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[Cover Page](#), [Introduction](#), [Contents](#), [Call for Papers](#), [All papers in one file](#)

Contents

1. [Practical Technique of Western Blotting](#)
Jenny Young, Ma Hongbao 1-3
2. [Prevalence of Enterohaemorrhagic Eschericia Coli 0157:H7 Causing Severe Urinary Tract Infection in Abeokuta, Nigeria](#)
Akinduti P.A, Akinbo J.A, Ejilude O.A, Mannie-Udoh M.I, Umahoin K.O, Ogunbileje J.O, Folarin B.O 4-9
3. [The Role Played By Blocking over the Northern Hemisphere in Hurricane Katrina](#)
Y. Y. Hafez 10-25
4. [Anthropogenic Impacts on Protected Area of Burundi. Case Study of Ruvubu National Park](#)
Ntovenimana Remegie, Gu Yansheng 26-33
5. [Feeding behaviour of wild Asian Elephants \(*Elephas maximus*\) in the Rajaji National Park](#)
Ritesh Joshi, Rambir Singh 34-48
6. [Comparison Of Dac-Elisa And Dot-Blot-Elisa For The Detection Of Cucumber Mosaic And Banana Streak Viruses Infecting Banana](#)
P. Rajasulochana, R. Dhamotharan & P. Srinivasulu 49-57
7. [Research on the New Accounting Control Based on the Environment of IT](#)
TAO Ping, LI Wen-hua 58-64
8. [The Truth about Global Warming](#)
Willie J. McDonald 65-67
9. [Assessment *In Vitro* Of The Biological Effect Of A Herbal Product Extract: Morphological And Radiolabeling Analysis](#)
G. Diré, E. Lima, M. Gomes, D. Mattos and M. Bernardo-Filho 68-77
10. [Deterioration of Soil Organic Components and Adoptability of Green fallows for Soil Fertility Replenishment](#)
E.U. Onweremadu: E.C. Matthews-Njoku, F.C. Nnadi², F.C. Anaeto: F.O. Ugwuoke, D.O. Onu M.A.C.A. Odii 78-84
11. [Know Thyself](#)
Kees Beukering 85-87
12. [Sterol Regulatory Element Binding Proteins \(SREBPs\)](#)
Ma Hongbao, Cherng Shen 88-94
13. [Study on the Influence of wetland Media on the Purifying the micro-polluted Raw Water](#)
Xu Yang, Shuli Yu, Yongsheng Ma, Yan Zhao, Xiaoju Yan, Cunhai Xiu 95-100

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Practical Technique of Western Blotting

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Abstract: Western blotting is a widely used in protein detection method. This article describes the principle theory and technique for Western blotting procedure. [The Journal of American Science. 2008;4(2):1-3]. (ISSN 1545-1003).

Keywords: protein; SDS-polyacrylamide gel electrophoresis (SDS-PAGE); Western blotting

1. Introduction

Western blotting is powerful technique that is widely used in the protein detection. Since 1979, protein blotting has evolved greatly (Kurien, 2006). Western blotting analysis can detect the protein in a solution and give the protein information sensitively (Dechend, 2006; Ma, 1994; 2004; Peter-Katalinic, 2005; Sakudo, 2006; Westermeier, 2005). As the general process: 1. Separate the proteins using SDS-polyacrylamide gel electrophoresis (SDS-PAGE); 2. Transfer the protein from SDS-gel to a nitrocellulose membrane (electric transfer); 3. Put the primary antibody on the membrane; 4. Use the secondary antibody (this antibody should be an antibody-enzyme conjugate, e.g., horseradish peroxidase (HRP)); 5. Use the dye and read the result. As an application, Western blotting method revealed a significant decrease in endothelin-A receptor protein in left circumflex coronary arteries (Knudson, 2006). This article describes the principle theory and technique for Western blotting method.

I. Tissue Sample Preparation:

1. Isolate tissue (about 1 gram).
2. Put tissue in 3 volume of extract buffer.
3. Extract buffer (Table 1): The half-life of a 0.02 mM aqueous solution of PMSF is about 35 minutes at 8.0 pH. PMSF is usually stored as a 10 mM or 100 mM stock solution (1.74 or 17.4 mg/ml in isopropanol) at -20°C .
4. Homogenize sample under ice.
5. Centrifuge sample at 10,000 rpm for 10 minutes at 4°C .
6. Keep supernatant at -70°C until usage.

II. SDS PAGE:

1. Use 12% SDS gel. 12% SDS gel preparation is shown in Table 2 and an optional 12% SDS gel preparation reagent amount is shown in Table 3.
2. Take 50 μl of sample and add an equal volume of 2 x SDS gel-loading buffer. 2 x SDS gel-loading buffer is shown in Table 4. 2 x SDS gel-loading buffer lacking dithiothreitol can be stored at room temperature. Dithiothreitol should then be added, just before the buffer is used, from a 1 M stock (Dissolve 3.09 g of dithiothreitol in 20 ml of 0.01 M sodium acetate (pH 5.2). Sterilize by filtration. Dispense into 1-ml aliquots and store at -20°C).
3. Boil the sample (in loading buffer) at 100°C for 3 – 5 minutes.
4. Load the sample for electrophoresis: 8 V/cm ($6 \times 8 = 48$ volts) before the bromophenol blue (dye) front has moved into the resolving gel and 15 V/cm ($6 \times 15 = 90$ volts) until the bromophenol blue reaches the bottom of the resolving gel.
5. Make the gel for transfer in transfer buffer: 0.65 mA/cm² (about 100 volts) for 1.5 – 2 hours, or 30 volts overnight, on ice.
6. Western blotting transfer buffer (Table 5).
7. Block the filter with blocking buffer for 1 – 2 hours at room temperature (0.1 ml blocking solution per cm² filter), with gentle agitation on a platform shaker. Blocking solution is shown in Table 6 and Phosphate-buffered saline (PBS) (pH 7.4, 1000 ml) is shown in Table 7.

8. Discard blocking solution and immediately incubate filter with primary antibody.
9. Add 10 ml (0.1 ml of blocking solution per cm² of filter). Blocking solution is shown in Table 8.
10. Add 0.005 ml of primary antibody (1:2000) in to blocking solution.
11. Incubate at 4°C for 2 hours or overnight with gentle agitation on a platform shaker.
12. Discard blocking solution and wash filter 3 times (10 minutes each time) with 250 ml of PBS.
13. Incubate the filter with 150 mM NaCl, 50 mM Tris-HCl (pH 7.5) (phosphate-free, azide-free blocking solution) for 3 times for 10 minutes each time.
14. Immediately incubate the filter with secondary antibody.
15. Add 10 ml of phosphate-free, azide-free solution (150 mM NaCl, 50 mM Tris-HCl, 5% nonfat dry milk pH 7.5). Phosphate-free, azide-free blocking solution (pH 7.5, 1000 ml) is shown in Table 9.
16. Add 0.005 ml of secondary antibody solution (1:2000).
17. Incubate 1 – 2 hours at room temperature with gentle agitation.
18. Discard secondary and wash with 150 mM NaCl, 50 mM Tris-HCl (pH 7.5) (phosphate-free, azide-free solution) for 3 times for 10 minutes each time.

