also be investigated. For example, the diterpene (1) and (2) were isolated from the bark of *Xylopia aethiopica* and was investigated against the enzymes prolyl endopeptidase (PEP) and α-thrombin and was found to have inhibitory activity against them [15].

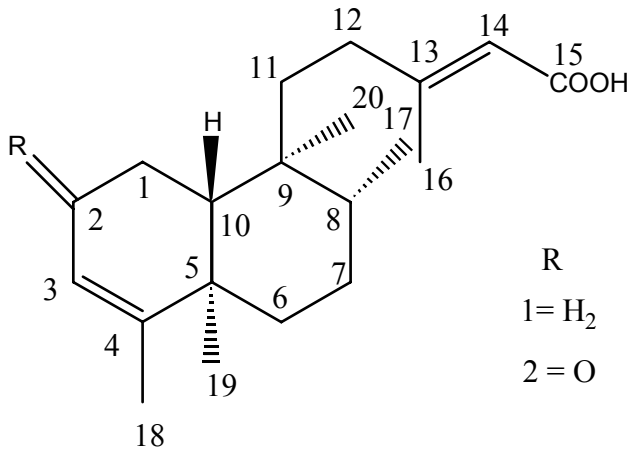

Fig. 1.0. Structure of Diterpene (1) and (2).

Research in herbal medicine and isolated drug discovery need to be continued, considering the threat of new emerging disease such as SARS, bird flu, not to mention the killer HIV AIDS. Plants are a good source of herbal medicine and natural products/ phytochemicals[1-15]. Many synthetic drugs owe their discovery and potency as a result of a mimic of structures from natural products isolated from plants rather than to the creativity and imagination of contemporary organic chemists. For example, the drug taxol (a diterpenoid), first isolated from the bark of the yew tree *Taxus brevifolia* has yielded two approved drugs for breast and ovarian cancer[6,14]. In Guyana, there are many medicinal folklore practises but most are without scientific research. For example, drinking water extract of *Momordica Charantia* is a good remedy for diabetes. Thus, there exist an urgent need to correlate folklore herbal practices with scientific evidences. With an increasing emphasis on scientific research, Guyana stands well in this area. Its our scientific endeavour, to correlate antimicrobial activity of *Momordica Charantia* with its folklore practices.

Momordica Charantia commonly called bitter melon belongs to the family *cucurbitaceae* and grows in tropical areas, including parts of the Amazon, East Africa, Asia, and the Caribbean, and is cultivated throughout South America as a food and medicine[16-18]. It's a slender, climbing annual vine with long-stalked leaves and yellow, solitary male and female flowers borne in the leaf axils. The fruit looks like a warty gourd, usually oblong and resembling a small cucumber. All parts of the plant, including the fruit, taste very bitter. In Guyana traditional medicine, a leaf tea is used for diabetes, to expel intestinal gas, to promote menstruation, and as an antiviral for measles, hepatitis, and feverish conditions. It is used topically for sores, wounds, and infections and internally and externally for worms and parasites.

Escherica. coli can cause several intestinal and extra intestinal infections such as urinary tract infections, meningitis, peritonitis, mastitis, septicemia and gram-negative pneumonia[19]. *Staphylococcus aureus* can cause furuncles (boils), carbuncles (a collection of furuncles)[20]. In infants, *Staphylococcus aureus* can cause a severe disease Staphylococcal scalded skin syndrome (SSSS). Staphylococcal *endocarditis* (infection of the heart valves) and pneumonia may be fatal. *Candida Albicans* is a diploid fungus (a form of yeast) and is a casual agent of opportunistic oral and genital infections in humans[21].

Fig. 2.0: Momordica Charantia

2.0. Procedure:

2.1: **Collection of Plant materials**: The leaves of the above plant was collected off the coastal plain of Guyana. The detached plant leaves were subjected to aerial drying for two weeks, removed and placed in separate conical flasks. It was then extracted with the required solvents.

2.2 Extraction: The leaves were first extracted in hexane thrice over a period of five days[1-13].Water was removed from the accumulated extract by stirring over anhydrous Na_2SO_4 and extract filtered.Solvents were removed in *vacuo* using a rotor vapor. The extracts was placed in sample vials and allow to evaporate. Further drying was done in a dessicator to remove residual solvents. Extracts were stored in capped vials and were weighed. The above procedure was repeated with the same leaves but with different solvents of increasing polarity: dichloromethane, ethyl acetate, and then ethanol.

2.3. Antimicrobial activity tests

2.3.1. **Making up extract solution**

A pproximately 0.03g of dried crude extract of *Momordica Charantia* was weighed and transferred to a 10 mL volumetric flask. The respective solvent was then added to make up the 10 mL solution.

2.3.2. Microorganisms:

Micro organisms: *Staphylococcus aureus, Escherichia coli and Candida albicans* were obtained from the Georgetown Public Hospital (GPH) microbiology laboratory and was stored in a refrigerator at the Food and Drug microbiology lab.

2.3.3. **Potato dextrose agar (PDA)** [23]

The potato *was peeled* and 100g was measured, finely chopped and boiled to a mash in distilled water.The dextrose was measured (12.5g) and placed in a 1L measuring cylinder. Agar was measured (12.5 g) and added to the measuring cylinder (with the dextrose). The potato mash was stirred and strained into the cylinder. Hot distilled water was added to make up 500mL.The contents was continuously poured and stirred until consistency was achieved. The content was then poured into a conical flask, plugged with cotton wool, over which aluminium foil was tightly wrapped. The flask was then autoclaved at 121 ^{0}C for 24hrs.

2.3. 4. Reference and Control:

The references were antibiotic in nature. *Ampicillin* and *Nyastatin*. *Ampicillin* was choosen as the reference for all bacterial species used: *E.Coli* and *S.aureus*. *Nyastatin* was used as the reference for the fungus, *Candida.albicans.* The Control experiment consists of a plate of solidifying agar onto which was inoculated pure solvent with microorganism mixed in a 1:1 portion [24].

2.3.5. Aseptic conditions:

The aseptic chamber consists of a wooden box (1m x 1m x 0.5m) with a door, was cleaned with 70% ethanol and irradiated with short wave UV light (from a lamp).

2.3.6. Mother plates:

These were made by culturing *C.albicans* on PDA. A sterilized 6mm cork borer was used to cut agar discs in the plate.

2.3. 7. Nutrient Agar:

Nutrient agar was purchased from the International Pharmacy Association in Guyana. 500ml of nutrient agar was made by placing 14g of the powdered mixture in a 1L flask, stirred, boiled and then autoclaved for 15 minutes at 121^{0}C. The plates were poured in a sterile environment and allowed to cool for 2 hours. Under aseptic conditions, the micro organisms were streaked onto separate plates and the discs were applied with a forceps.They were labeled and placed in an incubator at 37 ^{0}C for 24 and 48 hours for bacteria and fungi respectively.

2.3.8. **Colonies Counting**:

Colonies were estimated with the assistance of a colony counter. The number was estimated for 1cm^2 and then calculated for the entire plate. The plate radius was determined.

2.3.9. Retention Factor: R_f = Distance moved by sample

$$\frac{\text{Distance moved by sample}}{\text{Distance moved by solvent front.}}$$

In general, the most polar compound has the lowest R_f value.

2.3.10. Disc diffusion: Stokes Disc diffusion sensitivity technique [23].

Using Stokes Disc diffusion sensitivity testing technique[24] , an inoculum containing bacterial or yeast cells was applied onto nutrient agar plates. On each plate, a reference antibiotic was also applied.The reference antibiotic disc contained 10mg of antibiotic/disc. The discs were made by cutting discs (5-6mm) from a filter paper with a perforator, placing 5 of these discs in a vial and adding 0.2mL of each extract solution. These were left to dry. Discs were also made for the controls: ampicillin for the bacteria and nystatin for the fungus. Each disc was impregnated with the anticipated antimicrobial plant extract at appropriate concentration of 200 mg/ml using a microlitre syringe. This was then placed on a plate of sensitivity testing nutrient agar which was then incubated with the test organism:Bacteria/fungi. Incubation was done at 37ºC for 24 hr and 48 hr for the bacteria and *Candida albicans* species respectively. The antimicrobial compound diffuses from the disc into the medium. Following overnight incubation, the culture was examined for areas of no growth around the disc (zone of inhibition). The radius of the inhibition zone was measured from the edge of the disc to the edge of the zone.The end point of inhibition is where growth starts. Larger the inhibition zone diameter, greater is the antimicrobial activities. It is anticipated through the antimicrobial activity of plant extract, no area of growth will be induced around the disc. Bacteria or fungal strains sensitive to the antimicrobial are inhibited at a distance from the disc whereas resistant strains grow up to the edge of the disc. Discs applied to the plates already streaked with bacteria and the fungus

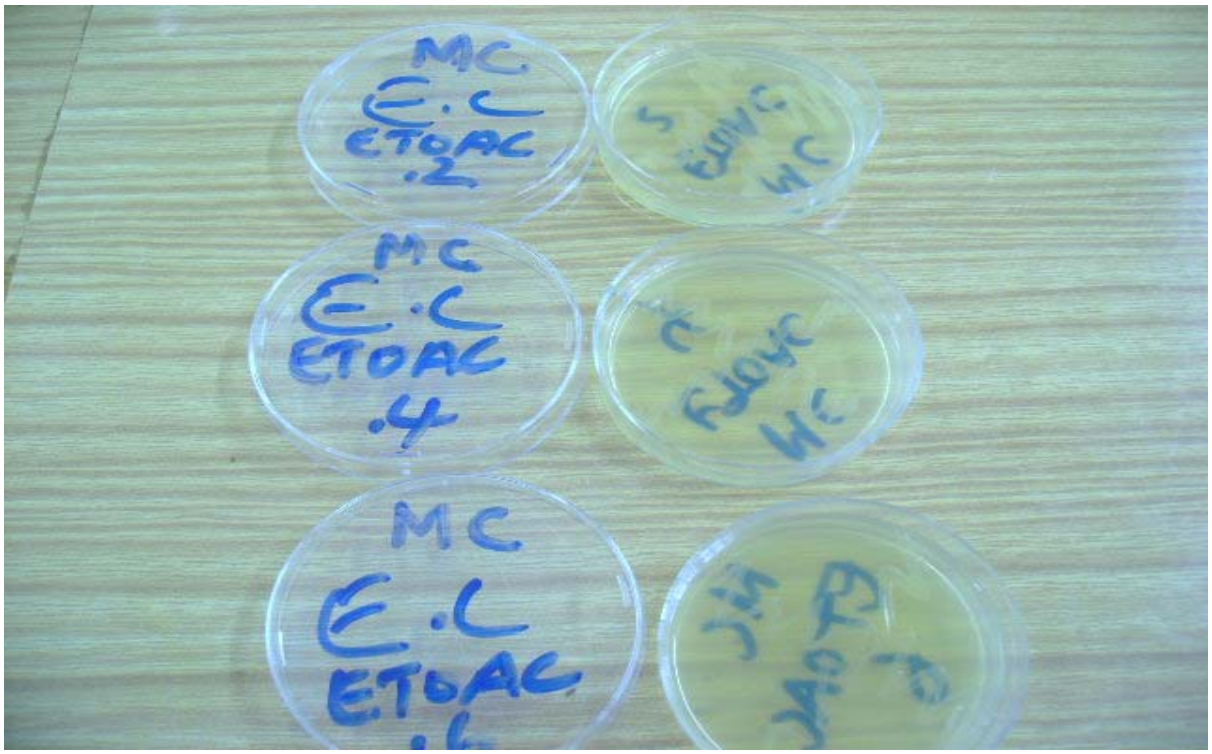

Fig. 3.0. Streak plate after 24 h: The ethyl acetate extract of *Momordica. charantia against E. coli*.

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2.3.11. Diffusion plate (well diffusion):

The fungus (*Candida albicans*) was mixed with the warm, melted, autoclaved PDA and poured into plates under aseptic conditions.The plates were covered and allowed to cool. As soon as the agar was partly solidified, the plates were inverted and left for 2h. When cooled, a well was made at the centre of the plate. The well was made by using a 6mm cork borer that was sterilized with alcohol and flame. The extracts were applied to different wells in volumes of 100-600uL using a micro liter syringe. The four solvents (hexane, dichloromethane, ethyl acetate and ethanol) were used as control whereas nystatin was used as the reference.The plates were labelled, covered, inverted and placed in a fume hood (no incubator was available) for 48h.

2.3.12. Pour Plate Method [23].

After the nutrient agar was placed in the autoclave at 120^{o}C for one and half hour, it was taken out and left to semi cool in a sterilized environment. 0.1mL of each solvent type extract and control were measured and placed in separate sterile glass plates plates (100mm diameter). 10mL of nutrient agar was then poured into the 100 mm plate, with an even depth of 4mm on a level surface shaken and allowed to cool. A sterile glass rod was used to uniformly stir the mixture into the nutrient agar which was left to solidify in the glass plate. The microorganisms were then streaked onto the plates and placed in an incubator at 37 ^{0}C for 24 and 48 hours for bacteria and fungi species respectively.

The inoculated plates were incubated in an inverted position (lid on bottom) to prevent collection of condensation on the agar surface. The plates were examined for the appearance of individual colonies growing throughout the agar medium. The number of colonies were counted so as to determine how effective the plant extract were against bacterial and fungi.

2.3.13. Streak plate for bacteria:

Nutrient agar was prepared as described above and 10mL was poured into plates. The plates were treated with the extracts and reference compound ampicillin in varying volumes of 100-600uL.The plates were allowed to cool and then the bacteria were streaked onto the surface.These plates were left for 24 hours.The plates with inhibition were used in further experiments.

2.3.14. Thin Layer Chromatography (TLC):

A baseline was drawn on the TLC plate. A spot of the plant extract was placed on the baseline with use of the pipette and allowed to dry.The plate was placed in the developing jar with the solvent. When taken out of the jar, the solvent front was drawn.They were examined under the UV/Vis lamp and the specks were circled with a pencil. The plates were then held in the iodine jar for a few seconds, shaken and taken out.The plate was further examined under UV lamp and any new specks were marked. The specks were labeled and their distances from the baseline were measured. The distance between the baseline and the solvent front was measured.The R_f values were determined.

2.3.15. **Results**:

Mass of dried leaves used for , *Momordica .charantia species was* 8.55g respectively. The physical state of the dried extract are shown in Table 1.0.

Table 1.0 Shows physical properties of the dry extracts.

Solvent	**Plant**	**Dry extract**
Hexane	*Momordica.charantia*	Yellow, brown, oily.
Dichloromethane	*Momordica.charantia*	Crystalline, black..
Ethyl acetate	*Momordica.charantia*	Soft, black ..
Ethanol	*Momordica.charantia*	Brown, powdery.

These extracts were in the concentration of 0.03g in 10ml of solvent except for *M.charantia* with ethanol which was 0.5g in 25ml. This works out to 0.0003mg/uL and 0.02mg/uL of crude extract respectively. The ampicillin and nystatin controls were in concentration of 250mg in 10ml.

Disc diffusion:

Table 2.0. Antimicrobial activity of Plant extract as shown by the inhibition zone diameter**.**

Area of inhibition. (mm^2) using *E.Coli*	**Area of inhibition. (mm^2) using** *S.aureus*	**Area of inhibition. (mm^2) using** *Candida albicans*	**Plant Extracts**	**Reference compound (Ampicillin) (mm^2)**	**Control Experiment**
			Momordica Charantia		No zone of inhibition
< 5	**< 5**	**< 5**	Hexane extract	**27**	No zone of inhibition
< 5	**< 5**	**< 5**	Dichloromethane extract	**28**	No zone of inhibition
< 10	**< 5**	**< 5**	EtOAc extract	**28**	No zone of inhibition
21	**18**	**20**	Ethanol extract	**30**	No zone of inhibition

Table 3.0. Results of the Well diffusion for plant extracts against *C.albicans.*

Zones of inhibition (mm^2)	**Extract**	**Volume of Extract**	**Observations**
0	*Momordica.charantia* with Hexane	100-600 uL	No zones of inhibition visible, scattered colonies.
0	*Momordica.charantia* with ethyl acetate	100-600 uL	No zones of inhibition visible, scattered colonies.
0	*Momordica.charantia* with dichloromethane	100-600 uL	No zones of inhibition visible, scattered colonies.
75x45	*Momordica.charantia* with ethanol	600 uL	Complete zones of inhibition.
20x30 = 600 30x50 = 1500 50x70 = 3500	Nystatin	200 uL 400 uL 600 uL	Zones of inhibition
Controls	Diffusion well with four solvents		Scattered colonies
Reference(Nystatin)		600 uL	Complete zones of inhibition

Pour Plate:

Table 4.0 showing the number of visible colonies when viewed under a colony counter.

Bacterium	**Extract**	**Volume**	**# of colonies**
S.aureus	*Momordica charantia* with ethanol	300 uL	80
S.aureus	*Momordica charantia* with ethanol	400 uL	60
S.aureus	*Momordica.charantia* with ethanol	500 uL	55
S.aureus	*Momordica.charantia* with ethanol	600 uL	50
E.coli	*Momordica.charantia* with ethyl acetate	600 uL	$12/cm^2$x $0.5(63.6cm^2)$= 381.
Controls: *S.aureus* *E.coli*	Nutrient agar only	600 uL	Excess growth observed. $26/cm^2$x $63.6cm^2$=1653. $20/cm^2$x $63.6cm^2$=1272.

These colonies were estimated with the assistance of a colony counter. The number was estimated for $1cm^2$ and then calculated for the entire plate. The plate radius was 45mm, therefore the area was 63.6 cm^2

Table 5.0. Results of the Well diffusion for plant extracts against *C.albicans.*

Zones of inhibition (mm^2)	**Extract**	**Volume of Extract**	**Observations**
0	*Momordica.charantia* with ethyl acetate	100-600 uL	No zones of inhibition visible, scattered colonies.
0	*Momordica.charantia* with dichloromethane	100-600 uL	No zones of inhibition visible, scattered colonies.
75x45 =	*Momordica.charantia* with ethanol	600 uL	Complete zones of inhibition.
20x30 = 600 30x50 = 1500 50x70 = 3500	Nystatin	200 uL 400 uL 600 uL	Zones of inhibition
Controls	Diffusion well with four solvents		Scattered colonies
Reference(Nystatin)		600 uL	Complete zones of inhibition

Streak plate:

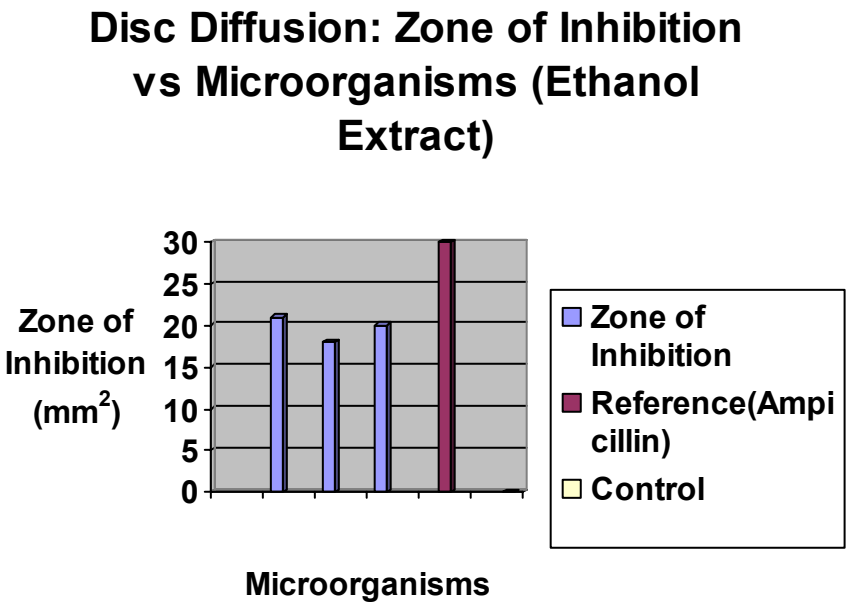
Table 6.0. showing the results of the streak plate method.

Bacterium	Solvent Extract	Volume of Extract	Observations
E.coli	*Momordica.charantia* with ethanol	600uL	Limited growth
E.coli	*Momordica.charantia* With ethyl acetate	200-600uL	Limited growth
S.aureus	*Momordica.charantia* with ethyl acetate	200-600uL	Limited growth
S.aureus	*Momordica.charantia* with ethanol	200-600uL	Limited growth
Controls:	The four solvents: hexane,dichloromethane, ethyl acetate and ethanol	200-600uL	Growth of microorganism
Reference (Ampicillin)		200-600uL	Inhibition

Table 7.0. showing the results of the TLC for all the extracts.

Solvents	**Plants**	**No of spots visible by UV**	**R_f value= d moved by sample / d moved by solvent**
Hexane	*Momordica Charantia*	1	0.67
Dichloromethane/hexane, 90: 10, v/v)	*Momordica Charantia*	5	0.05 0.08 0.18 0.40 0.50
Ethylacetate/dichloromethane, 90: 10, v/v)	*Momordica Charantia*	1	0.11 0.39 0.58 0.97
Ethanol/hexane, 90: 10, v/v)	*Momordica Charantia*	1	0.14

Graphs: Bar graphs are shown in Fig. 3.0 (a) and 4.0 (a) whereas a corresponding line graph for Fig. 3.0 (a) is shown in Fig. 5.0 (a).



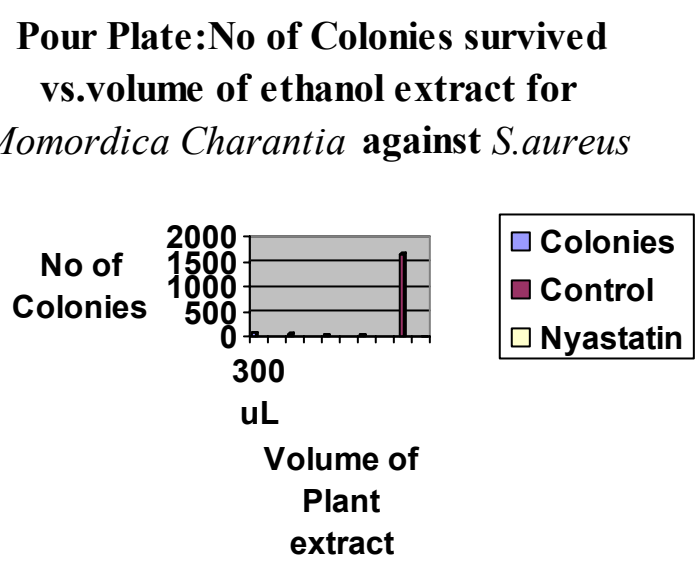
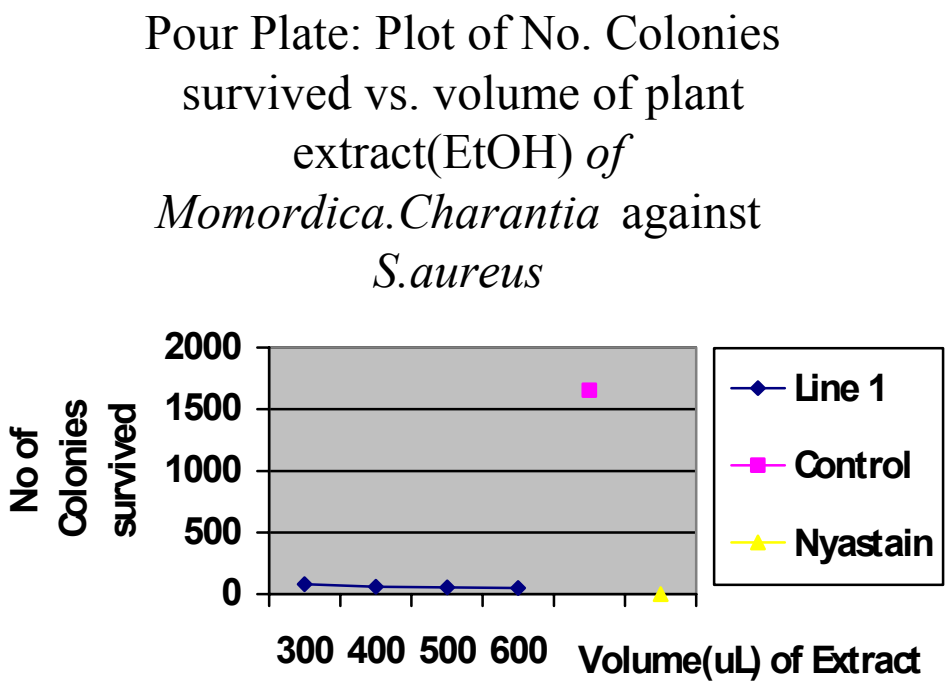


Fig. 4.0. (a)



(a)

Fig. 5.0. (a).

2.3.17. Discussion:

All four methods: Stokes disc diffusion sensitivity techniques, Pour plating, Well diffusion and Streak plate were successful in determining *Momordica Charantia's* antimicrobial activities. Antimicrobial activity follow the sequence: Ethanol extract > EtOAc extract > dichlormethane extract > hexane extract. Stokes disc diffusion indicates that the hexane, dichloromethane, ethylacetate extract of *Momordica Charantia* had neglible antimicrobial activity. Only a maximum zone of inhibition of 10 mm^2 was observed against *E.coli* for the EtOAc extract. However, the plant ethanol extract was effected against all three microorganisms studied. Significant zones of inhibition were observed. This range from 18 mm^2 to 21 mm^2. The former was obtained for *S.aureus* whereas the latter was obtained for *E. coli*.

The Well diffusion method was used primarily against *C. albicans*. It indicates that the EtOAc extract and dichloromethane extract of *Momordica Charantia* at volume of 100-600 uL induce no zones of inhibition. Instead scattered colonies were observed i.e microbial.This is consistent with the results obtained for the disc diffusion method. The ethanol extract of *Momordica Charantia* showed complete zones of inhibition. The zone of inhibition was observed to be: 75 x 45 mm^2. *Momordica Charantia's* hexane and dichloromethane extracts at 100-600ul showed zero zone of inhibition. The reference antibiotic: Nystatin showed zone of inhibition of 600 mm^2 to 3500 mm^2 as the volume of the extract increase from 200uL to 600uL respectively. For the control experiment, the well with the four solvents induced scattered colonies i.e negative inhibition. This indicates that the inhibition induced are truly due to the plant's active constituents rather than to the solvents used in the extraction process.

The Pour plate method indicate that the ethanolic extract of *Momordica Charantia* showed inhibition against the growth of bacteria, *S.aureus*. With increasing volume of extract from 300 uL to 600 uL, the number of colonies decreased from 80 to 50 against *S.aureus,* Table 4.0. In constrast, the EtOAc extract of *Momordica Charantia* at a volume of 600 uL induce the formation of 381 colonies i.e it was microbial. Control experiments for Pour plate indicate that the solvents induce growth of bacteria: *S.aureus and E.coli*, Table 4.0. However, the reference compound Ampicillin and nyastatin completely inhibit the

growth of microorganisms. For the pour plate method, the number of colonies were estimated using a colony counter.

For the Streak plate method, the ethanol extract of *Momordica Charantia* at a volume of 600 uL showed limited growth against *S.aureus and E.coli*. The EtOAc extract of *Momordica Charantia* at 200-600uL also showed limited growth against *E.Coli* and *S.aureus*. The control experiment indicated that all four solvents: hexane, dichloromethane, ethylacetate and ethanol showed growth of the two bacterial species whereas the reference compound Ampicillin showed complete inhibition at 200-600uL.

Fig. 3.0 (a) represent for the Disc diffusion method using the ethanol extract, a plot of the zone of inhibition vs. type of microorganism, Fig. 4.0 (a) represent for the pour plate method, a plot of the number of colonies survived vs. volume of *Momordica Charantia* ethanol extract against *S.aureus*. Fig. 4.0 (a) represent plots of the corresponding line graphs. For the disc diffusion technique, using the ethanol extract, the largest zone of inhibition was observed for *E.Coli*. As the volume of plant extract increased so too is the zone of inhibition for the well diffusion method. For the pour plate result, as the volume of the plant (*Momordica Charantia*) ethanolic extract increased, the number of colonies decreased

TLC analyses in various solvent system for each solvent type extract revealed the presence of spots that range from one to a maximum of five, Table 6.0. Each spot is presumbably due to a pure natural product or phytochemical. Each also has a specific R_f value. The larger the R_f value, the lower the polarity of natural product/phytochemicals The number of spots and R_f value for each spot is recorded in Table 5.0. For example for the dichloromethane extract of *Momordica Charantia*, five spots with R_f values of 0.05, 0.08, 0.18, 0.40 and 0.50 in dichloromethane/hexane, 90: 10, v/v) respectively were seen.

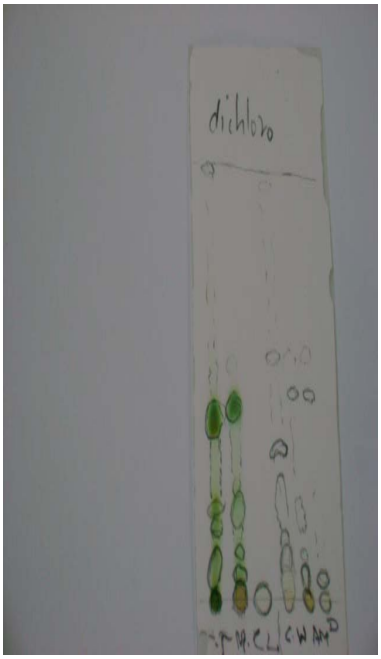


Fig. 6.0**. TLC analyses:** *Momordica Charantia* **etc.**

Conclusions:

It is clearly seen that *Momordica Charantia* has antimicrobial properties. However, antimicrobial activity is solvent dependent with the ethanol extract, the most potent and hexane the least. In general, the order of antimicrobial activity follow the sequence: Ethanol extract > EtOAc extract > dichlormethane extract > hexane extract. Thus, the ethanol extract of *Momordica Charantia* can be used as the active constituent of an antimicrobial cream. Future work such as isolation and purification of bioactive constituents should target the ethanol extract of *Momordica Charantia*. In Guyana's culture, *Momordica Charantia fruit* is edible and is used as "cooked carylla" eaten with rice. Also, an overnight aqueous extract of the plant leaves is used in the control of diabetes.

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Riboflavin profile in Nigerians with *Schistosoma heamatobium* infection

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ABSTRACT: Riboflavin profile and the degree of infection were studied among 100 volunteers comprising 65 children and 35 adults with *Schistosoma haematobium* infection. Light infection of < 50 ova/10ml was reported among 35 volunteers while heavy infection of > 50 ova/10ml was observed in 65 patients. The infected participants had mean riboflavin (22.0 ± 4.5 nmol/L), flavin mononucleotide, FMN (16.44 ± 2.8 nmol/L), flavin adeno dinucleotide, FAD (63.07 ± 0.75 nmol/L) . The control subjects had higher mean riboflavin (108.8 ± 10.2 nmol/L), FMN (102.8 ± 3.5 nmol/L) and FAD (404.9 ± 8.7 nmol/L). These differences between the mean control and the infected volunteers for mean riboflavin, FMN and FAD were statistically significant (χ^2 = 68.45, P> 0.05; χ^2 = 72.63, P> 0.05; χ^2 = 288.58, P> 0.05). The relationship between egg counts, riboflavin, FMN and FAD was negatively correlated (r = -0.30, r = -0.41, r = -0.38) respectively. The mean riboflavin (19.0±4.6 nmol/L), FMN (14.7±1.65 nmol/L) and FAD (56.58±12.49 nmol/L) in children were lower than the 35 infected adults. These differences for riboflavin, FMN and FAD were not statistically significant (P<0.05; χ^2=0.47, P<0.05; χ^2=1.69, P< 0.05; χ^2=0.66) respectively. We deduce that the depressed riboflavin status among the *S. haematobium* infected volunteers than their control subjects implicated riboflavin and its metabolites in the pathogenesis of this parasite. There is the need to incorporate riboflavin in the management of urinary schistosomiasis. [Nature and Science. 2008;6(1):15-18]. ISSN: 1545-0740.

INTRODUCTION

Schistosomiasis remains an important parasitic infection in many tropical areas, especially Africa. Six hundred million people are thought to be at risk and 200 million are estimated to be infected (Chan *et al*., 1996). Recent analysis suggest that the morbidity due to schistosomiasis is grossly underestimated (King *et al*., 2005), resulting in an estimated 280,000 deaths annually in sub-Saharan Africa (Hoetez *et al.*, 2006). Nutritional status has been implicated as one of the factors associated with schistosomiasis morbidity (WHO, 1992).

Riboflavin is an essential nutrient in human nutrition has flavin mononucleotide (FMN) and flavin adeno dinucleotide, (FAD) as its precursor or metabolites. Ingested riboflavin enters the blood stream as FMN (Combs *et al.,* 1998), and inadequate riboflavin supply results in low circulating concentration (Capo-Chichi *et al.,* 2000). Circulating FAD also was reported to be decreased in malnutrition (Capo-Chichi *et al.,* 1999; Capo-Chichi *et al.,* 2000). Schistosomiasis and riboflavin deficiency have been associated with anaemia (Antony *et al*., 2006; Vanden Broek *et al*., 2000). However, the existence of an association between nutritional status and schistosomiasis is still not clear. Several studies have tried to correlate the nutritional status of the host with prevalence/intensity of infection (Coutinho 1976, 1980, Stephenson 1986, Coutinho *et al*., 1992, Ferreira *et al*., 1993, Continho *et al.,* 1997) or severity of clinical manifestations in schistosomiasis. However, conflicting results have been reported which could be due to differences in local, epidemiological features and in part, to different methodologies (Costa *et al*., 1988, Projetti *et al*., 1992).

We therefore investigate riboflavin and flavin nucleotides concentration and the degree of schistosomiasis in our locality for which information is previously lacking. This present communication correlates riboflavin and flavin nucleotides status of infected volunteers and intensity of infection.

MATERIALS AND METHODS

This study was carried out in Ihieve-Ogben; a rural community in Owan East local government area of Edo State. It is located at Latitude 6ºN and longitude 6ºE. Ihieve-Ogben is located within the guinea savanna region of the State. Agriculture especially farming and hunting are their predominant activities while a few of them, mostly women, are traders. The village has a stream which the inhabitants use as their source of water and recreational activities. There are about 1,000 inhabitants in this community.

The investigation commenced with a community mobilization campaign at Ihieve-Ogben. This involved educating them on the significance of the study as well as seeking their consent. Ethical permission was obtained from the State Ministry of Health, Benin City, Nigeria.

The Ova found in the urine of the 100 participates with schistosomiasis were quantified and classified as light infection <50 ova/10ml and heavy infection >50 ova/10ml according to WHO standards (WHO, 1983). Thirty control volunteers were without the *S. haematobium* eggs in their urine. Malaria, intestinal parasites, HIV and other overt febrile illness were ruled out in these volunteers using standard procedures and kits. The plasma riboflavin, FMN and FAD were determined by a high performance liquid chromatography (Traunmüller et al 2003).

The data obtained in this study were subjected to statistical analysis namely correlation and chi-square tests using Microsoft Excel statistical package.

RESULTS

Riboflavin, the flavin neuclotides profile and intensities of infection are presented in table 1. Light infection of < 50 ova/10ml was reported among 35 volunteers while heavy infection of > 50 ova/10ml was observed in 65 patients. The mean infected participants had mean riboflavin (22.0 ± 4.5 nmol/L), flavin mononucleotide, FMN (16.44 ± 2.8 nmol/L), flavin adeno dinucleotide, FAD (63.07 ± 0.75 nmol/L) . The control subjects had higher mean riboflavin (108.8 ± 10.2nmol/L), FMN (102.8 ± 3.5 nmol/L) and FAD (404.9 ± 8.7 nmol/L). These differences between the mean control and the infected volunteers for mean riboflavin, FMN and FAD were statistically significant (χ^2 = 68.45, P> 0.05; χ^2 = 72.63, P> 0.05; χ^2 = 288.58, P> 0.05). The relationship between egg counts, riboflavin, FMN and FAD was negatively correlated (r = -0.30, r = -0.41, r = -0.38) respectively.

Table 2 shows riboflavin and flavin nucleotides status of infected children and adults. Sixty five children were infected with *S. haematobium* for which their mean riboflavin (19.0±4.6 nmol/L), FMN (14.7±1.65 nmol/L) and FAD (56.58±12.49 nmol/L) was lower than the 35 infected adults. These differences for riboflavin, FMN and FAD were not statistically significant (P<0.05; χ^2=0.47, P<0.05; χ^2=1.69, P< 0.05; χ^2=0.66) respectively.

Table 1: Riboflavin and flavin nucleotides profile and intensity of infection

Intensity of infection	No Infected		Riboflavin (nmol/L)	FMN (nmol/L)	FAD (nmol/L)
	Children	Adult			
Light Infection <50 ova/10ml	15	20	27.0±6.27	17.0±1.69	66.1±0.81
Heavy Infection >50 ova/10ml	50	15	17.0±2.1	15.87±0.47	60.04±0.81
Mean			22.0±4.5	16.44±0.28	63.07± 0.75

Table 2: Riboflavin and flavin nucleotides status of infected children and adults

	No infected	Riboflavin (nmol/L)	FMN (nmol/L)	FAD (nmol/L)
Children	65	19.0±4.6	14.7±1.65	56.58±12.49
Adult	35	25.5±7.5	18.08±1.36	69.50±6.37
Mean	-	22.25±5.5	16.39±1.4	63.07± 4.0
Control	30	108.8±10.2	102.8±3.5	404.9±8.7

DISCUSSION

We reported depressed levels of riboflavin and flavin nucleotides in participants infected with schistosomiasis than the control subjects. Similar report on riboflavin deficiency in schistosomiasis had been documented earlier (Coutinho *et al.,* 1997, Rohner *et al.,* 2007). Of pathological importance is the negative correlation between the intensities of infection and the concentration of the riboflavin and its metabolites. Similar correlation between the nutritional status and prevalence/intensity had been reported earlier (Coutinho, 1976, 1980, Stephenson, 1986, Ferreira *et al.,* 1993). These observations and the lower

concentration of this micronutrient in the infected participants with heavy intensity of infection demonstrate the effects of *S. haematobium* on the riboflavin pool of the infected Nigerian. These implicate this micronutrient in the disease pathogenesis and the morbidity of urinary schistosomiasis in this locality. Since (Kawanaka *et al.,* 1983) documented a link between *Schistosoma* eggs survival and vitamin uptake, we deduce that riboflavin could be one of the essential vitamins required for the survival of *S. haematobium* eggs, which probably explains the low levels of riboflavin and flavin nucleotides in the infected volunteers.

Our result shows higher FAD in the control participants than the infected volunteers. The relatively higher FAD in the control volunteers than the infected participants reflects the impact of *S. haematobium* on the riboflavin pools in these Nigerians. This observation supports the earlier report of (Asahi *et al.,* 1984) who documented that the extracts of *Schistosoma* eggs exhibit hemolytic activity. Also this hemolysis has been documented to cause the release of flavin metabolites such as FAD and FMN from the intracellular compactment into plasma and mobilization of riboflavin from tissues into the circulation during febrile illness (Bamji *et al*., 1987). Our data which revealed three times the concentration of FAD to the riboflavin among the *S. haematobium* infected volunteer further supports the effect of haemolysis of this parasites in our investigated infected host.

In conclusion, we deduce that the depressed riboflavin status in the infected participants than their control counterparts implicates riboflavin and its metabolites; FAD and FMN in the pathogenesis of urinary schistosomiasis. This strongly supports our recommendation for the inclusion of nutrition program especially aimed at checking riboflavin deficiency in the management of this parasitic infection in the face of low socioeconomic and nutritional status which abounds in developed nations like Nigeria where urinary schistosomiasis still occur in endemic proportion.