III. Alkaline phosphatase stain:

1. Add 5 ml of the substrate 5-brono-4chloro-3-indolyl phosphate/nitro blue tetrazolium (BCIP/NBT) solution (Sigma).
2. Observe the filter for the blue color on the filter (about 20 minutes).
3. Discard BCIP/NBT solution when the bands are clear (about 20 minutes).
4. Immediately stop the enzymatic reaction by add water.
5. Cover the filter with plastic membrane and keep the filter.
6. Analyze the blue bands and compare the color.

The half-life of a 0.02 mM aqueous solution of PMSF is about 35 minutes, at 8.0 pH. PMSF is usually stored as a 10 mM or 100 mM stock solution (1.74 or 17.4 mg/ml in isopropanol) at –20°C.

1X SDS gel-loading buffer lacking dithiothreitol can be stored at room temperature. Dithiothreitol should then be added, just before the buffer is used, from a 1 M stock (Dissolve 3.09 g of dithiothreitol in 20 ml of 0.01 M sodium acetate (pH 5.2). Sterilize by filtration. Dispense into 1-ml aliquots and store at –20°C).

IV. Overall of Western blotting solutions

Tissue Extract buffer, 100 ml
50 mM Tris-HCl (pH 8.0), 0.6 g (Or 50 mM HEPES (pH 7.0), 1.19 g)
150 mM NaCl, 0.88 g
0.02% sodium azide, 0.02 g
0.1% SDS, 0.1 g
0.1 mg/ml phenylmethylsulfonyl fluoride (PMSF), 0.01 g
0.001 mg/ml aprotinin, 0.1 mg
1% Nonidet P-40 (NP-40), 1 ml (Or 1% Triton X-100, 1 ml)
2 X SDS gel-loading buffer, 100 ml
100 mM Tris-HCl (pH 6.8), 1.21 g
200 mM dithiothreitol
4% SDS, 0.4 g
0.2% bromophenol blue, 0.2 g
20% glycerol, 20 ml
SDS 5x Running Buffer, pH 8.3, 1000 ml
Tris, 125 mM, 15.14 g
Glycine, 1.25 M, 93.84 g
SDS, 0.50%, 5 g

Western Transfer Buffer, 1000 ml
Tris, 48 mM, 5.814 g
Glycine, 39 mM, 2.928 g
SDS, 0.04%, 0.37 g, 3.7 ml of 10% SDS
Methanol, 20%, 200 ml
Blocking solution, 100 ml, in 100 ml phosphate-buffered saline (PBS, pH 7.4)
Nonfat dried milk, 5%, 5 g
Antifoam A, 0.01%, 10 ml
Sodium azide, 0.02%, 20 mg
Phosphate-buffered saline (PBS), pH 7.4, 1000 ml (adjust to pH 7.4 with HCl)
NaCl, 8 g
KCl, 0.2 g
Na ₂ HPO ₄ , 1.44 g
KH ₂ PO ₄ , 0.24 g
Phosphate-free, azide-free blocking solution, 1000 ml (adjust pH with 12 N HCl about 3.35 ml)
150 mM NaCl, 8.766 g
50 mM Tris-HCl (pH 7.5), 6.057 g
5% (w/v) nonfat dried milk

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Prevalence Of Enterohaemorrhagic Escherichia Coli 0157:H7 Causing Severe Urinary Tract Infection In Abeokuta, Nigeria

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ABSTRACT: Increasing renal debility among various age groups in recent past has shifted public attention on the prevalence of renal diseases caused by enterohaemorrhagic escherichia coli serotype 0157:H7 (EHEC 0157:H7) in Abeokuta, Nigeria as a result of consumption of improperly cooked meal especially of bovine source. This study spanned through a period of 23 months between February 2006 to November 2007 with total urine sample of 1205 from various age group including known UTI patients and related diseases (988) and Non-UTI patient 217 which serves as control group. All the urine samples were investigated for EHEC 0157:H7. E.coli serotype accounted for 372(46.4%) of all the isolates like *Pseudomonas aeruginosa* 74(9.2), *Staphylococcus albus* 122(15.2), *Proteus mirabilis* 51(6.4%), *klebsiella specie* 147(18.3%), *staphylococcus aureus* 36(4.5%). Age group 41-50 male shows highest prevalence of 1/3(33%) , while female age group 6-10 having 1/11(9.0%), 11-20(6.7%). 21-30, 6/8(7.5%), 41-50 ½(8.3%), suggesting that female were more predisposed to UTI and its related diseases. Incidence of E.coli0157:H7 in chronic PID and gynaecological condition having 1/9(11.1%) inferred that E.coli0157:H7 could as well be responsible to this disease condition and foetal debilities. Most of the isolates were verocytotoxin producers with 112 out of 223(5.4%) in severe UTI and all produce type-1 and 2 verocytotoxin and likewise in chronic PID and gyaenecology conditions. This study shows a very high prevalence of E.coli0157:H7 in this area which could be a major aetiological cause of renal diseases. Attention should be more paid to undiagnosed hemolytic colitis and hemolytic uremic syndrome caused as a result of severe UTI in developing countries with aggressive strategic public health campaign in preventing secondary transmission .The study is statistically significant (p<0.05). [The Journal of American Science. 2008; 4(2):4-9].

Keyword: enterohemorrhagic *Escherichia coli*0157:H7, UTI, Verocytotoxin, chronic PID

INTRODUCTION

Increasing renal debility in young adult and elderly from complication of urinary tract infection in recent years has shifted public attention on the prevalence of the illness and its declining effective therapeutic management. *Escherichia coli* is accounted for the majority of urinary tract infection in young adult and pregnant women, but other Gram negative rods of different genera such as *Proteus* spp,

Enterobacter aerogene, Pseudomonas aeruginosa and Enterococci spp can also be the cause, particularly in hospitalized patients or those with predisposing condition such as bladder catheterization or diabetes (3,5). The symptoms and signs always include urinary frequency, dysuria, haematuria and pyuria but none is absolutely specific for E.coli infection. Flank pain is associated with upper urinary tract infection while it was reported that nephropathogenic E.coli typically produce hemolysin(5). Enterohaemorrhagic E.coli causing hemolytic colitis (HC), a severe form of diarrhea; has been associated with hemolytic uremic syndrome (HUS) which progress in some patients to renal failure (1,14). Hemolytic uremic syndrome is life threatening condition especially among children and elderly. The death rate associated with HUS was reported to be 3-5% in USA (1,6).

Of the E.coli serotype that produces verocytotoxin, O157:H7 is the most common cause of HUS illness or severe thrombotic thrombocytopenic purpura which affect all ages(1,5). Increasing acute renal failure reported as a result of UTI prevalence in this part of the country motivated this investigation. High consumption of roasted beef locally known as "suya" and particularly pasteurized milk, yoghurt etc were common vehicle of E.coli O157:H7 transmission (1,2,19). Their low infection dose, unusual acid tolerance and their apparent special association with ruminant that are used for food could as well serve as a major point of contraction (1,14).

This study, therefore examine a two years prospective investigation to determine the prevalence of severe UTI caused by enterohaemorrhagic E.coli O157:H7 in Abeokuta, Nigeria.

MATERIALS AND METHODS

Sample collection: 988 suspected urine samples were collected from Federal Medical Center, Sacred Heart Hospital and General Hospital, all in Abeokuta township. These hospitals were among the largest hospital in south-western Nigeria (one of the geo-political zone in Nigeria). These urine samples were among those submitted to the hospital laboratory for routine culture and sensitivity. 217 urine samples were collected as control from subjects that have not had episode of UTI in the past previous 6 weeks but were visiting the hospital for other reason other than UTI.

Bacteriological procedure: Specimen were cultured on Blood Agar and MacConkey agar; and incubated at 37c for 24hrs aerobically. Each colonial characteristic like colony size, consistency, shape, pigmentation and lactose fermentation on MacConkey were noted in addition to Gram stain reaction. Isolates were identified to specie by standard biochemical methods according to the protocol of Cowan and Steel(7,8). Colonial morphology that conformed to E.coli identity (7), was further tested for E.coli O157:H7 by sub-culturing the colony onto Sorbitol MacConkey Agar(9) for sorbitol fermentation. Non-sorbitol fermenting organism were further tested for verocytotoxin production i.e shiga-like toxin.

Detection of verocytotoxin: virulent factor exhibited by the E.coli O157:H7 were identified using Reversed Passive Latex Agglutination VTEC-RPLA test kit produced by OXOID TD960

Statistical analysis: All data were analysed by chi-square test for statistical comparison between the group and a p-value <0.05 was considered significant.

RESULT:

Table 1 shows the pattern and distribution of some pathogens, UTI E.coli isolates of 372 (46.4%), having highest frequency with Klebsiella oxytoca 147 (18.3%) then Staphylococcus albus 22 (15.2%), Pseudomonas aeruginosa 74 (9.2%), Proteus mirabilis 51 (6.4%) and Staphylococcus aureus 36 (4.5%). Chronic pelvic inflammatory disease (PID) which its aetiological source could be traced to E.coli having 21(39.6%) then Staphylococcus albus 20 (37.8%) and Streptococcus pyogene 12 (22.6%), all were isolated in association with severe cystitis. Gynaecological conditions which include preterm labour, intra-uterine foetal distress and miscarriage in association with pyelonephritis and severe cystitis shows an increase of E.coli 93 (69.9%), Staphylococcus albus 22 (16.5%) and Pseudomonas aeruginosa 18

(13.5%). Non-Escherichia coli 0157:H7 urine samples which serve as control group shown no E.coli isolates but increase Proteus mirabilis 151 (69.7%), Klebsiella oxytoca 52 (24.0%), Pseudomonas aeruginosa 8(3.7%) while Staphylococcus albus and staphylococcus aureus have equal occurrence of 3 (1.4%) but no Streptococcus pyogene was isolated. Pattern and distribution of bacteria pathogen isolated from severe UTI (Table 1), Chronic PID associated with cystitis and Gynaecological conditions associated with pyelonephritis and cystitis. Non-E. coli 0157:H7 infected control group.

A very high incidence (Table 2) of E. coli 1/3(33%) was found in male of age group 41-50. Recurrent occurrence of E. coli 0157:H7 in 1/11(16.7%) in female age group 6-10, 11-20, 21-30, 41-50, 61-70 respectively. Chronic PID associated with cystitis in female subjects shows 1/9(11.1%) and 1/3(33.3%) in age group 31-40 and 41-50 respectively that were of reproductive age group. Gyneacological conditions with severe pyelonephritis and cystitis show 1/9(11.1%) in 21-30 age group.

Verocytotoxin vt was detected from 12 e.coli 0157:h7 in 223(5.4%) serotypes isolated from UTI (Table 3). VT-1 from 2 of 12(33.3%), VT-2 from 6 of 12 (50%) and VT-1&2 in 4 of 12 (33.3%) isolates. In Chronic PID associated with severe cystitis, 2 out of 24(8.3%) while VT-1 from 2 of 2(100%), nil from VT-2 and VT-1&2 respectively. Gyneacological conditions show VT-1 1 out of 16 (6.3%) and nil from 1(0%) for VT-1 and VT-2 respectively but 100% for VT-1&2 isolates.

Table 1: Pattern and distribution of bacteria pathogens.

Isolate	UTI	Chronic PID	Gyneacological Condition	Non-E.coli 0157:H7
	No(%)	No(%)	No(%)	No(%)
E.coli	372(46.4)	21(39.6)	93(69.9)	0(0)
Pseudomonas aeruginosa	74(9.2)	0(0)	18(13.5)	8(3.7)
Staphylococcus albus	122(15.2)	20(37.8)	22(16.5)	3(61.3)
Proteus mirabilis	51(6.4)	0(0)	0(0)	151(69.7)
Streptococcus pyogene	0(0)	12(22.6)	0(0)	0(0)
Klebsiella oxytoca	147(18.3)	0(0)	0(0)	52(24.0)
Staphylococcus aureus	36(4.5)	0(0)	0(0)	3(1.4)
Total	802(66.6)	53(4.4)	133(11.0)	217(18.0)

Table 2: Distribution of E.coli0157:H7 in various disease conditions according to age group