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Karyotypic diversity of some tilapia species

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Abstract: This study present cytogenetic analyses of three fish species in Egypt, *Oreochromis niloticus, sarotherodon galilaeus* and *Tilapia zillii* belonging to cichlids, with a mean objective of contributing for a better understanding of the relationships between these species and the probability of their hybridization. The karyotypes of these species have been investigated by examining metaphase chromosomes spreads obtained from headkidney cells. The diploid chromosome numbers of all three species were 2n=44. The karyotypes of *Oreochromis niloticus* were one pair of submetacentric; 13 pairs of subtelocentric and 8 telocentric ; *Sarotherodon galilaeus* showed one pair of metacentric, 6 pairs of submetacentric; 7 pairs of subtelocentric and 8 pairs of telocentric chromosomes . The chromosomes of *Tilapia zillii* were,10 pairs of submetacentric ;5 pairs of subtelocentric and 7 pairs of telocentric chromosomes . There was a significant difference between the mean lengths of the haploid sets of chromosomes of fishes under investigation. The cytogenetic characteristics partaken by the species analyzed in the present study reinforce and the probability of hybridization between *Oreochromis niloticus* and *sarotherodon galilaeus* ,but less chance between *Tilapia zillii and Oreochromis niloticus* or *, sarotherodon galilaeus*. [Nature and Science. 2008;6(1):19-27]. ISSN: 1545-0740.

Keywords: karyotypic diversity, tilapia, chromosomes.

Introduction

The importance of fish taxonomy is not only with description of new forms, but also with placing each form within taxonomic system that shows it's relationships to other forms .For more than a century, systematists have sought to organize this diversity by studying aspects of their external and internal morphology which have been especially successful in defining species and in organizing these species into genera. These groupings have usually been confirmed when examined with cytogenetically approaches.

Tilapia is a generic term used to designate a group of commercially important food fish belonging to the family Cichlidae; Cichlids are classified in the large order Perciformes, which consists of three aquacultural important genera- *Oreochromis niloticus, sarotherodon* and *Tilapia,* they inhabit the fresh and brackish waters .

Tilapia have been receiving increased scientific study as they have important species in tropical aquaculture. The classification of the Tilapiines relies heavily on the differences in breeding and brooding behavior to discriminate between species at the generic or subgeneric level.

Karyological studies of fishes can contribute significantly to the solution of many problems in areas of research ranging from taxonomy, systematic or genetics to phylogenetics, or environmental toxicology (Alsabti ,1985) .

In the last few decades works have been focused on the field of cytogenetic investigation of fishes, especially in the area of systematics, mutagenesis and aquaculture. The karyotype is the chromosome complement of an individual or related group of individuals, as defined by chromosome size, morphology and number. Though for all somatic cells of all individuals of species, the number of chromosomes is used as an indicator of classification of species of chromosomes and interrelationships within families. The studies of these characters help to investigate the aquatic structure for the investigate the aquatic structure for the population of each species population in each habitat, so it can determine what

species are related to each other in an accurate manner?. This may help to facilitate the hybridization between them in the future to improve the strains.

As a first step in establishing the fish taxonomy for this important aquaculture species we have analyzed the chromosomal karyotype in three common tilapia species, *Oreochromis niloticus, sarotherodon galilaeus* and *Tilapia zillii*

Material and methods

I- **Chromosomal preparation**

A- Collection of samples:

Twenty individuals from each species (*Oreochromis noiloticus*, *sarotherodon galilaeus and Tilapia zillii*) were collected from the freshwater canals at Giza and Kafr El- Sheikh governorates in Egypt. Each specimen was injected intrapertioneally with 0.01% of freshly prepared colchicine solution per gram of body weight of fish. The specimen was then placed in a well aerated holing tank for 2-4h (The fish larger than 20cm was held for at least 6hrs. after injection).

B- Slide preparation and Cell Harvest:

Clean slides are critical for high quality preparation. dipped in 95 % ethanol and then swirled in distilled water (Yu et al., 1981).

The specimen was killed by pithing, or decapitation, The anterior headkidney was taken after sacrificing the specimen, then washed with isotonic solution of NaCl. Small pieces of tissues were) transported to hypotonic solution of (0.56% Kcl) using Pasteur pipette in centrifuge tube and homogenized, then centrifuged for 5-7 minutes at about 1000 rpm, then, the supernatant was removed.

Fixation was carried out by the addition of 8 ml of cold mixture of absolute methanol acetic acid (3:1) at 4°C for about 30 minutes. Then centrifugation was carried out at 800 - 1000 rpm for 10 min. and the supernatant was removed. Refixation for about 10 minutes was carried out twice as above (Bertollo, 1978).

C- Spreading of cells and Staining :

Cells suspension were concentrated, and spread by Pasteur pipette on slides. Slides were dried on flame, after 24 hr, they were stained with 10% Giemsa (10 ml Giemsa stock solution and 90 ml Sorensen's buffer pH = 6-8) for 40 minutes.

Examination, photography, and chromosomes karyotypc:

50 fields from each specimen were examined, photographed on light microscope (Dialux model 22B), with an oil immersion leitez magnifying lens (1000x), and photographed by an automatic camera (wild photoautomate, modal Mps 45) fixed on the microscope. The total length of each chromosome was measured, and finally chromosomes were arranged descendly in pairs according to their length, where the longest pair at first and the shortest one at last. Classification of chromosomes followed Levan, et al.,(1964).Metacentric (C.I. > 0.39 %); submetacentrics (C.I.>0.27%) are described as two arm chromosomes, and subtelocentrics (C.I. > 0.09 %); teloacentrics (C.I. < 0.009 %) as one arm chromosomes.

Results

The metaphase spread of the chromosomes of three species under investigation (Fig.1, 2, 3), showed that the diploid chromosome numbers of all three species were 2n=44. The karyotypes of *Oreochromis niloticus* (Fig.4-A) were one pair of subetacentric (chromosome no. 2); 13 pairs

of sub-telocentric (1, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14) and 8 pairs telocentric chromosomes (numbers15,16,17,18,19,20,21,22).

The type of chromosomes of *Sarotherodon galilaeous* (Fig.4-B) was submetacentric (numbers 3, 4, 5, 6, 8, 9); 7 pairs are subtelocentric (l, 2, 10, ll, 12, 14, 15) and 8 pairs of telocentric chromosomes (13, 16, 17, 18, 19, 20, 21 and 22).

The karyotypes of *Tilapia zillii* (Fig.4-C) were, 10 pairs of submetacentric ;5 pairs of subtelocentric and 7 pairs of telocentric chromosomes .

The length of the haploid set of the chromosomes in *Oreochromis niloticus* ranged between 3.46 µ m to 0.59 µ m for the longest and the shortest chromosomes respectively, the arm ratio ranged between 0.19 µ m to 0.00 µ m for the longest and the shortest chromosomes respectively (Table 1), where table 2 shows the length of the haploid set of chromosomes which ranged between 3.92 to 0.63µ m for the longest and shortest chromosomes respectively; the arm ratio ranged between 0.13µ m and 0.00 µm for the longest and shortest chromosomes respectively; and table (3) shows the length of the haploid set of chromosomes which ranged between 3.27 to 0.59µ m for the longest and shortest chromosomes respectively; and the arm ratio ranged between 0.23 µm and 0.00 µm for the longest and shortest chromosomes respectively.

These results revealed a significant difference between the mean length of the haploid sets of chromosomes of *sarotherodon galilaeus* and *Tilapia zillii* in chromosome numbers 11 and 21 only while no difference was found between other chromosomes, they are longer in *sarotherodon galilaeus* .In case of *Oreochromis niloticus* and *sarotherodon galilaeus* there is a significant difference in mean length of chromosomes numbers 1,2,4,5,17,18 and 22, they were longer in *sarotherodon galilaeus* while other chromosomes were longer in *oreochromis niloticus*, chromosomes numbers 15,19,20 and 21 were equal in the two species .

In case of *Tilapia zillii* and *Oreochromis niloticus* ,the difference was in chromosomes numbers 6,8,11,14 and 21, they were longer in *Oreochromis niloticus* than *T ilapia zillii* . These results led to the probability of hybridization between *Oreochromis niloticus* and *sarotherodon galilaeus* ,but less chance between *Oreochromis niloticus* and *Tilapia zillii* .

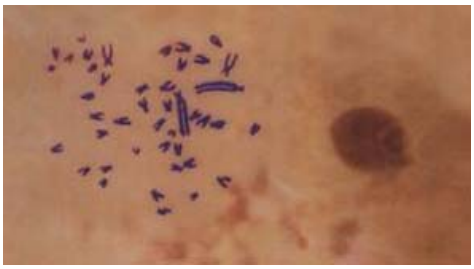

Fig.(1): Chromosome metaphase spread
Of *Oreochromis niloticus*

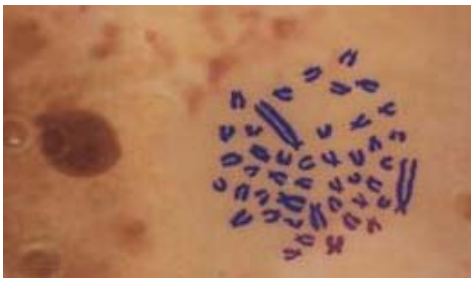

Fig.(2): Chromosome metaphase spread
Of *sarotherodon galilaeus*

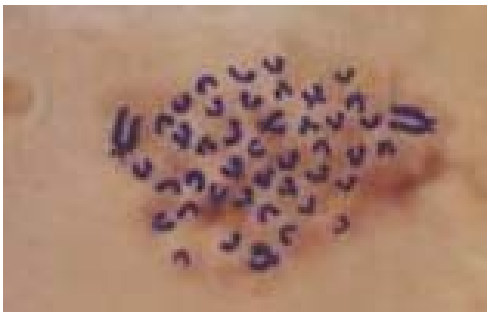

Fig.(3): Chromosome metaphase spread
Of *Tilapia zilli*

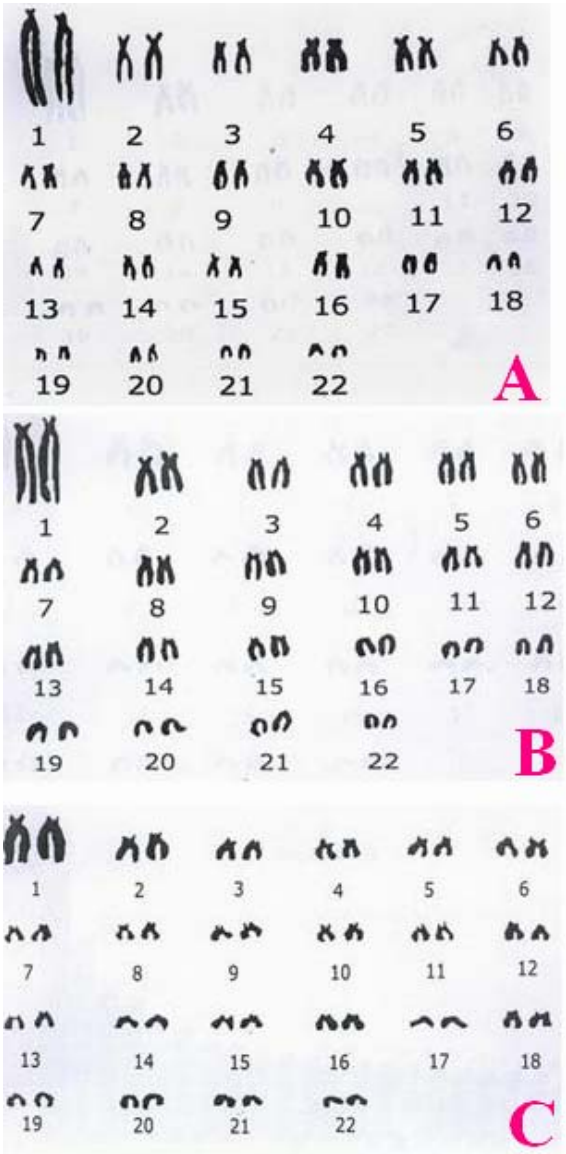

Fig. 4: Giemsa-stained karyotypes of:
A (Oreochromis niloticus); B(sarotherodon galilaeus); and C(Tilapia zilli

Table(1):Range, mean and arm ratio of the chromosomes set (n = 22) of Oreochromis niloticus

Chromosome No.	Range	Mean + S.D	Arm ratio	Type
1	5.190 - 1.730	3.46 ±1.02	0.19	S.T
2	3.290 - 1.300	2.29 ±1.50	0.41	S.M
3	2.290 - 1.950	2.12 ±0.36	0.31	S.T
4	1.993 - 1.490	1.83 ±0.21	0.33	S.T
5	1.993 - 1.460	1.66 ±0.32	0.29	S.T
6	1.230 - 1.530	1.46 ±0.33	0.31	S.T
7	1.675 - 0.952	1.39 ±0.29	0.23	S.T
8	1.632 - 0.950	1.36 ±0.29	0.30	S.T
9	1.615 - 0.800	1.38 ±0.25	0.28	S.T
10	1.596 - 0.910	1.21 ±0.19	0.18	S.T
11	1.596 - 0.793	1.19 ±0.23	0.22	S.T
12	1.596 - 0.712	1.19 ±0.21	0.22	S.T
13	1.481 - 0.730	1.09 ±0.18	0.18	S.T
14	1.485 - 0.636	1.06 ±0.24	0.13	T
15	1.361 - 0.611	0.98 ±0.24	0.05	T
16	1.360 - 0.608	0.97 ±0.25	0.04	T
17	1.198 - 0.690	0.85 ±0.18	0.00	T
18	1.059 - 0.521	0.83 ±0.20	0.00	T
19	1.059 - 0.495	0.81 ±0.19	0.00	T
20	1.049 - 0.413	0.80 ±0.32	0.00	T
21	0.911 - 0.521	0.76 ±0.21	0.00	T
22	0.915 - 0.532	0.59 ±0.21	0.00	T

M = Metacentric
T = Telocentric
S.M = Sub Metacentric
S.T = Subtelocentric

Table(2):Range, mean and arm ratio of the chromosomes set (n = 22) of sarotherodon galilaeus

Chromosome No.	Range	Mean + S.D	Arm.ratio	Type
1	7.381 – 2.610	3.92 ±1.41	0.13	S.T
2	7.013 – 1.220	3.05 ±1.63	0.23	S.T
3	2.992 – 1.183	2.64 ±0.78	0.35	S.M
4	2.651 – 1.093	2.21 ±0.45	0.37	S.M
5	1.937 – 1.092	1.65 ±0.35	0.28	S.M
6	1.893 – 0.901	1.30 ±0.31	0.28	S.M
7	1.762 – 0.812	1.19 ±0.29	0.35	M
8	1.652 – 0.800	1.15 ±0.36	0.32	S.M
9	1.581 – 0.850	1.12 ±0.28	0.26	S.M
10	1.523 – 0.792	1.12 ±0.25	0.24	S.T
11	1.534 – 0.785	1.10 ±0.52	0.22	S.T
12	1.493 – 0.772	1.06 ±0.31	0.25	S.T
13	1.427 – 0.756	1.01 ±0.21	0.21	T
14	1.431 – 0.703	0.98 ±0.19	0.07	S.T
15	1.391 0.703	0.96 ±0.21	0.21	S.T
16	1.389 – 0.691	0.93 ±0.15	0.01	T
17	1.201 – 0.662	0.89 ±0.13	0.01	T
18	1.140 – 0.650	0.86 ±0.23	0.00	T
19	1.064 – 0.632	0.81 ±0.13	0.00	T
20	1.062 – 0.631	0.75 ±0.11	0.00	T
21	0.925 – 0.591	0.68 ±0.13	0.00	T
22	0.805 – 0.399	0.63 ±0.10	0.00	T

M = Metacentric
T = Telocentric
S.M = Sub Metacentric
S.T = Subtelocentric

Table(3):Range, mean and arm ratio of the chromosomes set (n = 22) of Tilapia zilli

Chromosome No.	Range	Mean + S.D	Arm ratio	Type
1	3.390 – 1.410	3.27 ±1.21	0.23	S.T
2	3.160 – 1.420	2.43 ±0.75	0.25	S.T
3	2.630 – 1.401	1.80 ±1.39	0.30	S.M
4	2.015 – 0.921	1.68 ±0.30	0.32	S.M
5	1.995 – 0.861	1.41 ±0.29	0.32	S.M
6	1.813 – 0.703	1.31 ±0.27	0.21	S.T
7	1.795 – 0.685	1.16 ±0.27	0.34	S.M
8	1.783 – 0.631	1.09 ±0.28	0.34	S.M
9	1.632 – 0.662	1.02 ±0.29	0.38	S.M
10	1.551 – 0.813	1.00 ±0.23	0.35	S.M
11	1.432 – 0.750	.95 ±0.25	0.31	S.M
12	1.406 – 0.731	0.930 ±0.24	0.33	S.M
13	1.401 – 0.780	0.90 ±0.24	0.32	S.M
14	1.218 – 0.690	0.88 ±0.22	0.29	S.T
15	1.211 – 0.671	0.87 ±0.21	0.21	S.T
16	1.211 – 0.625	0.86 ±0.21	0.08	T
17	1.073 – 0.617	0.82 ±0.24	0.07	T
18	1.072 – 0.591	0.81 ±0.19	0.05	T
19	1.072 – 0.590	0.80 ±0.21	0.01	T
20	1.991 – 0.589	0.76 ±0.21	0.00	T
21	0.910 – 0.389	0.64 ±0.15	0.00	T
22	0.910 – 0.388	0.59 ±0.13	0.00	T

M = Metacentric
T = Telocentric
S.M = Sub Metacentric
S.T = Subtelocentric

DISCUSSION

Among the various fish groups, the family Cichlidae occupies the fourth place in number of species comprising about 85 genera and 700 species (El serafy et al.,1993), the greatest diversity is encountered in Africa . Techniques such as chromosomal analysis, DNA sequencing, amino acid

sequencing and protein electrophoresis have made it possible for systematic to utilize new sets of data for phylogenetic studies for this diversity (Duellman, 1985).

Tilapia are a group of Cichlid fishes of major economic importance in aquaculture, their uncontrolled and prolific breeding at a small size in mixed sex culture constitutes a constraint on their efficient production. Although interspecific hybridization of these species leading to all made stocks has been proposed as a possible solution to this problem (Beveridge and McAndrew,2000). In general, these fish are appropriate for both intensive and extensive piscicultre because one of there positive aquacultural characteristics of tilapia are their tolerance to poor water quality and the fact that they eat a wide range of natural food organisms, therefore, the present work is planned to study the cytological characteristics of three species of fishes , *Oreochromis niloticus*; *Sarotherodon galilaeus*, and *Tilapia zillii,* to elucidate the genetic relationship, similarity between them and the probability of their hybridization.

The study of chromosomes receives the interest for classification of species and understanding of evolution. In spite of being used extensively in taxonomic research of invertebrates and even in vertebrates, it is incomplete in most animals because of technical difficulties. Among vertebrates, fishes from the group for which cytologically data are mostly lacking (Alves, 2000; Artoni and Bertollo, 2001), in this concept we utilized the cytogenetic and karyotypic study to examine the similarity relationship among three tilapia species and the probability of there hybridization.

The cytogenetic data obtained from this study suggesting that this group has a conservative karyotypic structure , the diploid number of *Oreochromis niloticus*, *Sarotherodon galilaeus* and *Tilapia zillii* was 2n= 44 which is in agreement with Kornfield et al., (1979), and Sherwood & Patton (1982).

On comparing the mean length of the haploid set of chromosomes in both *Sarotherodon galilaeus* and *Tilapia zillii* showing no significant difference in the mean length of all chromosomes with the exception to chromosomes numbers 11& 21, where there was a significant difference between the two species was found. Also, the comparison between *Oreochromis niloticus* and *Sarotherodon galilaeus* showed no significant difference between chromosome pair number, where the chromosomal numbers 1, 2, 4, 5, 17, 18 & 22 those of *Sarotherodon galilaeus* were longer than another of *Oreochromic niloticus*. and the chromosomal mean length 3, 6, 7, 8, 9, 10, 11, 12, 13 & 14 of *oreochrorms. niloticus* were longer than other ones of *Sarortherodon galilaeus* and other numbers 15,19, 20, 21 are equal in both species but in comparing the range and mean length of chromosomes of *Oreochromis niloticus*, and *Tilapia zillii* its clear that there is a significant difference of the mean length of chromosomal numbers 6, 8, 11, 14 and 21 and there is no significant difference was found between the remaining chromosome pairs numbers.

Nijjhar et al., (1983) in his study on twenty species of *Tilapia* were analyzed karyologically ,there was a high homogeneity appeared, 2n =44 with 2 pairs of marker chromosomes , much larger than the others, and minor differences in the number of biarmed chromosomes, he demonstrated that the 1st longer pair. Of chromosome in the karyotypes of Tilapia was suspected to be the sex chromosomes. On the other hand, El serafy et al.,1993, found that male *Oreochromis niloticus* has seven submetacentric and fifteen subtelocentric from Serow and four submetacentric and eighteen subtelocentric from Kanater and Manzalla regions .While the female has three submetacentric and nineteen subtelocentric , six submetacentric and sixteen subtelocentric at the same regions .

In the studied species the relative length of the individual chromosomes between the species, show that the chromosome length varies little except in chromosome number one which is the longest in the whole karyotype

It is suggested from this study that, some of the observed intraspecific karyotype differences resulted from the evolutionary modification in genetically isolated populations , there are some evidence for inter-population variation having occurred at the molecular levels for a number of tilapia species ,so, further studies on a molecular level is important to establish this suggestion, this in agreement with Majumber ,1984.

Chew et al.,2002 suggested that chromosome number 1 , which is larger than all other chromosomes in the karyotype, was produced by the fusion of three chromosomes and explain the overall reduction of chromosomal number from ancestral teleost karyotype 2n=48 to 2n=44 observed in tilapia . Harvey et al.,2002, suggests that the difference in chromosome number dose not prevent the production of interspecific hybrids between *Oreochromis niloticus* 2n=44 and *Oreochromis karongae* 2n=38 under the suggestion that these consists of Robertsonian fussions of a more complex nature .

From this study , it can be concluded that there is a close similarity between *Sarortherodon galilaeus* and *oreochromis niloticus* giving a probability of hibridization ,where the comparison between *tilapia zilli*

and Sarortherodon galilaeus ,or *oreochromis niloticus* put a less chance of hybridization due to less similarity between them .

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Understanding the Physiology of Heterocyst and Nitrogen Fixation in Cyanobacteria or Blue-Green Algae.

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Abstract: Ever since the man started thinking about his origin and evolution, he has sought answer on scientific facts and findings. In nature there are certain tiny microscopic blue-green algae or Cyanobacteria that have immense importance from many aspects ranging primarily from Nitrogen Fixation to the most coveted query – the origin of animal life on this planet. This paper is a peer review into the various physiological aspects of the nitrogen fixation and simultaneous Oxygen evolving mechanisms of blue-green algae. This paper makes the understanding of cyanobacterial nitrogen fixation in the Heterocyst easy, and also links the evolution of free Oxygen (g) from the splitting of water by its vegetative cells and ultimately, the role of Cyanobacteria in altering the primitive earth's reducing atmosphere into present day oxygenating and thus making animal life possible on earth. [Nature and Science. 2008;6(1):28-33]. ISSN: 1545-0740.

Key Words: Cyanobacteria; Blue-Green Algae; Heterocyst; Nitrogen Fixation.

Introduction:

The blue-green algae or Cyanobacteria are the most primitive form of algae under plant kingdom. These are basically a type of autotrophic bacteria, which are prokaryotic in their cellular structure. These are called as blue-green algae because they contain the photosynthetic pigments- ***c phycocyanin*** **(dominant pigment)**, ***c phycoerythryin***, and ***chlorophyll a***, which are responsible for their characteristic **blue-green colour**. These are known by different names such as, ***Blue-Green Algae / Cyanobacteria*** **(Stainer and Cohen-Bazire, 1977),** ***Schizobacteria / Myxobacteria, Myxophyceae*** and ***Cyanophyceae***. These blue-green algae are the first plant forms, which got the power of chlorophyll in their thylakoids and started the life supporting process of photosynthesis in the planet earth. The common examples of blue-green algae are ***Nostoc, Anabaena, Rivularia, Gleotrichia, Gleocaspa, and Camptylonema*** etc.

The primitive environment of the earth was reducing i.e. Hydrogen rich (H_2 plus) or Oxygen deficient (O_2 minus). This primitive atmosphere was totally dominated by Hydrogen gas, and Oxygen gas was not present in free state. That time life was present in the form of simple primitive ***archaebacteria*****,** such as iron bacteria, sulphur bacteria, chemosynthetic bacteria and methane producing ***methanogens*** etc. They used to generate energy not from the sunlight, but from the different chemicals only, and there was no photosynthesis at all in the primitive earth due to the absence of any photosynthetic pigment. They were these blue-green algae or Cyanobacteria, which initiated the process of photosynthesis and started giving off free-Oxygen gas as a by-product of photosynthesis, by the splitting of water molecules with the help of **Photosystem II (PS II)**. And thus we can say that the blue-green algae were the pioneer in transforming the early earth's reducing atmosphere to present day oxygenating atmosphere, which ultimately made it possible for animals to breathe. Although, the evolution was also going on simultaneously, and the whole plant and animal kingdom was in a state of establishing itself.

Later on in the evolutionary process, the blue-green algae migrated inside a eukaryotic cell and got the designation of Chloroplast. This is how chloroplasts were formed, and that is why the chloroplasts are prokaryotic **(Endosymbiont hypothesis, Margulis, 1971;Bogorald, 1975;Mahler and Raff, 1975;Saccone and Quagliarello 1975;Bucher *et al*., 1977; DeRobertis and DeRobertis 1984)**. Now, the resultant cell was the first unicellular, eukaryotic alga of the class Chlorophyceae **(F.E.Fritsch, 1944)**. Similar migratory Endosymbiont hypothesis is also given for the origin of mitochondria, where the free-living aerobic, ATP generating bacteria migrated inside a eukaryotic cell (cell with nucleus and other cell organelles) and transformed in the present day mitochondria. That is why both, chloroplast and

mitochondria are prokaryotic in nature and have their own independent genetic material and specific protein synthesis.

Many of these blue-green algae have the power of nitrogen fixation, and for this nature has provided them a very special enlarged cell, which is called as **Heterocyst** ***(Gr. Hetero=different; Cyst=swollen and encapsulated cell), (Fay et al.*** **1968),** ***(Stewart, 1967).*** This Heterocyst is very much enlarged than its other body cells (vegetative cells).

Interestingly this Heterocyst is very unique in itself as it is specially designed by nature and has many important features to facilitate the nitrogen fixation. There are different factors, which control the Heterocyst formation, for example: The production of Heterocyst increases in the conditions of low light intensity and increase in the amount of phosphate in the medium **(Fay et al., 1968).** It has also been reported that the Heterocyst formation depends upon the availability of carbon intermediaries and ATP. The former are supplied by photosynthesis and the later by oxidative metabolism **(Tyagi, 1973). Singh and Trehan (1973)** found that the Heterocyst differentiation is inhibited in the presence of combined sources of nitrogen (nitrate and ammonium nitrogen), but is induced in the presence of nitrogen gas. However they further declared that the differentiation of Heterocyst, hormogones and spores in blue-green algae is genetically controlled. It was also found that in ***Camptylonema lahorensis***, Heterocyst differentiation is genetically controlled but its phenotypic expression is dependent on growth conditions in the medium.

According to **Tyagi (1973)**, apart from microbial nitrogen fixation, which is its prime function, the Heterocyst is also credited with many other biological functions of Cyanobacteria or blue-green algae. **Geitler (1921)** supposed that originally Heterocyst were developed for reproduction. This claim has also been backed by the occurrence of endospore formation in the germinating Heterocyst of *Anabaena cycadeae* **(Spratt, 1911)**. But of all these above discussed functions, the first and foremost work assigned to Heterocyst is the **cyanobacterial nitrogen fixation**.

Characteristic features of Heterocyst:

1. The Heterocyst is the site for cyanobacterial nitrogen fixation which is an enlarged cell, and may be present terminally or intercalary in the filamentous cyanophycean algae.
2. In the process of cyanobacterial nitrogen fixation, hydrogen gas (H_2) is also evolved as a by product and 40% of it is recycled by the ***hup*** gene (hydrogen uptake gene), **(Margheri *et al* 1990), (Howarth and Codd, 1985)** whereas remaining 60% hydrogen gas can be used by biotechnologists as a source of future clean fuel. **(Dutta *et al.* 2005)**
3. The Heterocyst is made up of three (3) different cell wall layers- the outer fibrous and middle homogenous layers are made up of ***non-cellulose polysaccharide***. Whereas, the inner laminated layer is made up of ***glycolipids***. **), (Lang, 1968)**
4. On one hand, these special cell wall layers permit the atmospheric N_2 (g) to diffuse inside, whereas on the other hand they stop the atmospheric O_2 (g) to come inside.
5. This is a **damage-control mechanism** for the enzyme ***nitrogenase***, as the *nitrogenase* is sensitive to O_2 and cold, and cannot function in the presence of O_2 (g).
6. Moreover the **Photosystem II (PS II), is also absent** from the Heterocyst, because PS (II) does the Photolysis of water and generates free O_2 gas.

   That is why **only PS (I) is present here**, which generates assimilatory powers ATP, which helps in nitrogen fixation. Had there been PS (II) also in the Heterocyst, then it would have done the Photolysis of water in the Heterocyst and free oxygen gas (O_2) would have stopped the functioning of ***nitrogenase*** enzyme, thus ultimately checking the whole cyanobacterial nitrogen fixation.
7. Absence of PS (II) helps in maintaining the O_2 (-), or H_2 (+) internal environment of Heterocyst.
8. If by chance, some oxygen gas (O_2) also enters the Heterocyst from polar plugs, then the enzyme ***Oxidase*** present inside Heterocyst executes reaction between this entering oxygen gas and hydrogen molecules, and ultimately makes the water, thus helping in maintaining the internal environment of Heterocyst as reducing and not oxygenating.

And this is how the marvel-cell Heterocyst of Cyanobacteria performs its unique biochemical mechanism of nitrogen fixation and is valuable in the present life status of the mother planet earth. First in making the Precambrian earths reducing environment, to present day oxygenating, and also functioning as bio-fertilizers. So, the whole biota of the earth is indebt to the blue-green algae for helping in establishing both, the plant and animal kingdom.

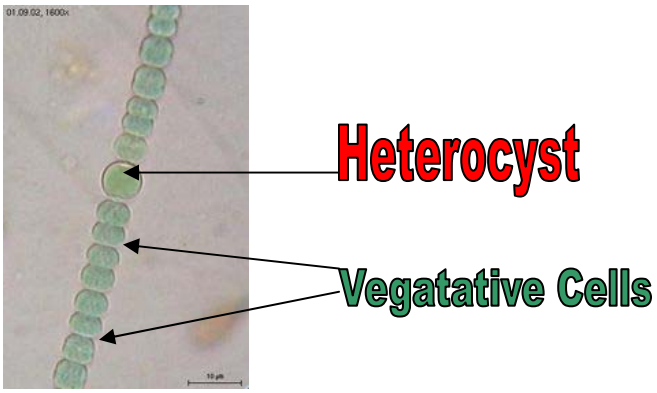

Fig 1: Cyanobacteria: *Anabaena sperica*

Cyanobacteria belong to the family Cyanophyceae (Algae), and as prokaryotes they represent the most primitive group of living organisms. They are extremely simple organisms that can exist as single cells, as slender filaments like the ones seen here, or as simple colonies. Cyanobacteria are capable of enduring a wide variety of environmental conditions ranging from freshwater and marine habitats to snowfields and glaciers. They are capable of surviving and flourishing even at extremely high temperatures.

Table: Enzymological comparison between Heterocyst and Vegetative cell in ***Anabaena variabilis***, grown aerobically **(after C.P. Wolk, 1973)**

Activity mainly or entirely in Heterocyst	**Activity mainly or entirely in Vegetative Cell**
Enzymes 1. *Nitrogenase* (encoded by ***nif*** gene) 2. *Glutamine Synthetase* 3. *Glucose-6-Phosphate dehydrogenase* (in Oxidative Pentose Phosphate Pathway) 4. *Uptake hydrogenase* (encoded by ***hup*** gene) 5. *Bi-directional* or *Reversible hydrogenase* (encoded by ***hox*** gene) **6.** *Oxidase* in high concentration.	***Enzymes*** **1.** *Glutamate Synthase* (in GOGAT-Glutamine Oxalo gluterate Amino Transferase Pathway). **2.** *RUBP carboxylase* **3.** *Oxidase* in low concentration.
Main Metabolic Pathways 1.Nitrogen fixation Pathway 2. Oxidative Pentose Phosphate Pathway. 3.Only Photosystem I (PS I) present and Photosystem II (PS II) is absent from Heterocyst.	**Main Metabolic Pathways** **1.** Calvin Cycle (Carbon Fixation) or Reductive Pentose Phosphate Pathway **2.** Photosystem II (PS II) present. Photolysis of water and evolution of O2 gas occurs.

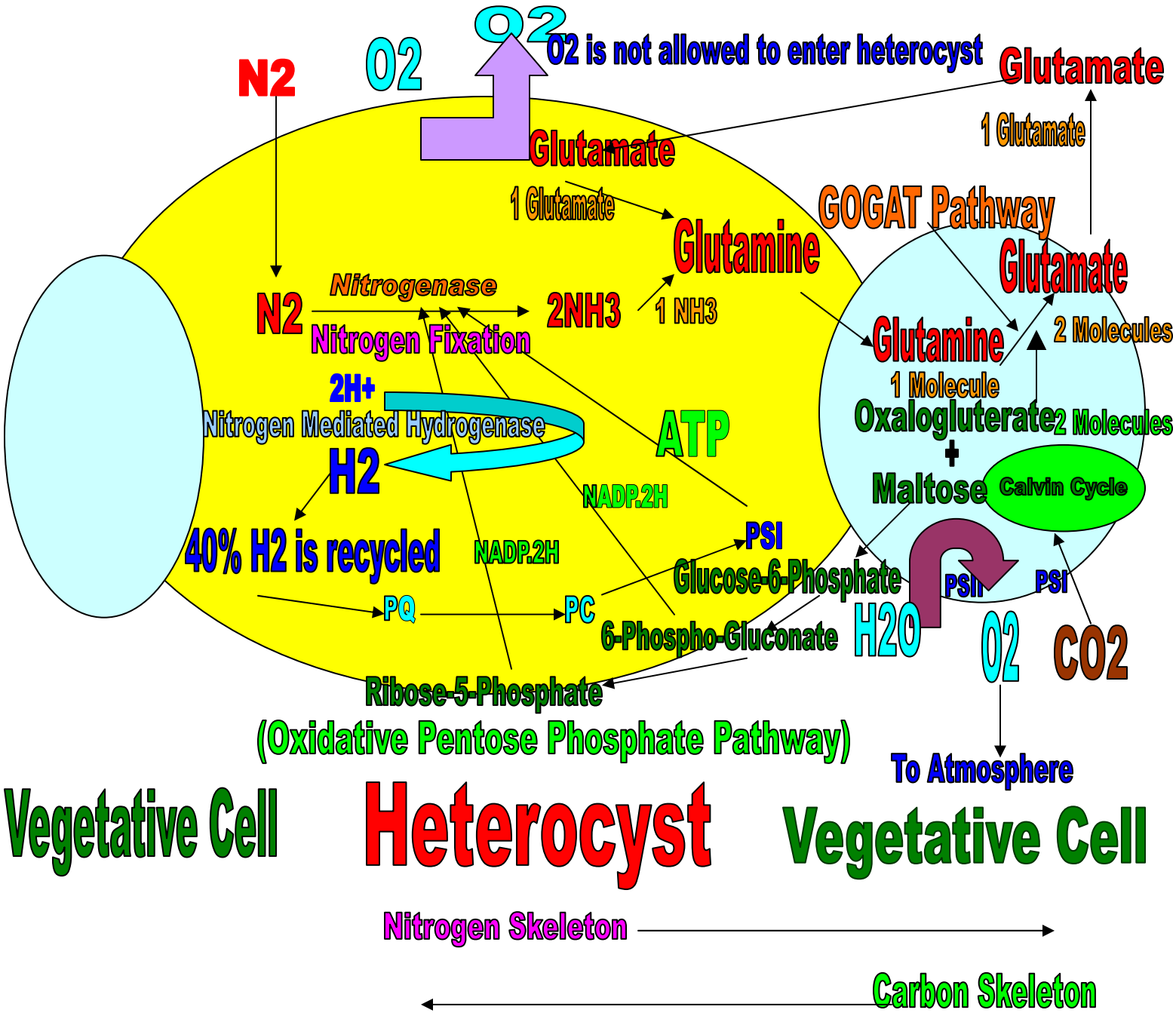

Figure 2: The central bulging cell is the Heterocyst, which is attached to two normal vegetative cells. The biochemical nitrogen fixation is going on in the Heterocyst, whereas in the vegetative cell the carbon fixation is in progress.

Different Biochemical Reactions of Heterocyst:

The different biochemical reactions going on, in the cyanobacterial Heterocyst and the adjoining vegetative (normal) cell are explained in the **Figure2**.

The Central bulging Yellow colored cell is the Heterocyst, which is the surrounded by two light blue colored vegetative cells. As mentioned earlier, the cell wall of Heterocyst is three layered, which gives the diffusion passage only to atmospheric Nitrogen (g) and stops the entry of Oxygen (g) inside the Heterocyst. So that it helps in maintaining the early earth's reducing environment inside the Heterocyst. As we know that the activity of ***Nitrogenase*** enzyme present inside the Heterocyst is stopped in the presence of Oxygen, so it's a defense mechanism evolved by the nature for the ***nitrogenase*** enzyme. Now the ***nitrogenase*** enzyme combines the atmospheric Nitrogen and Hydrogen molecules (present inside the Heterocyst) to form two molecules of Ammonia (NH_3). Now after this, one molecule of Ammonia is changed into Glutamine, which further migrates to the adjoining vegetative cell for other biochemical pathways.

In Vegetative Cell:

On the other hand, Carbon fixation cycle (Calvin Cycle) is going on simultaneously in the adjoining vegetative cell, which has got both PS (I) and PS (II) for this purpose. Atmospheric CO_2 is taken up by the ***RUBISCO*** **(*Ribulose Biphosphate Carboxylase*)** enzyme and changed into 3-PGA (3-Phosphoglyceraldehide), which further goes on in the Calvin Cycle, and in this way the Carbon is fixed in the adjoining vegetative cell of Blue-Green Algae. The PS (II) here splits the water (H_2O) molecules and generates Oxygen (O_2) (g), which is then released into the atmosphere. The Calvin Cycle also produces Maltose and Oxalo gluterate.

In Heterocyst:

Now after that the maltose and Oxalo gluterate are formed in the vegetative cell, two molecules of Oxalo gluterate react with one molecule of Glutamine and enter into the **GOGAT Pathway (Glutamine Oxalo Gluterate Amino Transferase).** Now as a result two molecules of Glutamate are formed and out of theses two, one molecule of Glutamate is cycled back to the Heterocyst and the cycle goes on.

On the other hand the Maltose is sent to the Heterocyst through different intermediate Carbon compounds as a Carbon Skeleton. These intermediate carbon compounds are: Glucose-6-Phosphate, which is changed into 6-Phospho-Gluconate, which is ultimately changed into Ribose-5-Phosphate. Now at each and every step of transformation of these different Carbon Compounds, they give rise to the sufficient amount of energy molecules i.e. **ATP (Adenosine Tri Phosphate)** to help in the fixation of atmospheric Nitrogen into solid Ammonia molecule (Nitrogen Fixation).

The Hydrogen ions (H^+) formed in the energy transfer process are taken up by the enzyme ***Nitrogen Mediated Hydrogenase/Bi directional/Reversible Hydrogenase*** encoded by ***hox gene,*** and are changed into molecules of Hydrogen (g) (H_2).