Age group	UTI		Chronic PID	Gyneacological conditions
	male	female	female	female
	n* (%)	n* (%)	n*(%)	n* (%)
0-5	0/2(0)	0/7(0)	nil	nil
6-10	0/6(0)	1/11(9.1)	nil	nil
11-20	0/3(0)	1/15(6.7)	nil	nil
21-30	1/10(10)	6/80(7.5)	0/7(0)	1/9(11.1)
31-40	0/3(0)	0/41(0)	1/9(11.1)	0/7(0)
41-50	1/3(33)	1/12(8.3)	1/3(33.3)	nil
51-60	0/3(0)	0/0(0)	0/3(0)	nil
61-70	0/0(0)	1/15(16.7)	0/2(0)	nil
71-above	0/2(0)	0/11(0)	nil	nil
Total	2/31(6.5)	10/192(5.2)	2/24(8.3)	1/16(6.3)

n*=number showing positive as numerator and total no of specimen as denominator

Table 3: Prevalence of verocytotoxin producers among E.coli 0157:h7 isolates by Reverse Passive Latex Agglutination(RPLA-OXOID TD 960)

Disease condition	E.coli0157:h7	VT-1	VT-2	VT-1&2
And no of samples	n* (%)	n* (%)	n* (%)	n* (%)
UTI(223)	12/223(5.4)	2/12(16.7)	6/12(50)	4/12(33.3)
Chronic PID(24)	2/24(8.3)	2/2(100)	0/2(0)	0/2(0)
Gynaecological(16)	1/16(6.3)	0/1(0)	0/1(0)	1/1(100)

DISCUSSION

This study confirms E.coli 0157:H7 as one of the major cause of severe UTI in Abeokuta affecting various age groups. From this study, enterohaemorrhagic E. coli 0157:H7 was isolated in chronic PID affecting most reproductive age group and gynaecological conditions associated with severe cystitis and pyelonephritis. Findings from this study indicate a very high prevalence of E.coli 0157:H7 among 41-50 male (33%) suffering severe UTI. Its prevalence spread across various female age groups. This suggest that female mostly adult were more predisposed to this infection.

Reproductive age group female subjects of 31-40 and 41-50 having chronic PID associated with cystitis have prevalence rate of 11.1% and 33.3% respectively. Gynaecological conditions which include preterm labour, intra-uterine foetal distress and miscarriage show prevalence rate of 11.1% only in 21-30 age group.

Studies have shown that VTEC strain is commonly isolated in haemolytic colitis and haemolytic uremic syndrome belonging to serogroup 0157 and they possess flagella antigen H7 while VT-2 toxin is common to both (1,13,20). Following this assertion, VTEC is on the increase in UTI subjects with 16.7%, while chronic PID of 100% VT-1 suggest an absolute prevalence and also gynaecological condition given an inference of 100% VT-1&2. Then if, VT-1 and VT-1&2 could give such an increasing prevalence, then many of these patients could as well be suffering from undiagnosed life threatening haemorrhagic colitis (HC) and haemolytic uremic syndrome(HUS)(1). Very scanty documentation of E.coli 0157:H7 prevalence in UTI and other renal related diseases could not be obtained. Comparison of data was not possible from other part of the country due to poor documentation.

Symptomatic and asymptomatic persons were assumed to transmit E.coli0157:H7 to susceptible individuals in the same manner since we have no data to prove otherwise (11,14). The prevalence rate from the study is statistically significant $p > 0.003$.

PUBLIC HEALTH IMPLICATION

With the evidence of E.coli0157:H7 appearance, thousands of people are at risk locally as a result of secondary transmission through contact with faecal materials of infected cattle or cow products and poor hygiene (11). Intensive public health strategies in prevention of E.coli0157:H7 transmission could include public media campaign targeting a high risk group such as children, elderly and immunocompromised individuals (11,15,16). Considering the potential public health benefit to be gained by these actions and low cost of associated with its implementation, these strategies would be relevant in prevention of hemolytic colitis and hemolytic uremic syndrome outbreak and other highly infectious pathogenic organisms (11,17).

CONCLUSION

E. coli 0157:H7 is the major cause of HUS illness that affect all ages (17). From this study, renal diseases associated with severe UTI were majorly caused by E.coli0157:H7 and also chronic PID, miscarriages and preterm labour could be linked with severe cystitis and pyelonephritis. Physicians and other health professional should pay more attention on any infection caused by E.coli serotype 0157:H7 in UTI and other related diseases. Public health campaign should be intensify to grassroot in preventing secondary transmission in order to curb its outbreak (12). Adequate personal hygiene and proper cooking of food material especially of bovine origin should be more emphasized (S18)

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The Role Played By Blocking over the Northern Hemisphere in Hurricane Katrina

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ABSTRACT: In 2005, there were 28 tropical Atlantic storms and hurricanes according to the Saffir-Simpson scale. Among these, there were three large hurricanes with surface winds of more than 150 knots: Katrina, Rita, and Wilma. This paper investigates the role played by a blocking system over the northern hemisphere in hurricane Katrina. The 6-hour and daily NCEP/NCAR reanalysis data composites for meteorological elements (surface pressure, surface wind, precipitation rate, and geopotential height at 500 mb level) over the northern hemisphere for August 2005 were used in this study. In addition, satellite images for hurricane Katrina and its damage have been used. These datasets have been analyzed using the methodology of anomalies. The results reveal that a diffluent block persisted over Siberia and was associated with a strong westerly air current aloft over North America from 22 to 31 August 2005. In addition, a strong westerly air current aloft existed over the North Atlantic region. Splitting of westerlies into two branches occurred over the North Atlantic; the first branch went towards the north while the second extended to the south towards the tropical Atlantic region. The splitting of the main air current over the North Atlantic caused an unusually strong northeast and easterly wind in the tropical Atlantic region. These unusual winds caused by the blocking system in the northern hemisphere circulated, accelerated, and controlled the track of hurricane Katrina from 23 to 31 August 2005. Analysis of the 10-day mean anomaly of the geopotential height at 500 mb for the northern hemisphere for August 2005 revealed that there was an outstanding positive anomaly of more than +200 m over North America simultaneously with positive anomalies of more than +150 m over Siberia during the last 10 days of August 2005). [The Journal of American Science. 2008; 4(2):10-25].

Keywords: Hurricane, Geopotential Height, Zonal, Meridonal, Blocking.

1. Introduction

The 2005 hurricane season will long be remembered for its tropical Atlantic storms. The season had 28 tropical Atlantic storms, including three large hurricanes with surface wind speeds of more than 150 knots: Katrina, Rita, and Wilma (NCDC, 2005). Katrina had sustained wind speeds of 150 knots, making it a Category 5 hurricane on the Saffir-Simpson scale, which is a rating scale from 1 to 5 based on a hurricane's intensity (Zebrowski and Judith, 2005). Hurricane Katrina initiated over the tropical Atlantic region on 23 August and lasted until 31 August. It caused about \$60 billion in damage, displaced 500,000 people, and killed 1,053, making it the deadliest hurricane to hit the United States since 1928. Katrina made landfall at Gulfport, Mississippi, and 80% of New Orleans was inundated with water up to 20 feet deep after several levees failed around Lake Pontchartrain. Katrina shut down an estimated 95% of crude production and 88% of natural gas output in the Gulf of Mexico.