However 40% of this Hydrogen gas is recycled through Plastoquinone and Plastocyanin, by ***Uptake Hydrogenase*** enzyme encoded by ***hup gene***.

In this way the nitrogen fixation is done in nature by the Cyanobacteria and their Heterocyst.

The whole humanity is indebted to the kindness and greatness of these tiny microscopic life forms which not only paved way for the establishment of animal and Human life but also make a promising Biofertilizer for Plants.

Had Cyanobacteria not been there in nature, only God knows what would have been our fate?

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Land Evaluation and Productivity of Organically-Fertilized Crop Mixtures in a Degraded Tropical Soil

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Abstract: We investigated the fertility status of degraded soil and consequently amended them with varying rates of poultry manure in 2005 and 2006 cropping seasons. Yam – maize –Cassava intercrop was used for the productivity assessment. Soil samples and amendment were characterized before the experiment which was laid out in a randomized complete block design (RCBO) using 5 rates of poultry manure, namely O, 5000, 10000, 15000 and 20000 kg ha^{-1}. These rates of poultry manure were replicated thrice. Performance indices were plant height and tuber and grain yield, and they were enhanced by poultry manure amendment. Maize and cassava had their optimum yield at 15000 kg ha^{-1} poultry manure while yam did not reach its optimum performance in the highest rate of 2000 kg ha^{-1}, suggesting increased rate of poultry manure for yam production. [Nature and Science. 2008;6(1):34-42]. ISSN: 1545-0740.

Keywords: Amendment, crop mixtures, degradation, soil quality, tropical soils

Introduction

Food is the most basic of human needs and the foundation for human and economic development (Smith et al., 2006). Farmers in large parts of the sub-Saharan Africa have no tradition of aiming at maximum production per hectare, possibly due to poor marketing facilities to dispose surpluses. Instead, most African farmers fight against crop failure (Verheij, 2003). Farmers still hold tenaciously to slash-and –burn method of land clearing which removes vegetal wastes and contributes to global warning and land degradation (Reich *et al.,* 2001). Land degradation causes declining yield and varying productivity (Holdren *et al.,* 2005). Declining productivity is worsened with increasing demographic pressure and conflictive land use types.

As most farmers prefer annual crops, there is need to put in place such types that can relatively protect soil against degradative forces in addition to practise fertility supplementation attributes. Van School (1998) suggested the use of intercropping among other agronomic practices to improve productivity. However, the status of these soils require organic manure input since organic matter improves physical, chemical and biological properties of soils (Linger and Critchley, 2007). The inherently

poor fertility characteristics of tropical soils have made nutrients availability in them to be largely controlled by organic matter (Agboola and Omueti, 1982). Mbagwu *et al*. (1994) reported that organic manures improved physical properties of soils; with poultry droppings enhancing soil fertility (Ajayi *et al*., 2003). However, there are reported incidences Oniawa *et al*., 1998) of pollution of nearby water bodies, hence the need to monitor effect of poultry waste on soil and water resources (Ajayi *et al* 2003). Poultry droppings increase soil hydraulic conductivity and reduced bulk density (Mbonu and Elenwo, 2006) thereby improving water infiltration and aeration necessary for optimum performance of crops. These benefits formed the premise for the recommendation of biological wastes so soil fertility enhancement by Scholars (Agele, 2000; Ojeniyi *et al*., 2000) although a combination of organic and organic fertilizers gave highest yield (Adediran and Banjoko, 2003).

Yams (Dioscrea spp.) is the most widely cultivated indigenous crop in West Africa (Onwueme 1978) with a daily consumption of 0.5 – to 1.0 kg (Purseglove, 1981). Maize (*Zea mays L*.) is a major cereal crop whose importance is underscored by competing, and sometimes conflictive use. Maize is grown on about 1.5 million hectares of soil (CIMMYT, 1994). Cassava (*Manihot esculenta* Crantz) is one of the dominant starchy staple crops in Africa with a root yield of 17. 4 to ha-1 (sole crop) and 14.7 t ha^{-1} (Intercrop)(John *et al*., 2006). Cassava grows n soils too poor for many other crops (Ahn, 1993) Traditionally, cassava is often grown as an intercrop with yam, maize, banana and melon (Usman *et al.,* 2001). They maintained that such intercrops protect soils, and permit optimum utilization of available soil nutrients. However, crop mixtures on a farmland may result to in soil mining, and consequent degeneration in soil quality. This calls for the fertility –enhancing technologies including the application of organic manures. The major aim of this study was to evaluate the current soil fertility status and determine optimal level of poultry droppings supplementation capable of promoting yam-maize-cassava mixtures.

Materials and Methods

Study Area: The study was carried out during the 2005 and 2006 wet seasons at the university farm of Federal University of Technology, Owerri Nigeria, lying on latitude $5^0$43'14.623'' and longitude $7^0$37'34.490''. The University farm cover about 600 hectares of land, and its soils are derived from coastal plain sands. Owerri is humid tropical, having a mean annual rainfall of about 2500 mm and mean annual temperature range of 26-29 ^{0}C. It is characterized by a depleted rainforest vegetation. Socio-economically, farming, fishing, hunting, cottage industrial activities, sand mining and construction works are major enterprises in the area.

Analysis of Poultry manure used for the Experiment: Poultry manure used for the study was cured and samples of cured manure were air–dried at room temperature for 3 days. Ground poultry manure was digested with nitric-perchloric-sulphuric acid mixture and resulting aliquot was used to chemically characterize the amendment. The manure contained the following: organic carbon (68 gk g^{-1}), total

nitrogen (14.2 gk g^{-1}) ,Ca^{2+} (69.5 g kg-1) ,Mg^{2+} (20.2g kg^{-1}), K^{+} (5.8 g kg^{-1}) and available P (13.6 g kg^{-1}) .Poultry manure was incorporated manually at 0, 5000, 10000, 15000 and 20000 kg ha^{-1}

Field Experiment: There were 5 levels of poultry droppings .namely 0,5000, 10000, 15000 and 20000 kg ha-1. These levels were replicated three times and arranged in a randomized complete bock design (RCBD). The varieties of crops grown included Okuocha (Yam: *Dioscorea rotundata*), 8341-6 (Maize: *Zea mays* L.) and 30555 (**Cassava:** *Manihot esculenta* Crants). Field work involved land preparation, planting, weeding and harvesting. Earlier 15 soil samples were collected, prepared and using for preplanting soil characterization.

Laboratory Analyses: Particle size analysis was determined by hydrometer method (Gee and Or, 2002). Exchangeable cations, Ca, Mg and K were measured using inductively coupled plasma atomic emission spectrometer (ICP-AES) (Integra XMP, GBC, Arlington Heights, IL).

Soil pH was estimated potentiometrically in a glass electrode in deionized water (pH water) at a soil/ solution ratio of 1:2:5. Total carbon and total nitrogen were measured on aliquots by dry combustion at 1060 ^{0}C and detection of evolved $C0_2$ and $N0_2$, was done with a Carlo Element Analyzer (Carlo Erba, Italy). Organic matter was obtained by multiplying total carbon by a factor of 1.724 while available phosphorus was determined by Olson method (Emteryd, 1989).

Data Analysis: Soil data were subjected to analysis of variance (ANOVA) using PC SAS version 8.2 (SAS Institute, 2001).

Results and Discussion

Land Evaluation: The status of soils in terms of selected properties are shown in Table 1. Soils were sandy, very strongly acidic and of medium organic matter content. Consequently, total nitrogen value (mean) was low. Similar low values were obtained in exchangeable basic cations and available phosphorus. Calcium – magnesium ratio was very low and below threshold limit of 3.0 (Landon, 1984). Results on particle size distribution and Ca – Mg ratio are consistent with the findings of Oti (2002) in the study of erosion-degraded lands of Otamiri watershed in the same agroecology. Sandiness in these soils deficient in optimal organic matter is indicative of poor water and nutrient retentivity as macropores inherent in the soils permit rapid horizontal and vertical movements within the pedosphere. However, the dominance of sand-sized fractions among other particle sizes is attributable to parent material land use and climate of the study area. High rainfall amount, duration and intensity promote leaching of basic cations leaving a preponderance of acidic cations hence soils are very strongly acidic,suggesting unavailability of essential plant nutrients especially phosphorus, boron and molybdenum. This condition is further confirmed by very low Ca/Mg ratio as Landon (1984) observed that soils having such low ratios of these basic cation ratios

suffer from calcium and phosphorus deficiencies. These cations are very low when compared with quality control (QC) standards (FDALR, 1985; Enwezor *et al.,* 1989). High temperature of the area accelerates organic matter mineralization, and this could account for low organic matter content of soils. However values of organic matter were higher and contrasted with findings of Osodeke *et al*. (2002). In the same agroecology. organic matter in the study area is associated with processes that influence vegetation, litter decomposition and those cause by land users (Isirimah *et al.,* 2003).

Effect of Poultry Manure on Soil Properties: Poultry manure significantly ($p \leq 0.05$) improved all the chemical properties of soils investigated (Table 2). However, percentage increase in soil property value differed among the measured soil properties and rates of application (Table 3). Greatest influence was recorded in total nitrogen (TN) .Similar findings were made by Duruigbo *et al.* (2007) on soils derived from coastal plain sands in southeastern Nigeria . Highest increases in TN implies high responsiveness of soils to its application, suggesting that such soils might have lost a great deal of their TN by leaching. Increases in the values of other properties could be attributed to rise in organic fractions. Pitram and Singh (1993) reported increase in pH due to poultry manure application, which was attributed to ion exchange reactions which occur when terminal OH^- of Fe or Al hydroxides are replaced by organic anions such as tartrate, malate and citrate (Besho and Bell, 1992). These anions compete with soil phosphorus (Liu and Huang, 2000), implying that their abundance in the exchange site increases P-availability in the soil system, and this depends on the concentration of legend and soil pH (Giesler *et al*., 2005).

Performance of Crop Mixtures: Table 4 shows growth and yield characteristics of yam, maize and cassava, indicating significant ($p \leq 0.05$) in these attributes. Maize and cassava had highest height increase between 15000 and 2000 kg ha^{-1} while yam growth increase was between 5000 and 10, 000 kg implying that as more poultry manure was added the tuber crop was translocation gains to the tuber, and this could be why greatest yield difference was at the same poultry rate interface. However, tillage method may have affected tuber development and yield (Ohiri, 1995). Increased plant height in maize is consistent with the findings of Ojo *et al.* (2003) that poultry manure produced greater vigour than other organic amendments. Optimum yields for maize and cassava were obtained at 15000 kg ha^{-1} while further increase in rate of application of poultry manure is required for yam in the degraded soils. In cassava production in the area, John *et al* . (2006) suggested the se of organ mineral fertilizer for improved growth and yield of cassava. Although some scholars obtained significantly higher yields in cassava-based intercrop (Eke-Okoro *et al.,* 2003; Jalloh and Daphnia, 2003), a involving sole crops in this experiment would be necessary for comparative purposes such studies would also use the same degraded soils.

Table 1. Preplanting characterization of soils (mean values) (n=15)

Property	Unit	Value	Fertility class
Sand	g kg^{-1}	830.0	NA
Silt	g kg^{-1}	40.0	NA
Clay	g kg^{-1}	130.0	NA
Ca^{2}+	Cmol kg^{-1}	0.95	Very low**
Mg^{2}+	Cmol kg^{-1}	0.6	Low**
Kt	Cmol Kg^{-1}	0.1	Low*
Ca/mg		1.5	
pH (H_20)		4.4	Very strongly acidic**
OM	g kg-1	23.4	Medium*
TN	g kg-1	0.8	Low*
Av.P	mg kg-1	9.8	Low*

(*sources* Enwezor et al, 1989, **FDALR, 1985)

OM = organic matter, TN = total nitrogen, Av. P = available phosphorus

NA = not applicable.

Table 2. Effect of poultry manure on some soil characteristics

Treatment (kg)	Ca^{2+}	Mg^{2+}	K^{+}	pH (water)	OM	TN	Av.P
	← (cmol kg^{-1}) →				← (g kg^{-1}) →		(mg kg^{-1})
0	0.8	0.5	0.18	4.6	23	0.8	8.8
500	1.3	0.7	0.20	5.2	28	1.6	11.9
10,00	1.5	0.8	0.30	5.5	30	1.7	13.6
15,000	1.7	1.1	0.42	5.8	31	1.8	14.2
20,000	2.1	1.2	0.53	6.1	34	2.1	14.8
LSD $_{0.05}$	0.5	0.3	0.08	0.9	3.8	0.7	0.8

OM > organic matter TN = total nitrogen, Av.P available phosphorus

LSD = least significance difference

Table 3. Percentage increases in selected soil properties due to poultry manure application

Property	500	Treatments (kg)		20000
		10000	15000	
Ca^{2}+	62.5	87.5	112.5	162.5
Mg^{2}+	40	60.0	120.0	140.4
K+	11.1	66.0	133.3	194.4
pH (water)	13.0	19.5	26.0	32.6
OM	21.7	30.4	34.7	47.8
TN	50.0	112.5	12.50	162.5
Av.P	35.2	54.5	61.3	68.2

OM > organic matter TN = total nitrogen, Av.P available phosphorus

Table 4. Growth and yield characteristics

Treatment	Plant height (cm)at 8 WAP			Yield		Kg ha^{-1}
				M	C	Y
	M	C	Y	(grain)	(tuber)	(tuber)
O kg	22.0	12.0	296.8	300	9500	8000
5000 kg	29.4	16.3	298.2	690	10500	9800
10,000 kg	34.2	20.5	340.2	1260	11800	10750
15,000 kg	38.8	26.4	341.8	1320	12160	11600
20,000 kg	49.2	35.2	345.2	980	11240	11,850
LSD $_{0.05}$	8.2	3.6	3.6	79.0	89.8	62.5

Conclusion

Soils of the study sites were highly degraded and marginally suitable for arable production. However, poultry manure improved the fertility status of soils and consequently increased performance of fest crops. But rates for optimum performance differed among crops. It is suggested that further studies should incorporate fertility enhancing crops adaptable to this agroecology characterized by increasing population.

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Spatio-Vertical Distribution Of Arsenic In River Slope Soils Proximal To An Automobile Servicing Station

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Abstract: This study investigated the distribution of arsenic (As) in arable soils near an automobile servicing station at Nekede, Southeastern Nigeria before the rains in 2006. Grid sampling at 0, 25, 50 , 75 and 100 m was done from soil profile pits located at each grid point along three physiographic positions, namely crest, midslope and footslope in the study site. Soil samples were subjected to routine and special analytical tools. Data were analyzed statistically using SAS computer program. Results showed total As concentrations ranging from 26.1 - 36.2 mg kg^{-1} in automobile soils when compared with 0.2 -0.4 mg kg^{-1} in non-automobile soils in its spatial distribution. Vertical distribution of total As showed a range of 37.8 – 62.3 mg kg^{-1} (Automobile soils) and 0.1-0.3 mg kg^{-1} (Non-automobile soils). Available phosphorus soil organic carbon, clay content and pH had a good relationship with the spatio -vertical distribution of As, suggesting possible usage of these soil attributes for future modelling of As activity in soils of the study area. [Nature and Science. 2008;6(1):43-47]. ISSN: 1545-0740.

Key words: Arsenic, automobile servicing, distribution, soil pollution, variability

Introduction

Arsenic has long been identified as a carcinogen, and its elevated concentration in an ecosystem threatens public health and environmental safety (Singh et al., 2007). Arsenic is ubiquitous in nature as it is present in soils, rocks, water biological chains of plant and animal lives as well as in air above thermally active areas. However, heightened levels of arsenic in soils result from various human activities including mining, combustion, wood preservation and pesticide application (Singh *et al.,* 2007).

In response to its toxic nature, USEPA (2001) reduced its maximum contaminant level (MCL) in drinking water form 50 to 10 mg L^{-1} to protect consumers against the effects of long –term, chromic exposure of the heavy metal, as elected concentration in humans is associated with bladder, kidney, liver, lung and skin cancers.

In urban and Peri-urban areas of southeastern Nigeria, automobile servicing centers popularly called mechanic villages are common (Onweremadu *et al*., 2007a), resulting in the dumping of wastes on nearby arable soils.

In addition to the above, municipal solid wastes arising from wood processing industries and several cottage industries are disposed on soils around these sites, some of which have rivers. Yet, vegetable farming and farming common practices (Onweremadu, 2007). One of such locations in southeastern Nigeria is Nekede mechanic Village where inhabitants cultivate on dump site soils, practice capture fishery on Otamiri River and domestically use water from this river when there is failure in punfeed urban water supply. The major objective of this study was to investigate arsenic concentrations spatially and in .soil profiles as this would help in estimating the toxicity levels in the area.

Materials and methods

The study was conducted on arable soil near Nekede Mechanic Village lying between latitudes 5^0 10' 55.51'' and 5^0 25'10.12'' N and longitudes 6^0 45'25.11'' and 7^0 05' 06.21'' E. the northern part of the town juts into Owerri Metropolis. A large expanse of the study site is occupied by automobile servicing enterprises and wood processing industries, who discharges wastes on adjoining arable soils. Soils are derived from Coastal plain sands and the study area is generally a lowland. It has a humid tropical climate, with an annual average rainfall of about 2500 mm and temperature range of 26-29 ^oC. the rainforest vegetation of the area is highly altered by human activity. Arable farming is a major socio-economic activity of the area in addition to automobile servicing and allied activities.

Field sampling

Five transects were field studies were conducted in 2006 established from soil nearest to the automobile service centre (50 m away from) the station) towards Otamiri River in Imo State, southeastern Nigeria. A base line was used to establish a grids at 0, 25, 50, 75 and 100 m with a instilled on each grid for separate determinations. Three physiographic positions representing land unit namely crest midslope and footslope were identified and five profile pits were dug and sampled from each land unit. This activity was respected in an undisturbed site 10 km away from the automobile service station (control) the control site has similar features including proximity

to the Otamiri River. A total of 75 soil samples were collected, air-dried and sieved using 2 – mm sieve for laboratory analysis.

Laboratory Analyses

Particle size analysis was determined by hydrometer method according to the procedure of Gee and Or (2002). After equilibrating for 30 min, soil pH was estimated potentiometrically in water with a soil-liquid ratio of 1:2.5 using a Beckman Zeromatic pH meter. Soil organic carbon was measured by combustion at 840 ^{o}C (Wang and Anderson, 1998). Cation exchange capacity (CEC) was determined by ammonium acetate leaching at pH 7.0 (Blakemore et al., 1987). Available phosphorus was estimated colourimetrically using Bray 2 method (Olsen and Sommers, 1982). Base saturation was calculated as the sum of exchangeable basic cations divided by the CEC, and multiplied by 100 percent. Total as was determined using an Atomic Absorption Spectrophotometer (AAS) as described by Palz *et al.* (1993) and three standards namely, 0.1 mgkg-1, 0.2 mg $^{-1}$ and 0.3 mg kg^{-1} were used.

Statistics: Data were subjected to mean, coefficient of variation (%) and correlations using SAS computer package (SAS Institute, 2001).

Results and discussion

Arsenic concentration in soils: Spatial distribution in total soil As is shown of Table 2, with arable soils near automobile servicing stations having values (26.1-36.2 mg $^{kg-1}$) higher than maximum permissible limit 0.5 mg kg^{-1} (FEPA, 1988) and 0.43 mg kg^{-1} (URS, 2002). However, As concentration increased downslope, which could be attributed to surface and subsurface movement of soil water in its join towards the natural valley (Otamiri River) indicating high susceptibility of the study area to surface water pollution. But, the As values in the polluted soils were higher than 6-60 mg kg-1 obtained in a similar site by Safiullah (2007) in Bangladesh. Lower concentrations of As (0.2-0.4 mg kg^{-1}) were obtained in soils of an unpolluted site located near the upper course of Otamiri River of the same study location, implying minimal influence of automobile wastes on the latter soils. Yet, higher values of As (37.8-62.3 mg kg^{-1}) were recorded in its vertical distribution in the same polluted soils (Table 3) when compared with the spatial distribution in the same soils suggesting pronounced intrapedal pedogenic processes of loss such as leaching and eluviation in the study area. In both polluted and unpolluted sites, As had greater vertical distribution variability (CV = 19.3 -77.8 % compared to its spatial variation (CV = 7.7-58.6 %), which could be attributed to higher variation in soil organic carbon. Generally, differences in slope percent may have had more influence on the vertical distribution of As in both polluted soils near automobile stations and their unpolluted counterparts. This result contrasts the findings of Mainville et al. (2006) in their study of another heavy metal that there was no slope effect in Hg distribution in the deforested Napo River valley in Ecuador.