Several papers have reviewed the tropical storms and its hazards and damage, (Sallenger, 2000; Gray, 2001; Zebrowski and Judith, 2005, Asbury, 2006; Verbout et al., 2007). Many studies have also investigated the formation and persistence of hurricanes, the effect of Atlantic blocking action upon European weather and climate, and the role of blocking systems in the northern hemisphere climate variability (Rex, 1950a, 1950b, 1951; Dole, 1978, 1982; Hafez, 1997, 2003; Cohen et al., 2001; Hasanean and Hafez, 2003; Hafez, 2007). According to these studies, blocking systems form from anomalies of the geopotential height at 500 mb over the northern hemisphere of more than +100 m and that persist for at least 7 days. The present work aims to uncover the role played by blocking systems that existed over the northern hemisphere through the period 22-31 August 2005 in hurricane Katrina.

2. Data and Methodology

The 6-hour and daily NCEP/NCAR reanalysis data composites for meteorological elements (surface pressure, surface wind, precipitation rate, and geopotential height at the 500 mb level) over the northern hemisphere for August 2005 (Kalnay et al., 1996) were used in this study. In addition, satellite images for

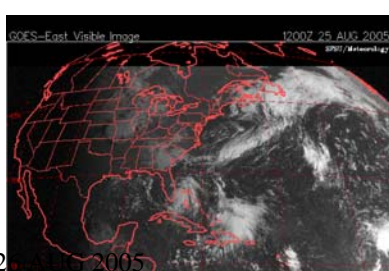
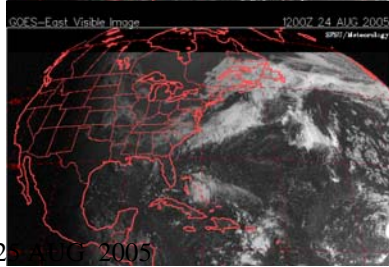
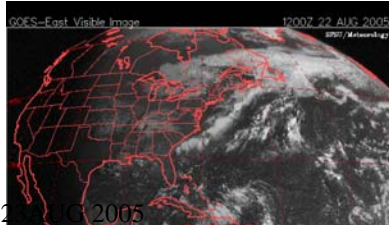
hurricane Katrina and its damage were used. Satellite images were obtained from NASA's Earth Observatory and from Jeff Schmaltz of the MODIS Land Rapid Response Team at NASA Goddard Spaceflight Center. In the present work, these datasets were analyzed using the anomalies methodology and linear correlation coefficient techniques (Spiegel, 1961).

3. Results

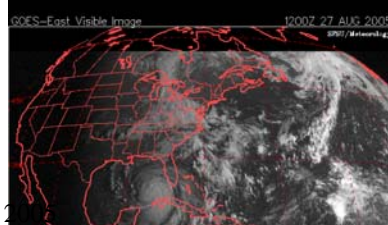
3.1 Study of the role played by blocking system over the northern hemisphere in Hurricane Katrina

Three violent category 5 Atlantic storms formed during the 2005 hurricane season. Hurricane Katrina initiated on 23 August and lasted until 31 August, with maximum surface winds reaching 150 knots and a minimum surface pressure of 902 mb on 28 August over the Atlantic Ocean at Lat. 26.3° N – Lon. 88.6° W. Figure 1 shows the daily GEOS satellite images for the tropical Atlantic region through the period of hurricane Katrina (22-31 August 2005), and illustrates the development stages of the hurricane. Hurricane Rita had a wind speed of 155 knots, a minimum surface pressure value of 897 mb, and lasted from 18 to 26 September. Hurricane Wilma had 160 knot surface wind speeds, a minimum surface pressure value of 882 mb, and lasted from 15 to 26 October 2005. The present work focuses mainly on hurricane Katrina. Figures 2-4 show the distribution of vector, zonal, and meridional surface winds over the tropical Atlantic region through the period of 22-31 August 2005. Figure 5 shows the daily distribution of the 500 mb geopotential height (m) composite mean in the northern hemisphere through that period. Analysis of the 10-day mean of the northern hemisphere geopotential height fields at the 500 mb level during August 2005 reveals that a diffluent block existed over Siberia through the last 10 days of August. The block over Siberia had maximum positive anomalies of +150 m in the north and minimum negative anomalies of -150 m in the south. During the last 10 days of August, a remarkable abnormal high pressure system existed over North America, with maximum anomalies of +200 m, which was accompanied by abnormal low pressure over the North Atlantic region with minimum anomalies of -200 m, as shown in Figure 6 and Table 1. Analysis of the daily mean of the northern hemisphere geopotential height anomalies at the 500 mb level for the period 22-31 August 2005 shows that the unusual pressure system over the northern hemisphere over Siberia, North America, and the North Atlantic persisted for 9 days (23 to 31 August). Table 2 illustrates the distribution of the geopotential height anomalies over the northern hemisphere through the period of study. The daily means of the anomalies of the meridional wind, zonal wind, and precipitation rate in the tropical Atlantic and America through the period of 22-31 August 2005 were analyzed. The results show that the tropical Atlantic region and America were below two abnormal types of zonal and meridional winds (Figures 3 and 4). In addition, a maximum anomaly of precipitation (+90 mm/day) occurred on 29 August. The correlation coefficients between the anomalies of geopotential height over the North Atlantic and the anomalies in the meteorological elements in the tropical Atlantic and America show significant correlations (+0.75 and +0.60) between the anomalies in the geopotential height at the 500 mb level over North America and Siberia, and the precipitation rate of the tropical Atlantic and eastern America, respectively, through the period of 22-31 August 2005. In addition, there is a significant negative correlation (-0.60) between the anomalies in the geopotential height at the 500 mb level over northern Siberia and the anomalies in the meridional wind over the tropical Atlantic region and eastern America, as shown in Table 4.

22 AUG 2005



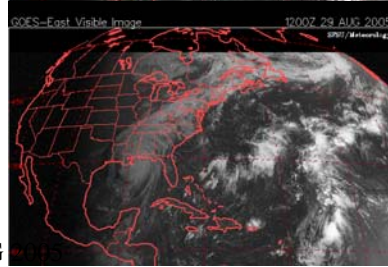
27 AUG 2005



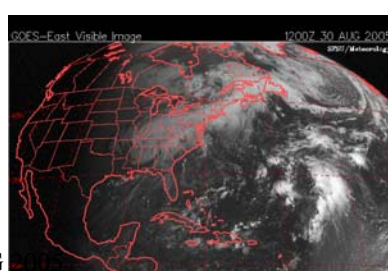
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29 AUG 2005



30 AUG 2005



31 AUG 2005



Figure (1): Daily GEOS satellite images for the tropical Atlantic region through the period of hurricane Katrina (22-31 August 2005).

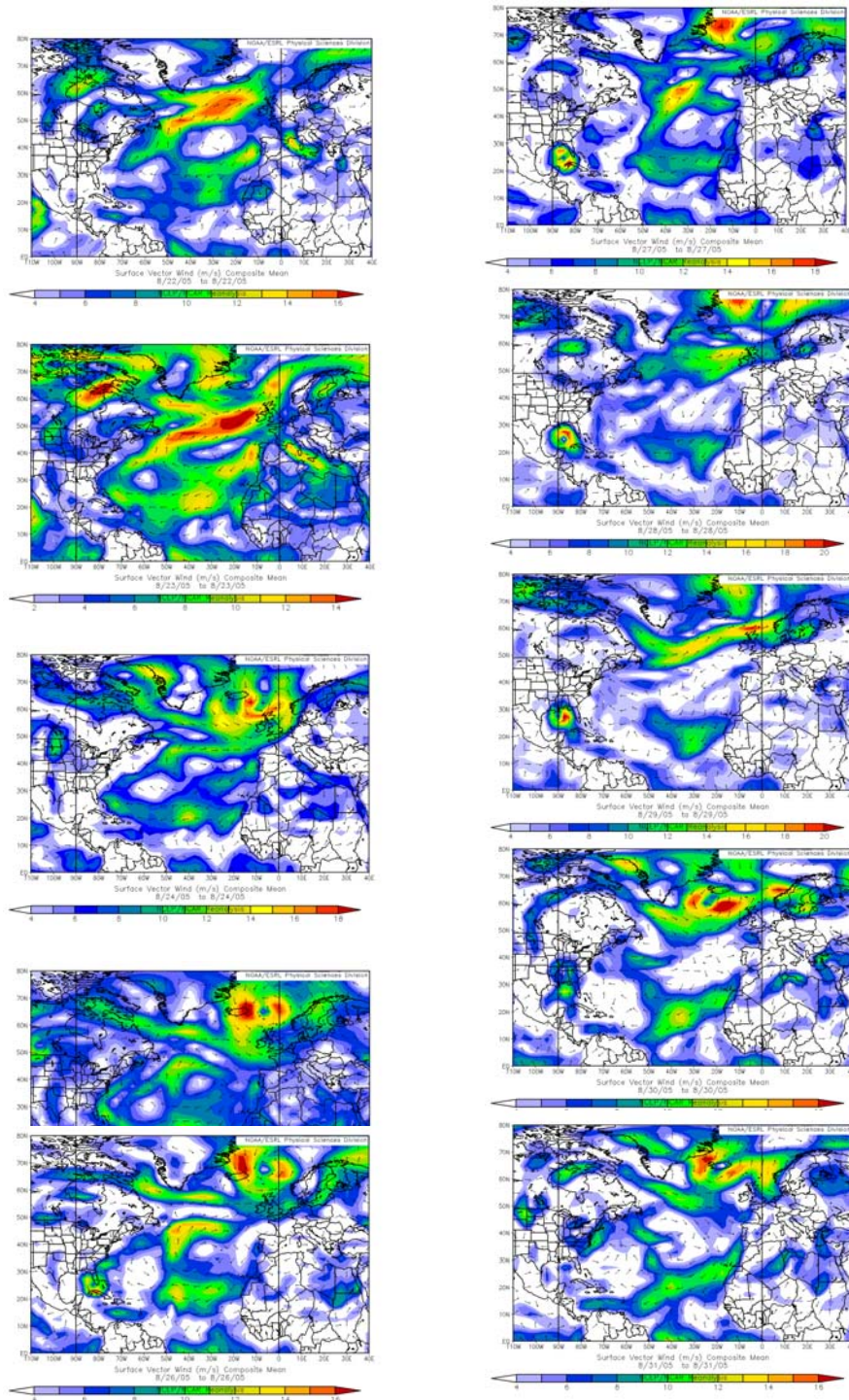


Figure (2): The daily distribution of surface wind vector (m/sec) composite mean in the North Atlantic region through the period of 22-31 August 2005.