Arsenic and soil properties: Spatio-vertical relationship between As and soil properties are presented in Table 4, indicating varying influences of the heavy metal on studied soil properties. There was a significant negative correlation (R = - 0.94, ; P = 0.01) between As and Available phosphorus (Av.P), suggesting that an increase in As results to a corresponding decrease in Av. P, which could be attributed to the complexation between them in the adsorption sites (Jain and Loeppert, 2000) as both elements possess similar properties (Gao *et al*., 2004). Significant negative correlation (R = - 0.89; P = 0.01) was also reported between As and soil organic carbon, implying that soil organic matter (SOM) reduces the availability of As in the soil solution. This could be why Singh *et al*. (2007) recommended the use of organic amendments in combination with *Vetiveria zizanioides* in removing arsenic in contaminated soils. However, there was a good relationship between As and clay content especially in the vertical distribution of the heavy metal (R = 0.96, P = 0.01). Similar findings were made by numerous investigators (Zhang *et al*., 2001; Avila-Perez *et al*.,2002; Che *et al*., 2003) that highest concentrations of heavy metals are associated with fine sized grains. This relationship was less significant in spatial distribution of heavy metals (Pariznaganch *et al.,* 2007).

Table 1. Brief description of study site

Physiographic position	Sampling point	Description
Crest (8-10%) slope	50 m away from automobile service station	Deforested site used for dumping automobile wastes very few scrubs
Midslope (5-7%) slope	500 m any from automobile service station	Open dump site with giant grasses
Footslope (1 % slope)	1000 m away from automobile service station	Sediments from open dump site very tall luxuriant grasses plants

Table 2. Spatial variability of soil properties in studied soils.

Statistical tool	Sand	Silt	Clay	Bsat	SOC	pH	CEC	Av.P	Total As
	←	g kg $^{-1}$	→			(water)	cmol kg^{-1}	mg kg $^{-1}$	←
	Crest (Typic Haludult Dystric Nitisol) Automobile								
Mean	765	30	205	28.7	26.2	4.8	5.6	5.6	26.1
CV (%)	21.3	46.5	81.4	17.6	73.5	27.2	28.5	31.6	52.6
	Midslope (Flenentic Dystrudept/Dystric fluvisol Automobile								
Mean	710	65	225	35.2	27.6	5.1	4.6	26	26.6
CV (%)	15.2	76.0	85.6	26.6	96.4	29.6	22.3	46.3	58.2
	Footslope (fluveaquentic Eutropept/Eutric fluvisol) Automobile								
Mean	650	80	270	21.3	29.2	5.5	4.8	6.5	22.8
CV (%)	33.4	29.2	91.2	52.6	116.6	55.6	31.6	44.8	36.2
	Crest (Typic Hapludult / Dystric Nitisol) non-automobile								
Mean	781	40	179	40.6	25.4	5.5	5.8	12.6	0.2
CV (%)	9.3	23.6	42.4	19.3	48.4	11.6	9.6	16.2	10.4
	Midslope (Typic Hapludult/Dystric Nitisol) non-automobile								
Mean	774	45	181	41.8	26.6	5.6	6.2	18.8	0.4
CV (%)	9.8	35.2	36.8	23.2	36.1	17.6	11.6	15.6	9.6
	Footslope (fluveaquentic Eutropept/Eutric fluvisol) non Automobile								
Mean	660	130	210	58.8	26.8	5.9	6.4	2.4	0.4
CV (%)	12.2	39.2	27.6	11.2	28.4	45.6	14.3	19.3	7.7

Bsat base saturation, SOC = Soil organic carbon, CEC = cation exchange capacity Av.P = available phosphorus.

Table 3. Vertical variability of soil properties in studied soil

Statistical tool	Sand	Silt	Clay	Bsat	SOC	pH	CEC	Av.P	Total As
	←	g kg $^{-1}$	→			(water)	cmol kg^{-1}	mg kg $^{-1}$	→
	Crest (Typic HaFludult /Dystric Nitisol) (Polluted)								
Mean	702	50	248	24.8	7.4	5.1	4.6	4.9	45.4
CV (%)	17.5	48.9	71.0	48.9	157.1	28	73.0	78.6	67.2
	Midslope (Flenentic Dystrudept/Dystric Fluvisol (Polluted)								
Mean	668	50	282	26.8	9.6	5.1	5.2	4.1	62.3
CV (%)	30.9	69.2	77.4	48.1	152.0	33.0	64.8	83.4	71.6
	Footslope (Fluvaquentic Eutropept/Eutric Fluvisol) (Polluted)								
Mean	631	68	296	52	11.6	5.4	6.2	3.6	80.7
CV (%)	9.4	8.6	21.6	11.6	131.0	26.7	33.7	62.5	77.8
	Crest (Typic Hapludult / Dystric Nitisol) (Unpolluted)								
Mean	770	34	196	33.6	10.6	5.4	6.2	11.2	0.2
CV (%)	11.7	32.2	49.2	28.1	151.2	6.6	19.6	26.2	29.2
	Midslope (Typic Hapludult/Dystric Nitisol) (Unpolluted)								
Mean	764	40	198	34.2	13.4	5.4	6.4	16.8	0.1
CV (%)	6.0	50.0	37.3	23.8	111.9	9.1	11.7	29.6	24.8
	Footslope (Fluvaquentic Eutropept/Eutric Fluvisol) (Unpolluted)								
Mean	644	152	204	53.6	14.4	5.9	6.8	17.2	0.2
CV (%)	18.3	54.6	29.0	14.0	178.8	8.6	11.0	19.9	19.3

Bsat = base saturation, SOC = Soil organic carbon, CEC = cation exchange capacity, Av..P = available phosphorus.

Table 4. Relationship between As and some soil properties (N = 150)

Soil properties	R	R^2	$1-R^2$	Level of significance
	Spatial relationship			
Sand	0.10	0.01	0.99	NS
Silt	0.22	0.04	0.96	NS
Clay	0.67	0.44	0.56	*
Bsat	0.31	0.09	0.91	NS
SOC	- 0.89	0.79	0.21	**
pH	0.62	0.38	0.62	*
CEC	0.28	0.07	0.93	NS
Av.p	- 0.94	0.88	0.12	**
	Vertical relationship			
Sand	0.16	0.02	0.98	NS
Silt	0.29	0.08	0.92	NS
Clay	0.96	0.92	0.08	**
Bsat	0.23	0.05	0.95	NS
SOC	- 0.75	0.56	0.44	*
pH	0.56	0.31	0.69	*
CEC	0.33	0.10	0.90	NS
Av. P	- 0.90	0.81	0.19	**

SOC = soil organic carbon, Bsat = base saturation, CEC = cation exchange capacity, Av. P = available phosphorus

Conclusion

The study revealed that As concentration in soils varied both spatially and vertically in the pedosphere.However,higher values of As are associated with soils under automobile wastes when compared with more tolerable concentrations in non-polluted sites.Arsenic distribution in soils of the study area are strongly influenced by available phosphorus, soil carbon, clay content and soil pH. There is need for the inclusion of more edaphic and non-edaphic properties in future studies involving As for the purpose of relating them to determine more influential factors for establishing As activity models.Such studies will surely increase reliability of models in predicting biotoxicity and bioaccessibility of As in the study area.

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Growth and Performance of *Glycine max* L. (Merrill) Grown in Crude Oil Contaminated Soil Augmented With Cow Dung

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Abstract: In an effort to enhance crop production in crude oil contaminated soils, the effect of the addition of cow dung on the growth and performance of *Glycine max* grown in soil contaminated with various concentrations of crude oil were investigated in this study. There was a general improvement on the growth, dry weight, chlorophyll content, leaf area and pod production of the crop by the addition of cow dung to crude oil polluted soil. The performance of the crop also improved as the period of study increased suggesting that the toxicity of crude oil to the crop reduced as the period of study increased. Statistical differences ($P>0.05$) were noticed among the days of sampling for some of the growth and performance indices measured suggesting that the period of study affected the performance of such indices. The findings of this study show that addition of cow dung to crude oil contaminated soil makes such contaminated soils useful for agricultural activities. [Nature and Science. 2008;6(1):48-56]. ISSN: 1545-0740.

Keywords: Crude oil, cow dung, *Glycine max*, augmentation, performance

Introduction

Various activities in crude oil exploration, exploitation, storage and transportation lead to spillage of oil to the environment (Nicolloti and Eglis, 1998). The spilled oil pollutes soils and the soils to be less useful for agricultural activities with soil dependent organisms being adversely affected Baker, 1970; Mackay, 1991; Gelowitz, 1995, Siddiqui and Adams, 2002; Lundstedt, 2003). The effects of crude oil on the growth and performance of plants have been reported in many researches. These effects have been observed to occur due to the interference of the plant uptake of nutrients by crude oil and the unfavourable soil conditions due to pollution with crude oil (Plice, 1948; Gudin and Syratt, 1975; McGill and Rowell, 1977). It has been reported that plants and soil microbes compete for the little nutrient available in soils that are not rich like that polluted with crude oil thereby suppressing the growth of plants in such soils. However it is generally known that when soils not suitable for plant growth are augmented with manure, growth and performance of plants in such soil are enhanced. Merkl *et al* (2005b) reported that addition of inorganic fertilizer in a crude oil polluted soil enhances the growth and performance of *Brachiaria brizantha* in crude oil polluted soil. Although, the performance of plants as reported by Merkl and co–workers can be enhanced in crude oil polluted soil with fertilizer, it also increases the cost of crop production in crude oil polluted soil. It is therefore necessary to investigate the impact organic manure like cow dung can make the growth of crops in crude oil polluted soil. This is because such manure is cheaper and is more affordable to farmers than the inorganic fertilizers. This study was therefore carried out to investigate whether addition of cow dung to crude oil polluted soil will enhance the growth and performance of *Glycine max* in such soil. The information obtained will serve as a good reference for using cow dung to augment soils contaminated crude oil so as to use such soils for crop production.

Materials and Methods

Sources of seeds and Crude oil

The seeds of the *Glycine max* (TGX 1440-1E) used in this study were obtained from the Gene Bank Section of IITA Ibadan, Nigeria. The crude oil used for the studies was the Well Head medium. This was obtained from the Shell Petroleum Development Company's Health Safety and Environment Laboratory, Port Harcourt, Nigeria. The manure used was cow dung obtained from the Oremeji cattle market, Ifako Gbagada, Lagos, Nigeria.

Pollution of Soil and addition of manure:

This was done by adding 0g, 25g, 50g, and 75g of crude oil in pots containing 4000g of sandy loam soil. Each quantity of crude oil was added to six pots and was thoroughly mixed with the soil using hand trowel. Each quantity of crude oil served as a treatment with the 0g treatment serving as the control.

100g of the partially decomposed cow dung were added to three of the six pots having same quantity of crude oil. The remaining three pots were left to serve as control for each treatment. The manure was properly mixed with the polluted soil using hand trowel

Planting of Seeds and Germination Studies:

This was done following the modified version of the method described by Vavrek and Campbell (2002). Seven seeds of the *G. max* were sown in sandy-loam soil treated with different concentrations of crude oil (25g, 50g, and 75g) and the control treatment. The number of seeds that germinated from each pot was summed up after ten days. The percentage germination in each treatment was calculated using the formula:

$$\text{Percentage germination} = \frac{\text{number of seeds that germinated}}{\text{Number of seeds sown}} \times 100$$

Growth and Performance Studies

Crop Samples collection

The crops were collected by carefully uprooting a crop from each pot to avoid loosing the root tips. The collection of the crop samples was done once every 21 days for 105 days. The collected crops samples were properly labeled to show the treatments

Growth and Performance of *G. max*

These were determined by measuring the plant height, biomass, leaf area, chlorophyll content and the number of pods produced by *G. max* in each treatment. The plant heights were measured with meter rule while the biomass of the plant was determined by measuring the dry matter content of the plant after oven drying the plant in an oven at 60^0C to a constant weight using a weighing balance (Merkl, *et al*., 2004). The leaf areas of the plants were measured following the method described by O'Neal *et al*. (2002) and the chlorophyll content was measured as was described by Saupe (2004).

The growth and performance of *G. max* in crude oil polluted soil were compared with those of the crop grown the contaminated soils and that were augmented with cow dung.

Statistical Analysis

The data obtained were statistically analysed using LSD at 5% level of significance after analysis of variance test with SPSS 13.0 software

Results and Discussion

The influence of cow dung on the height of *G. max* (TGX 1440-1E) grown in soil contaminated with crude oil (well-head medium) is shown in figure 1. The heights of *G. max* in soils contaminated and augmented cow dung were greater than those of *G. max* in contaminated soils that had no cow dung. However, on day 42, the height of *G. max* in soil with contaminated with 50g crude oil (50.17cm) was greater than the height of *G. max* in soil contaminated with 50g crude oil and that had cow dung (43.67cm). However, the addition of cow dung did not produce any significant difference on the crops at $P>0.05$.

The plants grown in the soil without crude oil contamination grew better than those from the contaminated soil irrespective whether cow dung was added to soil or not. This shows that crude oil contamination inhibits plant growth an it is similar to the findings of Baker (1970), Akinola *et al*. (2004), Merkl *et al.* (2004) and Agbogidi *et al*. (2006; 2007). However, the greater plant height for plants from soils treated with 25g crude oil observed on day 105 as against the height of the plants from the uncontaminated soil may be due to exhaustion of nutrients from the sandy-loam soil used in this study by the plants from the uncontaminated soil and the increased addition of organic carbon by the degrading crude oil (Osuji and Onokaje, 2004; Okolo *et al*., 2005).

The addition of cow dung to soils contaminated with crude oil led to increase of the dry matter content of the *G. max* grown in such soils (figure 2). Thus the dry weights of the *G. max* from soils that were contaminated with crude oil and had cow dung added to them were greater than those from crude oil contaminated soils that had no cow dung added to. The addition of cow dung to the crude oil contaminated soil had much influence on the dry matter content of plant grown in soil with 25g crude oil that the dry matter content of plants from soil with 25g crude oil and cow dung was greater that of the plant from the uncontaminated soil. However, the dry matter content of *G. max* from soils with crude oil alone did not

show any significant difference from those of *G. max* from soil with crude oil and cow dung (P>0.05). This is similar to the findings of Merkl *et al.* (2005) who did not observe any significant difference on the influence of fertilizers on the shoot biomass of tropical pasture grass (*Brachiaria brizantha*). The difference between the dry matter content of *G. max* from soil with crude oil alone and soil with crude oil and manure was highest between *G. max* from soils with 25g crude oil and least between *G. max* from soils with 50g crude oil. The increased in the dry matter content observed in this study could be attributed to continuous growth of the plant which was shown the earlier section of this report.

The leaf areas of *G. max* from soils with crude oil and cow dung were higher than those from soils with crude oil alone (figure 3). Although the addition of cow dung led to increase in the leaf areas, there was no significant difference between the leaf areas of *G. max* from soils with crude oil alone and those of *G. max* from soils with crude oil and cow dung (P> 0.05). Therefore we can infer that addition of cow dung to crude oil contaminated does not have any effect on the leaf area. The noticed increase in the leaf area could be due to the general better growth of the plants grown in contaminated soils that had cow dung added to it. Although the addition of cow dung did not affect the leaf area of the plant significantly, there were significant effects of the days of sampling on the leaf area of the plant (P>0.05).

The total chlorophyll content of *G. max* from crude oil contaminated soils mixed with cow dung like the growth and the dry matter content of the crop was generally higher than that of *G. max* from soils with crude oil alone (figure 4). Although the addition of cow dung to the contaminated soil increased the total chlorophyll production, the means of total chlorophyll of *G. max* from contaminated soils alone and those of *G. max* from contaminated soils mixed with cow dung did not show any statistical differences (P>0.05). This suggests that the difference in the chlorophyll content of the plants from contaminated soils with cow dung and those from soils without cow dung may only be due to improved soil condition by the cow dung application. In addition to the chlorophyll content being higher in plants from contaminated soils that had cow dung being higher than those from soils that had no cow dung, plants collected from contaminated soils that had cow dung on days 21 and 105 had more chlorophyll than the plants from the uncontaminated soil. The increase of the chlorophyll content of the plant after day 63 could an indication of the plant recovery from the toxicity of crude oil.

The influence of cow dung on the production of pod by *G. max* grown in crude oil polluted soil is shown in figure 5. Plants from contaminated soil mixed with cow dung produced higher number of pods than *G. max* from crude oil polluted soil without cow dung when the concentration of crude oil was high. However, there was no statistical difference between the number of pods produced by *G. max* grown in contaminated soils mixed with cow dung and those without cow dung (P>0.05)

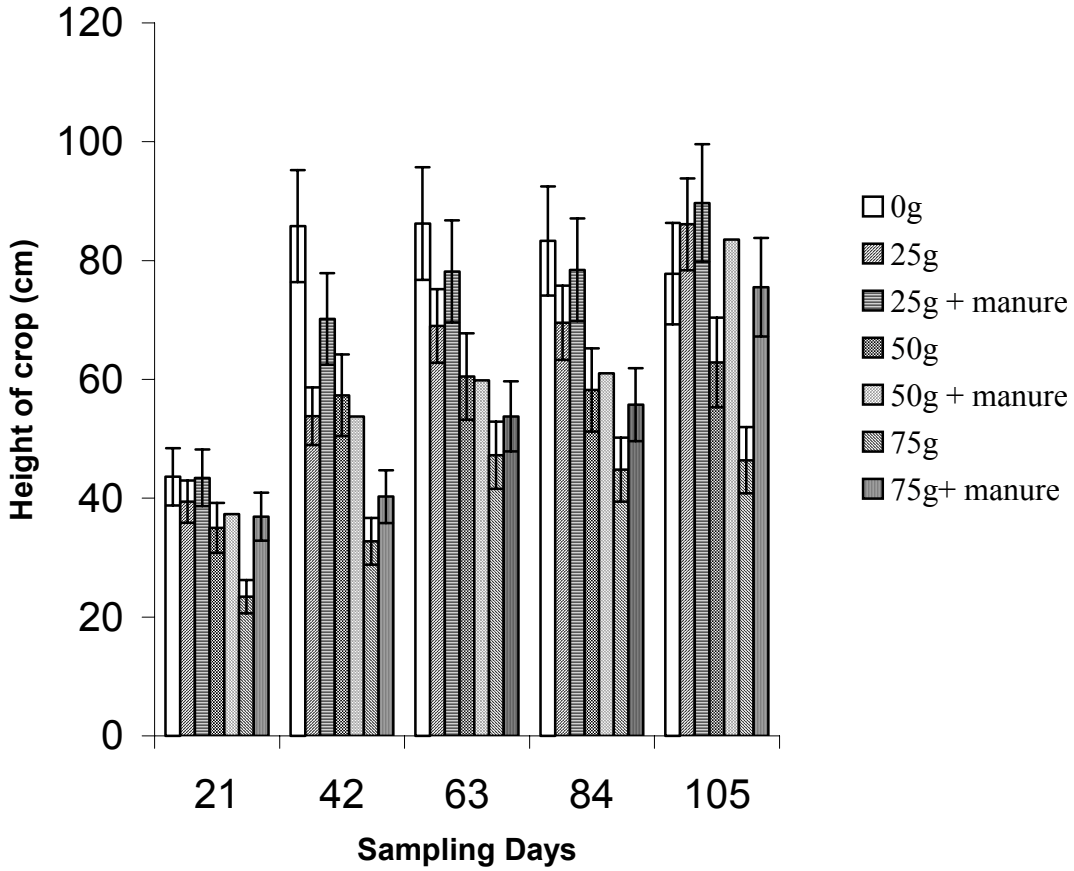

Fig 1: The effect of manure on the height of *G. max* grown in soils contaminated with varying concentrations of crude oil (well-head medium). The values are means ± S.E. of three replicate determinations. The errors bars show the difference in the level of response of the plants to the treatments in replicate buckets. 0g = control, 25g = soil with 25g crude oil and no cow dung, 25g+manure = soil with 25g cow dung and cow dung, 50g = soil with 50g crude oil and no cow dung, 50g + manure = soil with 50g crude oil and cow dung, 75g = soil with 75g crude oil and no cow dung, 75g + manure = soil with 75g crude oil and cow dung