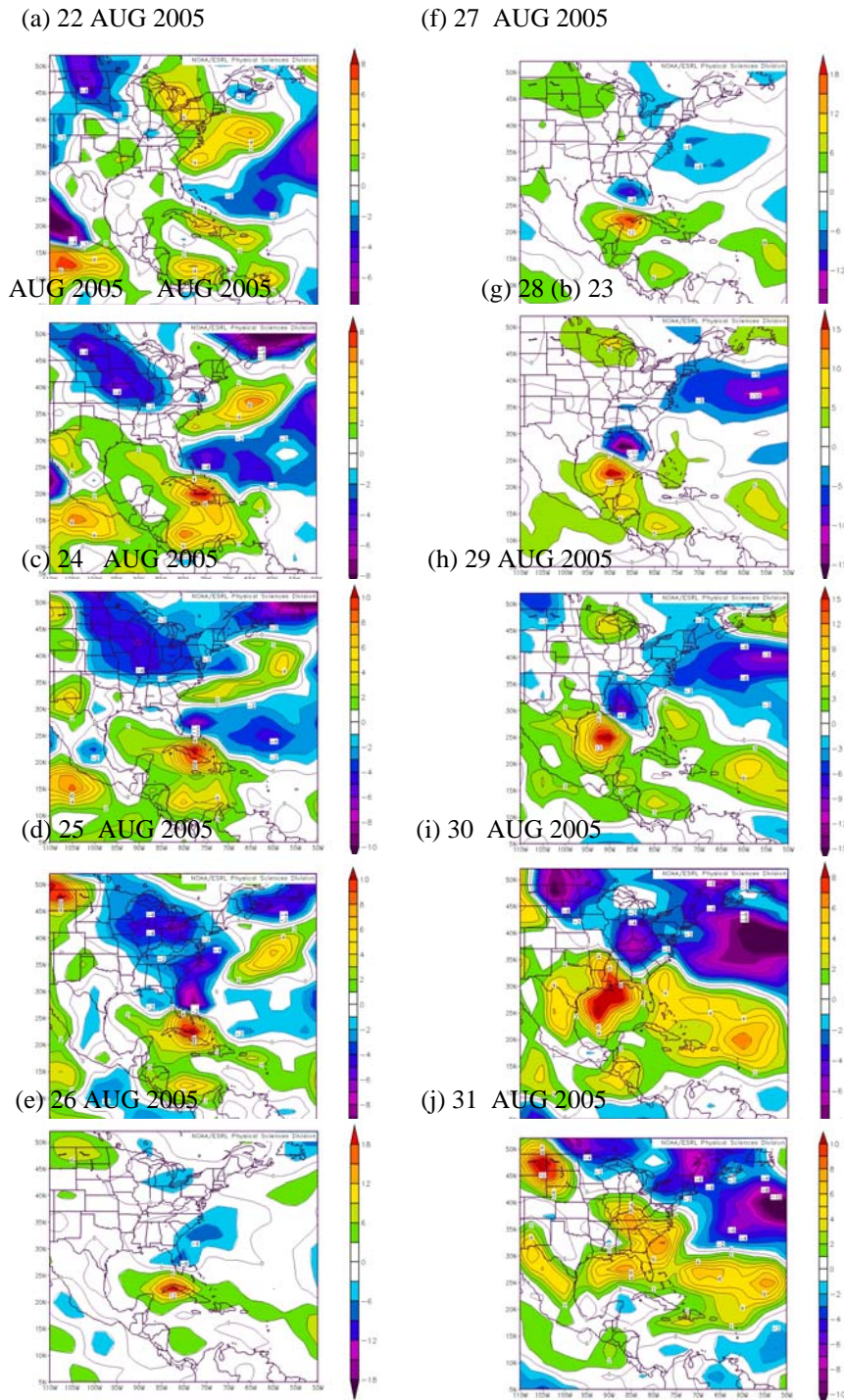


Figure 3: The daily distribution of surface zonal wind (m/sec) composite mean in the tropical Atlantic region through the period of 22-31 August 2005.

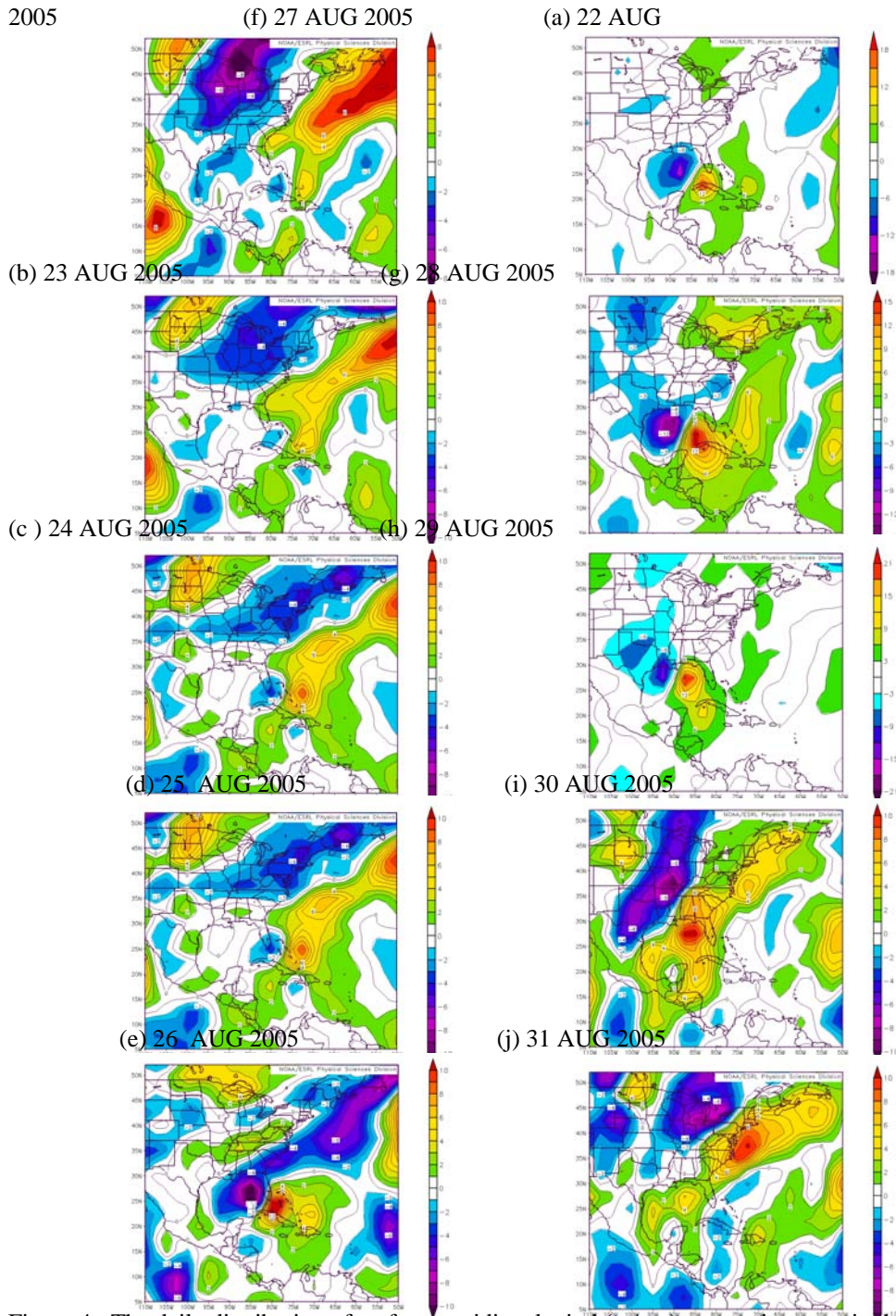


Figure 4: The daily distribution of surface meridional wind (m/sec) composite mean in the tropical Atlantic region through the period of 22-31 August 2005.