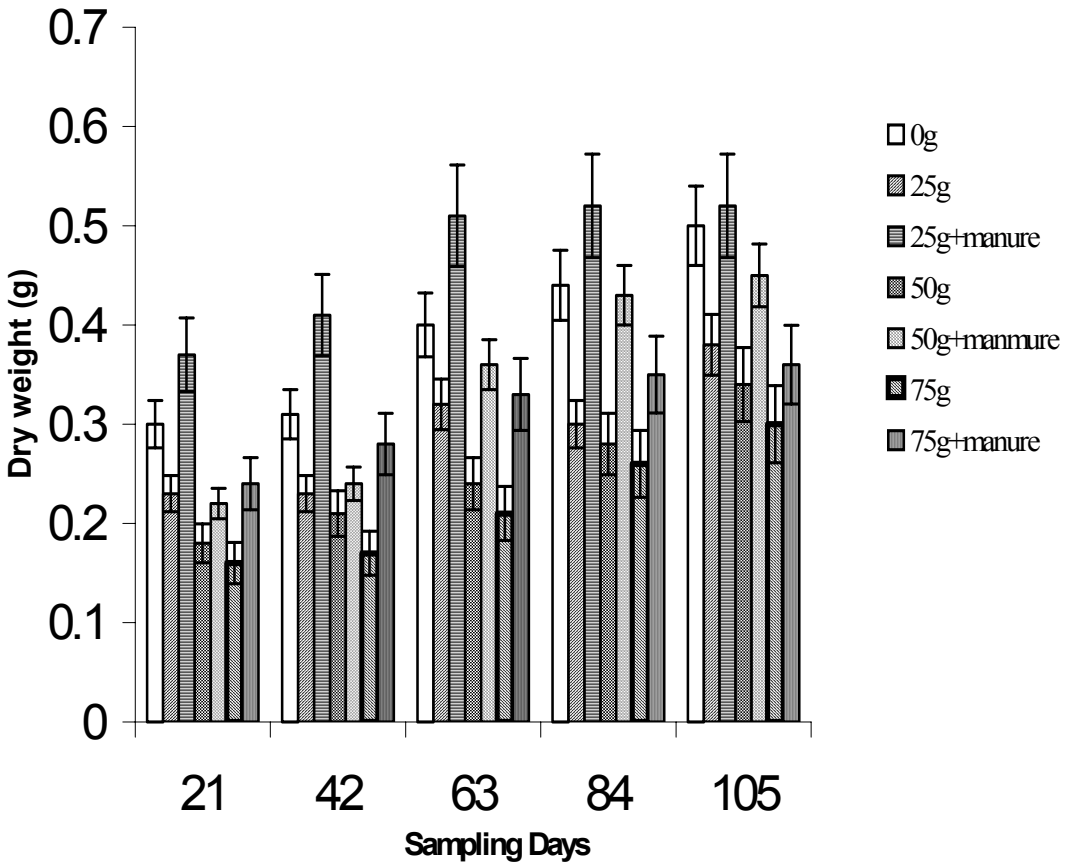

Fig 2: The influence of manure on the dry weight of *G. max* grown in soil polluted with varying concentrations of crude oil (well-head medium). Values are means ± S.E. of three replicate determinations. The errors bars show the difference in the level of response of the plants to the treatments in replicate buckets. 0g = control, 25g = soil with 25g crude oil and no cow dung, 25g + manure = soil with 25g cow dung and cow dung, 50g = soil with 50g crude oil and no cow dung, 50g + manure = soil with 50g crude oil and cow dung, 75g = **soil with 75g crude oil and no cow dung, 75g + manure = soil with 75g crude oil and cow dung.**

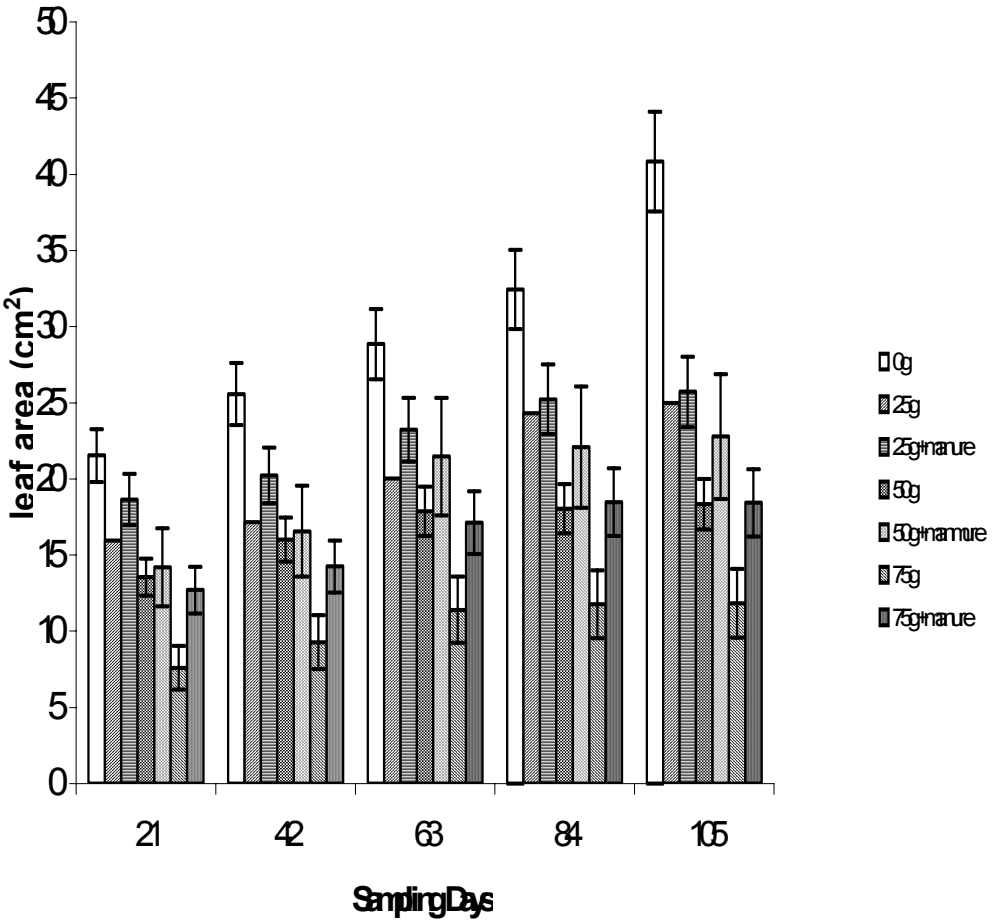

Fig.3: The effect of manure on the leaf area of *G. max* grown in soil contaminated with varying concentrations of crude oil (well-head medium). The values are means ± S.E. of three replicate determinations. The errors bars show the difference in the level of response of the plants to the treatments in replicate buckets. 0g = control, 25g = soil with 25g crude oil and no cow dung, 25g + manure = soil with 25g cow dung and cow dung, 50g = soil with 50g crude oil and no cow dung, 50g + manure = soil with 50g crude oil and cow dung, 75g = soil with 75g crude oil and no cow dung, 75g + manure = soil with 75g crude oil and cow dung

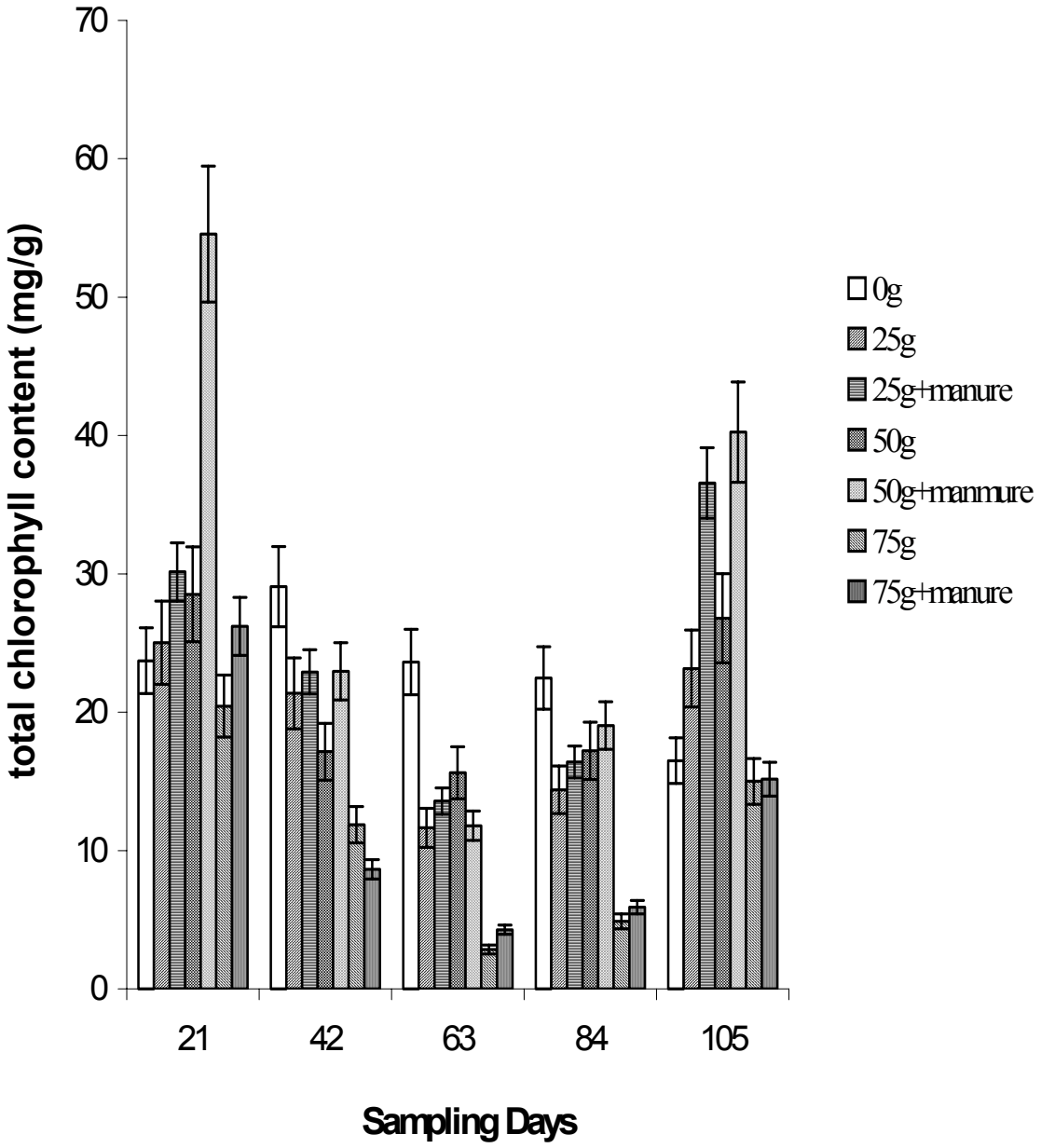

Fig 4: The influence of cow dung on the total chlorophyll content of *G. max* grown in soil contaminated with crude oil (well-head medium). The values are means ± S.E. of three replicate determinations. The errors bars show the difference in the level of response of the plants to the treatments in replicate buckets. 0g = control, 25g = soil with 25g crude oil and no cow dung, 25g = soil with 25g crude oil and cow dung, 50g = soil with 50g crude oil and no cow dung, 50g + manure = soil with 50g crude oil and cow dung, 75g = **soil with 75g crude oil and no cow dung, 75g + manure = soil with 75g crude oil and cow dung**

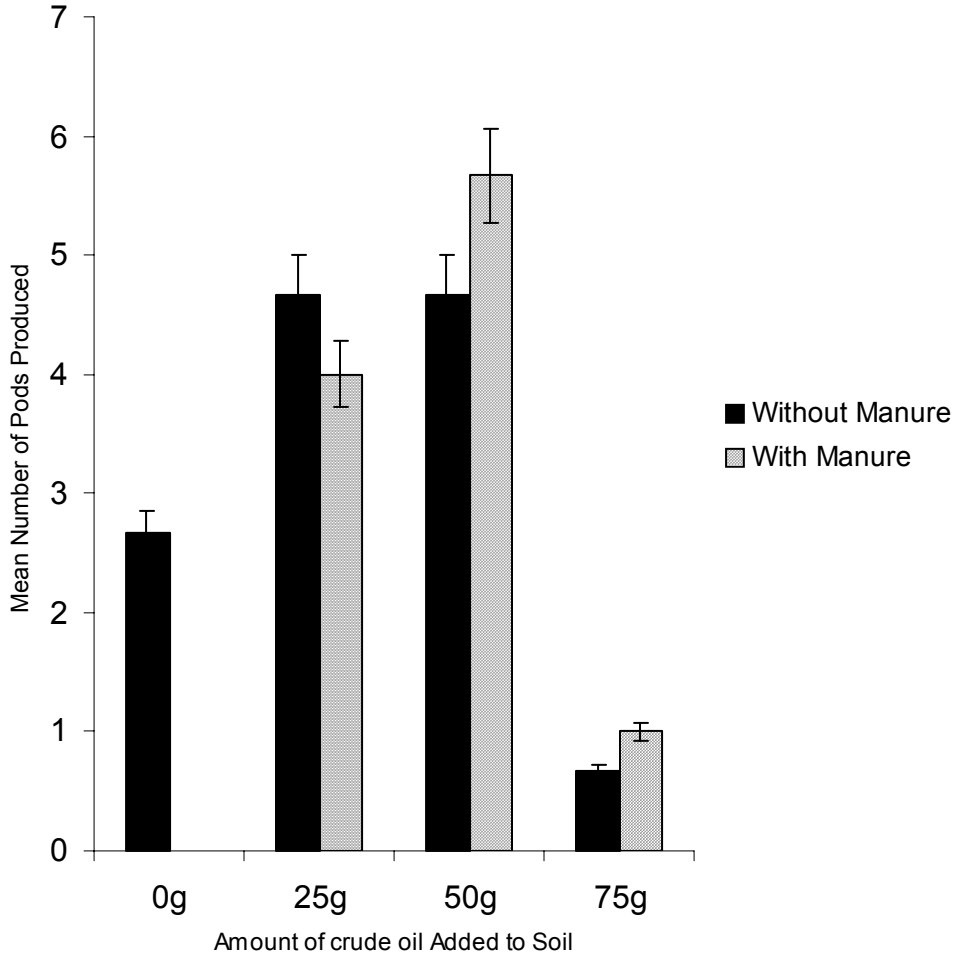

Fig 5: Influence of cow dung on pod production of *G. max* in crude oil polluted soil. The errors bars show the difference in the level of response of the plants to the treatments in replicate buckets.

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Can Lipid Lowering with Atorvastatin Reduce Plaque Disruption and Thrombosis?

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Abstract: Lipid lowering with diet alone in an atherosclerotic rabbit model has been shown to reduce the tissue factor, factor VII, matrix metaloproteinases and other elements responsible for arterial inflammation that can lead to thrombosis. This project design will reveal whether lipid lowering with atorvastatin reduces plaque disruption and thrombosis. [Nature and Science. 2008;6(1):57-62]. ISSN: 1545-0740.

Keywords: atorvastatin; lipid; plaque; thrombosis

Introduction

Atherosclerotic plaque rupture and/or disruption with subsequent arterial thrombosis are critical causes for acute coronary ischemic syndromes. We have previously reported on an atherosclerotic rabbit model of pharmacological triggering of plaque disruption and thrombosis (Abela, 1995). This is a modified model based on the earlier work by Constantinides who demonstrated that a vasoconstrictor agent, histamine and a procoagulant, Russell's viper venom (RVV) can trigger a platelet rich thrombus in an atherosclerotic rabbit model (Constantinides, 1981). This modified model has since been used to evaluate a viral agent causing atherosclerosis as well as imaging of thrombosis by magnetic resonance angiography. Although serum cholesterol has been well established as a major risk factor for cardiovascular events, the exact role of tissue cholesterol in the development of acute cardiovascular syndromes has not been defined. The purpose of this study is to determine if tissue cholesterol level influences cardiovascular events. This is performed by measuring tissue cholesterol content in an atherosclerotic model of plaque disruption and thrombosis.

The vulnerable plaque that is responsible for acute has been characterized by a thin fibrous cap and a rich lipid pool. Often, an inflammatory cellular component with few smooth muscle cells and low collagen content has been described (Kinlay, 1998). However, the role of the lipid pool in terms of its contribution to the unstable event has not been well defined. Speculation about the gruel content being highly thrombogenic has been suggested but it remains unclear what role the cholesterol content in the pool contributes to the severity of the acute cardiovascular event. It has also been suggested that reduction in the size of the lipid pool and/or its replacement by fibrous tissue reduces the vulnerability of the plaque (Monroe et al, 2003). Hence, a primary role for HMG-CoA reductase inhibitors is the alteration in the composition of the plaque content (Riessen et al, 1999; White, 1999). This study is conducted to evaluate the effect of the amount of cholesterol in the plaque and the severity of the event as defined by the amount of thrombus formation using an atherosclerotic model of plaque disruption and thrombosis. Furthermore, atorvastatin is used to evaluate the effect of antioxidation as a potential mechanism to reduce the risk of disruption and thrombosis.

Lipid lowering with diet alone in an atherosclerotic rabbit model has been shown to reduce the tissue factor, factor VII, matrix metaloproteinases and other elements responsible for arterial inflammation that can lead to thrombosis (Aikawa, 1999). However, the definitive test demonstrating that reduction of these elements will actually decrease thrombosis has not been demonstrated. It is no longer ethically possible to conduct controlled clinical trials between statin and placebo therapy in patients at high risk for cardiovascular events due to high cholesterol levels. However, in an atherosclerotic rabbit model of plaque disruption and thrombosis that we have developed, it would be possible to test the hypothesis that the reduction in tissue factor and other thrombogenic and inflammatory molecules will reduce the event outcome as measured by extent of thrombosis. Atherosclerosis is a disease characterized by inflammation, beginning with the earliest identifiable lesion (fatty streak) to the advanced vulnerable plaque. Clinical markers of inflammation, including C-reactive protein, modified low-density lipoprotein, homocysteine, tumor necrosis factor, and thermogenicity, have been identified as emerging risk factors that may add prognostic information in patient management. The model of plaque disruption and thrombosis can be triggered pharmacologically to evaluate the effect of various interventions on the outcome of thrombus formation in a prospective fashion (Abela, 1995). In a recent study of plaque disruption using this model

we have demonstrated that the extent of thrombosis is highly correlated with the content of tissue cholesterol (r=0.98) (Ma, 2007). Also, human data suggest that the reduction of inflammatory response by lipid lowering with statins has been associated with lower acute events (Ridker, 1999). Also, in an atherosclerotic rabbit model, atorvastatin is shown to reduce plaque inflammatory acitivity (Bustos, 1998).

Thus, it would be important to link the reduction of acute events is to a lowered inflammatory process. This could be demonstrated if atrovastatin by lowering the inflammatory activity and oxidative stress can reduce the thrombosis rate in our unique model of pharmacologically triggered plaque disruption and thrombosis (Ma, 2006).

Studies

For this project, atherosclerotic NZW rabbits are evaluated. Rabbits are made atherosclerotic by using a high cholesterol diet and balloon endothelial debridement. After feeding a cholesterol enriched diet for 6 months, the rabbits are thrombus triggered with Russell's viper venom (RVV) and histamine as previously reported (Abela, 1995). RVV activates clotting Factors V and X and histamine induces vasoconstriction in rabbits. Both these interventions are critical for the development of plaque disruption and thrombosis.

Atherosclerotic rabbits are thrombus triggered at 6 months. Group I rabbits are kept on a cholesterol enriched diet for 6 months prior to triggering. Group II rabbits are on a cholesterol enriched diet and atorvastatin for 6 months prior to triggering (Bustos, 1998).

After thrombus triggering, the rabbits are given heparin and then killed with euthanasia solution. The heparin prevents post-mortem thrombi from forming. The aortas will be exposed to evaluate the extent of thrombosis by counting the number of thrombi and planimetery of thrombus surface area. Tissues from the thoracic and abdominal aorta will be sampled for 1) measurement of tissue cholesterol levels by enzymatic techniques (Carr, 1993; Folch, 1975); 2) quantitative RT-PCR or Northern blot analyses of genes encoding the inflammatory molecules and thrombus; 3) Western blot and immuno-histochemistry study of inflammatory molecules and thrombus; and 4) lucigenin chemiluminescence determination of superoxide generation and plasma nitrite/nitrate levels using the griess reaction. Inflammation markers C-reactive protein, low-density lipoprotein and homocysteine are detected by immunohistochemistry methods in this project. These studies help elucidate the mechanism of reduction of plaque disruption and thrombosis.

The study details are as the following:

Study Groups: Eighty, male, New Zealand White rabbits weighing between 2.5 to 3.2 kg are divided into 4 groups. The method of establishing the atherosclerotic rabbit model and thrombus triggering are described previously (Abela, 1985; 1995).

Atherosclerosis Inducing: Briefly, the control-control group (Group I, 8=20) consisted of four normal rabbits that are fed a regular diet for 6 months. Rabbits in Groups II, III and IV (n=20, 20 and 20, respectively) underwent balloon deendothelialization and are then maintained on a 1% cholesterol enriched diet (Harlan-Sprague Dawley, Inc., Indianapolis, IN, USA) alternating with regular diet every month for a total of 6 months. Under general anesthesia (ketamine 50 mg/kg and xylazine 20 mg/kg, i.m.) balloon-induced deendothelialization of the aorta is performed using a 4F Fogarty arterial embolectomy catheter (Baxter Healthcare Corporation, Irvine, CA, USA) introduced through the right femoral artery cutdown. The catheter is advanced in a retrograde fashion to the ascending aorta and pulled back three times.

Pharmacological Triggering: Only the atherosclerotic rabbits (Groups II, III and IV) had pharmacological triggering since previous studies have not shown thrombosis to occur in normal arteries. Thrombus triggering is induced by RVV (0.15 mg/kg, i.p., Sigma Chemical Co., St. Louis, MO, USA) and histamine (0.02 mg/kg, i.v., Sigma Chemical Co., St. Louis, MO, USA) given at 48 and 24 hours prior sacrifice. In Group IV, atorvastatin (30 mg/kg) given 8 days prior to sacrifice. Heparin sulfate (1000 IU/rabbit, i.v., Sigma Chemical Co., St. Louis, MO, USA) is given 30 minutes prior to sacrifice to prevent postmortem clotting. Rabbits are sacrificed with an overdose of pentobarbital (50 mg/ml, i.v., Abbot Laboratories, North Chicago, IL, USA). Tissue samples from the heart, liver and kidney are stored immediately in liquid nitrogen until biochemical measurements. Procedures are performed according to Michigan State University's Animal Care and Use Committee approved protocol.

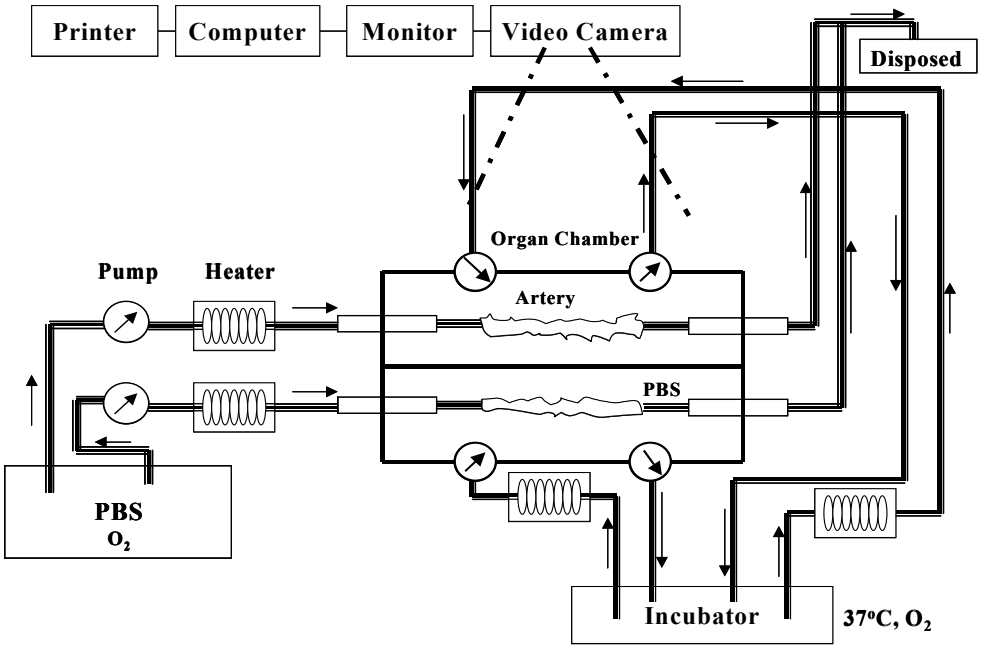

Figure 1. Dual organ chamber with separate perfusion using oxygenated physiological buffered solution at 37°C. Vessel diameter is measured by a computer planimetry system.

Quantitation of Thrombosis: The total surface area of the aorta, the surface area of aorta covered with atherosclerotic plaque, the surface area of aorta covered with *ante mortem* thrombus are measured. The surface area is measured from images obtained by a color charge-coupled device camera (TM 54, Pulnix, Sunnyvale, CA, USA) and digitized by an IBM PC/AT computer with a color image processing subsystem. The digitized images are calibrated by use of a graticule. Surface area is measured by use of a customized quantitative image analysis package. Also, the number of thrombi on the aortic arch to the distal common iliac branches is counted.

Artery Diameter Respond Evaluation: After rabbits are sacrificed both carotid arteries are isolated from each rabbit and placed in a dual organ chamber and perfused with oxygenated physiologic buffered solution (PBS) (NaCl 119 mM, KCl 4.7 mM, $CaCl_2$ 2 mM, NaH_2PO_4 1.2 mM, $MgSO_4$ 1.2 mM, $NaHCO_3$ 22.6 mM, glucose 11.1 mM and Na_2EDTA 0.03 mM) at 60 mmHg and 2.5 ml/minute flow rate at 37°C. Baseline vasodilation is determined using norepinephrine (NE, 1×10^{-6} M) preconstriction and pharmacological challenge is then performed with acetylcholine (Ach, 1×10^{-5} M) and sodium nitroprusside (SN, 1×10^{-5} M) successively. Vessel diameter is measured by a computer planimetry system (Figure 1). The data are calculated according to the formulas: Ach-NE (%)=(Ach-NE)/NE×100 and SN-NE (%)=(SN-NE)/NE×100 separately, where Ach, NE and SN represented the diameter (mm) of the arteries that are perfused by the PBS containing a corresponding chemical.

Metallothionein (MT): Metallothionein concentration as an index of oxidation is measured with Cd-hemoglobin saturation method (Eaton, 1991). Tissues are removed and rinsed in ice-cold Tris-HCl buffer (0.05 M, pH 8.6) then homogenized in 3 volume of the Tris-HCl buffer. The homogenate is centrifuged at 8,000×g for 10 minutes at 4°C and the supernatant fraction is heated for 90 seconds at 100°C. The heated samples are centrifuged at 8,000×g for 5 minutes at 4°C to remove precipitates. 100 µl of 400 ppm $CdCl_2$ solution is added into 200 µl of the supernatant and allowed to incubate at room temperature for 10 minutes. 150 µl of 2% bovine hemoglobin solution (w/v) is added into the sample, then the sample is mixed and heated in boiling water for 2 minutes. The boiled samples are placed in ice for 3

minutes and centrifuged at 8,000×g for 5 minutes at 4°C. Another 150 µl of 2% hemoglobin is added into the supernatant, then heating, cooling and centrifuging are repeated, and the supernatant is collected. The Cd concentration in the supernatant is measured using a flame atomic absorption equipment (Philips Electronics UK Ltd., Croydon, Surrey, England) and MT concentration is calculated from the Cd concentration measured in the supernatant (1 mg Cd represented 8.93 mg MT).

Glucose-6-phosphatase (G-6-Pase): Glucose-6-phosphatase measurement is followed Harper method (Harper, 1965). 0.1 ml of tissue homogenate (100 mg tissue/ml) in citrate buffer (0.1 M, pH 6.5) is added into a test tube and incubated at 37°C for 5 minutes. 0.1 ml of glucose-6-phosphate (0.08 M) is added and the sample is incubated at 37°C for 5 minutes, then 5 ml of trichloroacetic acid (10%, w/v) is added and centrifuged at 9,000×g at 4°C for 5 minutes. 1 ml of the supernatant is taken into a test tube and 5 ml of ammonium molybdate solution (2 mM) then 1 ml of reducing solution (42 mM 1-amino-2-naphthol-4-sulphonic acid, 560 mM SO_3) is added. The sample is incubated at room temperature for 30 minutes then absorption is measured at 660 nm.

Tissue Cholesterol: One cm^2 mid-thoracic and mid-abdominal aortic tissue samples are obtained. Total cholesterol (free and individual ester) in the tissue is measured by high-performance liquid chromatography (HPLC) (Kim, 1984). Each sample of aorta is ground to a fine powder with anhydrous sodium sulfate and extracted twice with 5 ml of chloroform and methanol mixture (2:1). The extract is dried under nitrogen and re-dissolved in 5 ml of isopropanol. A portion of isopropanol extract is filtered, dried and re-dissolved in the mobile phase. The sample (0.1 ml) is injected into the HPLC column and separated by using a Waters Radial-Pack C18 column eluted isocratically with acetonitrile:isopropanol (45:55 by volume) at 2 ml/min. The absorbance of the eluate is measured at 210 nm with a UV detector. Total cholesterol concentration is calculated by comparing the peak areas of samples with those obtained from the standard (Sigma Chemical Co., St. Louis, MO) (Witztum, 1985).

Serum cholesterol: Total serum cholesterol is obtained by enzymatic assays of blood samples collected from the rabbits prior to sacrifice. This is performed using a Sigma Diagnostics Kit for cholesterol (Sigma Chemical Co., St. Louis, MO).

Glucose Concentration: Sigma Glucose Diagnostic Kit (Sigma Chemical Co., St. Louis, MO, USA) is used for the serum and tissue glucose concentration measurements. The method of the instruction by Sigma is followed for this evaluation.

Light Microscopy: Arterial tissue specimen are embedded in paraffin, cut and mounted on glass slides. The sections are then stained with hematoxylin and eosin and Masson's trichrome stains. These are then examined using a light microscope.

Electron Microscopy: The tissue samples are fixed overnight in 4% glutaraldehyde (Fisher Scientific, Pittsburgh, PA, USA) with 0.1 M phosphate buffer (pH 7.4). Arterial segments (5 mm long) are subjected to critical point drying in liquid CO_2, mounted on stubs and gold-coated in a sputter coater. The intimal surface is examined using a JEOL scanning electron microscope (JEOL Ltd, Model JSM-6400V, Tokyo, Japan). Tissue sections are obtained and processed routinely for ultrastructural examination. Thin sections are stained with uranyl acetate and lead citrate and then examined with a transmission electron microscope (BEI preamplifier, Au Evirotech Company, Germany).

Statistics

Data analysis is performed using multivariate analysis comparing the two groups relative to the extent of thrombosis as related to cholesterol tissue content and presence and absence of tissue inflammatory markers. Using a 70% event rate we determined that 20 rabbits in each group could yield enough difference to detect a significant change between the two groups. With Jandel Scientific program, SigmaStat (Sigma Chemical Co., St. Louis, MO, USA) is used for data statistical analysis. $P<0.05$ is considered statistically significant difference. Measured data are reported as mean±SD. The student t-test is used for comparison.

Discussions

Atherosclerosis, or "hardening of the arteries", is the process that causes heart attacks and most strokes. It is characterized by the progressive build-up of fatty plaques in blood vessels. One major component of the atherosclerotic plaque is cells loaded with cholesterol called foam cells. It is currently believed that cholesterol, especially the low-density lipoproteins (LDL), must be modified or oxidized before they can be taken up to cause foam cells. Antioxidants such as vitamin C, vitamin E, and carotenoids can prevent the oxidative modification of LDL in the laboratory. This has given rise to the concept that these vitamins could decrease the risk of heart disease by preventing oxidation of LDL in the body. Myocardial infarction in human cases a triggering activity such as physical exertion precipitates the acute onset of the disorder (Mittleman 1993; Muller 1989; Tofle1990), but it is difficult to be studied in human. This study demonstrated that vulnerable plaques could be produced, the plaque disruption and platelet-rich arterial thrombus formation could be triggered pharmacologically in the atherosclerotic rabbits. Rabbits in the three groups which are balloon induced arterial injury and then maintained in an alternative 1% of cholesterol diet for a total of six months clearly caught atherosclerosis.

When cells use oxygen for energy purpose, they produce by-products called free radicals. Free radicals damage cells and tissues during a process called oxidation - a factor in many chronic illnesses, including some forms of cancer, cataracts, arthritis and cardiovascular disease. LDL, known as the "bad cholesterol", is actually a protein that carries cholesterol throughout the body. The cholesterol carried by LDL deserves its bad reputation, however. It often ends up in our arteries, causing clots that can lead to heart attacks. Oxidation of LDL-cholesterol contributes to the plaque build-up in arteries, a process called atherosclerosis that can cause blockages and reduced blood flow. The process also plays a role in the loss of elasticity in arteries. Antioxidants help to neutralize free radicals and prevent them from causing cellular damage. Once oxidized, the cholesterol is less apt to be expelled by the body's cleaning mechanisms and more likely to be stored in arteries.

In animal liver, glycogen is broken down into glucose-1-phosphate by liver phosphorylase and then converted into glucose-6-phosphate. Glucose-6-phosphate is dephosphorylated by G-6-Pase to yield free D-glucose, which passes into the systemic blood to be transported to other tissues.

MT is a ubiquitous class of low molecular weight and cysteine-rich proteins (about 1/3 of amino acid in MT is cysteine) binding unusually high amounts of metal ions, such as Ag, Cd, Cu, Hg and Zn. The most conspicuous biological feature of MT is its inducibility by a variety of agents and conditions. The biosynthesis of MT is induced by various factors such as heavy metals, certain hormones, cytokines, growth factors, tumor promoters, coldness, heat, hanger, radiation and diseases (Brady, 1982). Most of the inducing factors of MT biosynthesis are adverse factors. MT is thought to play an important role the homeostasis of metal ions and to be involved in the detoxification of heavy metals, scavenge free radical, etc. (Kagi, 1991).

The activity of G-6-Pase in liver of these four rabbit groups had the consistent magnitude result with glucose content in blood. This hinted that the alteration of glucose level in blood is adjusted by the activity of G-6-Pase in liver.

Carotenoid pigments are widely distributed in nature, where they play an important role in protecting cells and organisms against oxidation and free radical (Palozza, 1992).

This project design will reveal whether lipid lowering with atorvastatin reduces plaque disruption and thrombosis. Through the studies it will get valuable references for the atorvastatin researches and clinical application.

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MAASTRO lab has a vacancy for a Senior scientist, Head of Laboratory Research in molecular oncology (M/F)

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MAASTRO, Maastricht Radiation Oncology, is a co-operation between MAASTRO clinic, the University of Maastricht (UM) and the University Hospital Maastricht (azM) (see www.maastro.nl). MAASTRO consists of several division, including Maastro Clinic, which offers state-of-the-art radiotherapy to more than 3500 cancer patients each year from the Mid and South Limburg area in the Netherlands. MAASTRO clinic is also world-wide reference centre for Siemens Medical. In addition, research and training at Maastro is carried out in Maastro Physics, Maastro Trials, Maastro School, and Maastro Lab.

MAASTRO Lab is a basic and translational research laboratory embedded within the GROW research institute of the Faculty of Health, Medicine and Life Sciences at Maastricht University. Research carried out in the past has been focused on the tumour microenvironment and EGFR signalling pathways, both of relevance to radiation oncology. MAASTRO Lab has made several important discoveries in these fields, including demonstration that EGFR is up regulated by radiation and that hypoxia inhibits the initiation step of mRNA translation. In addition, we have initiated translational and clinical studies based on these results including both phase I novel treatment and molecular imaging trials as well as a Biobank project with more then 1500 patients included.

The lab has 4 permanent scientists, 5 technicians, more then 5 PhD students and is fully equipped for cell culture, molecular biology, flow cytometry, hypoxia, gene expression, proteomics and microscopy. Maastro lab has set up the necessary infrastructure for controlled exposures to hypoxia and hypoxia/reoxygenation, including development of novel equipment that allows rapid and precise changes in oxygenation. Access to expertise, equipment and resources within the much larger GROW research institute and other facilities in the University are also readily available, including the genome centre, advanced microscopy, and the animal facility with its imaging facility (Optical imager, MRI 7Tesla and micro CTPET to come). MAASTRO has a structural collaboration with the VU in Amsterdam on molecular PET biomarkers, with the TU/Eindhoven on Systems Biology and is initiating a new collaboration with the University of Toronto on research related to the Unfolded Protein Response and tumour hypoxia.

MAASTRO lab has a vacancy for a

Senior scientist, Head of Laboratory Research in molecular oncology (M/F)

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In this position you will be responsible for carrying out basic and translational research that is of relevance to radiation oncology in the broadest possible scope. You will initiate an independent research program based on demonstrated skills and expertise in fundamental aspects of biology. In addition, you will be chiefly responsible for the scientific research and training within the lab of experimental Radiation Oncology (MAASTRO lab). As head of research you will manage the laboratory scientific research, direct the research policy, and participate actively in ongoing and newly initiated research lines and projects. Successful grant applications to prestigious (inter)national organizations to support expansion of research activities will constitute an important part of your work.

Depending on experience, the process to appoint you as professor or associate professor at the faculty of Health, Medicine and Life Sciences from Maastricht University will be started. You will participate in research and educational activities within the faculty. The emphasis in this faculty appointment is on *microenvironment of solid tumours and cell signalling (EGFR)* but there is room for your specific area of expertise.

We are looking for a senior scientist with training and experience in basic molecular biology, biochemistry, cell biology or related area. Candidates should have a proven track record or demonstrate a strong potential to function as a principal investigator, with high impact scientific publications and several large operating scientific grants. Candidates should have experience and knowledge of molecular oncology and have a recognized expertise within a specific research area relevant to radiation oncology. Experience in radiation biology, collaboration with clinicians and ability to speak Dutch is a plus but is *not* a prerequisite. Preferably candidates will have experience in research group management. In addition, candidates should be capable of formulating strategic goals for their research program in line with the organisational strategy.

Conditions of Employment and salary are based on the Dutch Collective Labour Agreement for Hospitals (CAO-Ziekenhuizen). You will receive a permanent contract on a fulltime basis (36 hours/week), depending on your relevant experience.

Further information will be gladly given by Prof. Philippe Lambin, head of the Dpt of Radiation Oncology azM (e-mail: philippe.lambin@maastro.nl) or telephone number: +31-(0)88-4455666. Please also visit www.maastro.nl and www.grow-um.nl.

Your application letter, Curriculum Vitae and listing of publications can be sent before to the department of Personell to the attention of mrs. M.T.V. Vaessens, pbox 5800, 6202 AZ Maastricht, the Netherlands.

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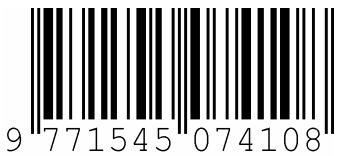

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