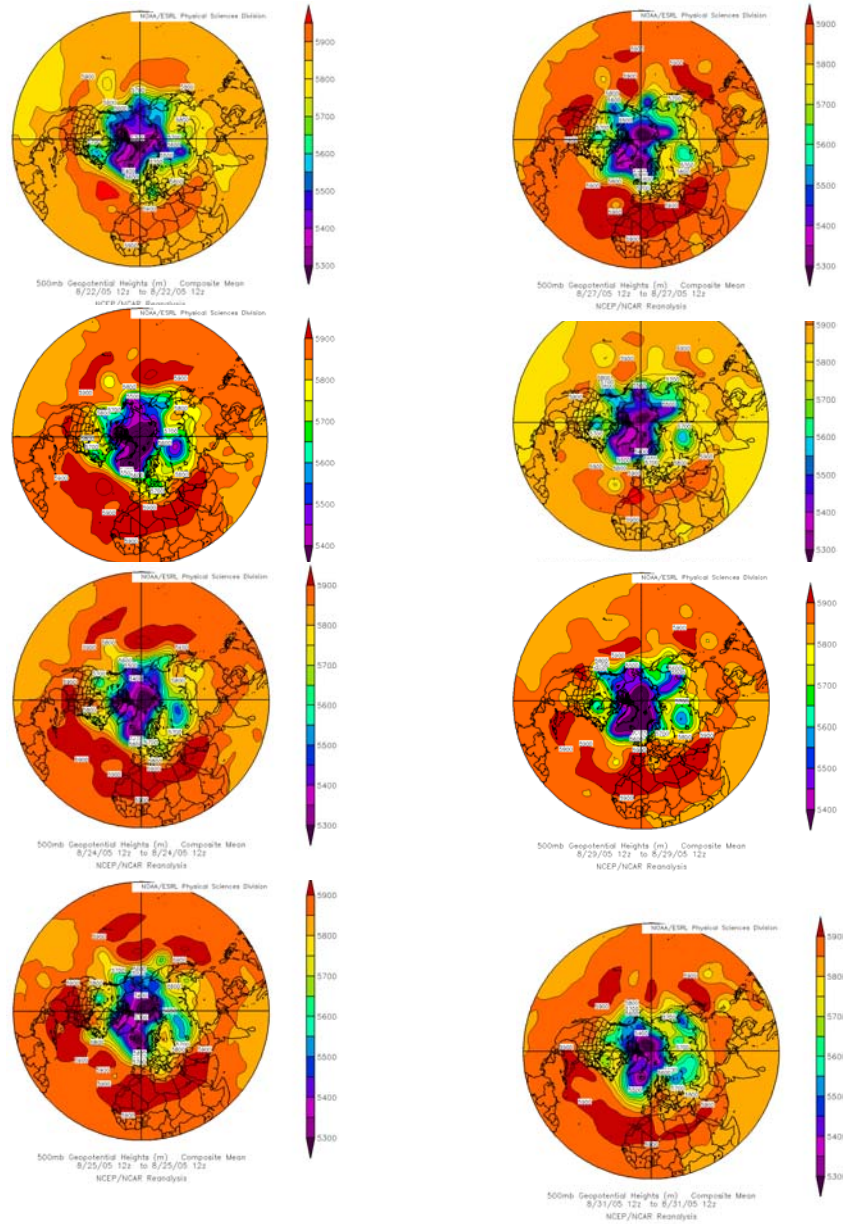


Figure 5: The daily distribution of 500 mb geopotential height (m) composite mean in the northern hemisphere through the period of 22-31 August 2005.

(a)

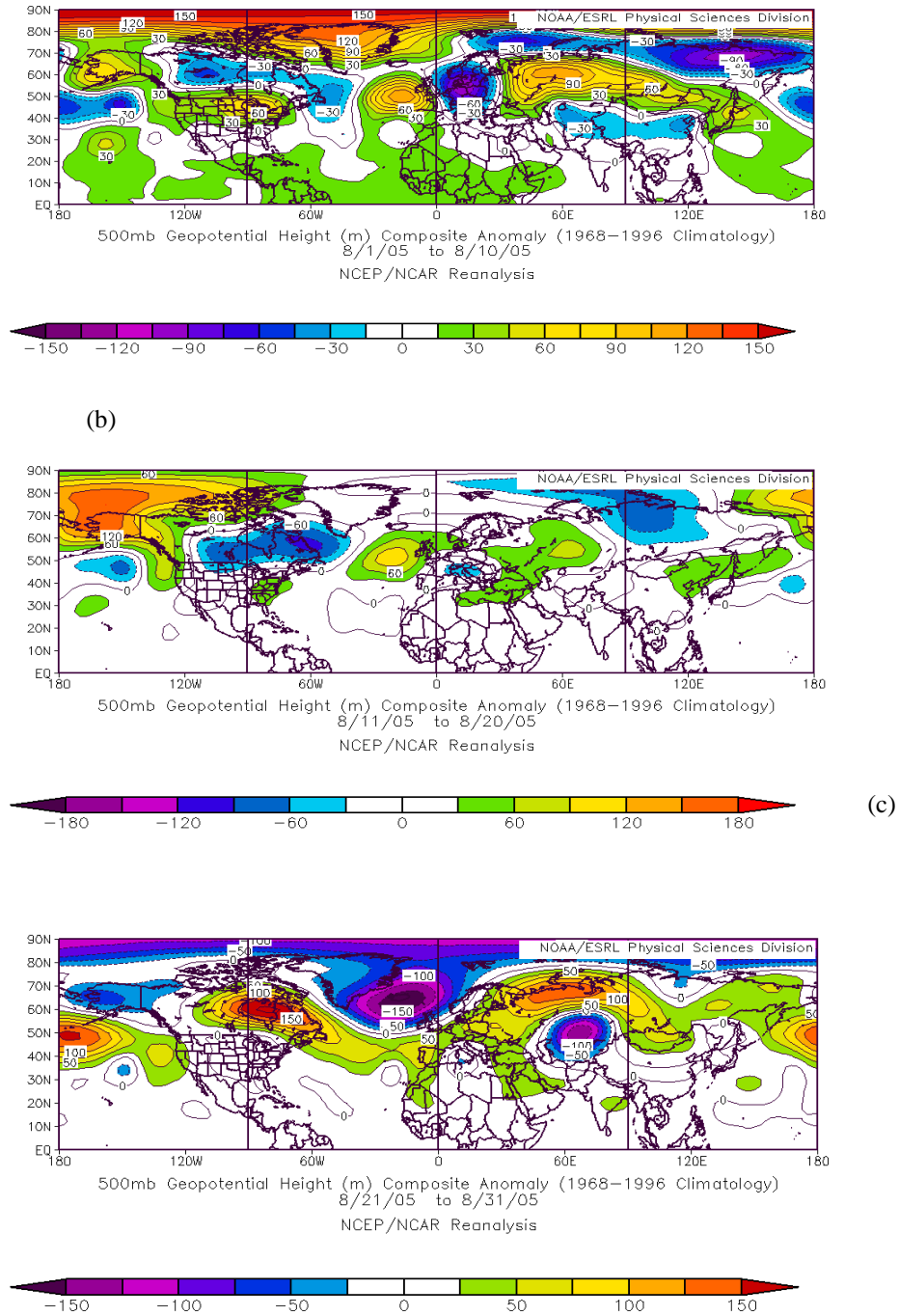


Figure 6: The 10-day distribution of the 500 mb geopotential height composite anomaly in the northern hemisphere for periods of (a) 1-10 August 2005, (b) 11-20 August 2005, and (c) 21-31 August 2005.

Table 1. The 10-day mean of the northern hemisphere geopotential height anomalies at the 500 mb level through the period of 22-31 August 2005.

Region	North America	North Atlantic	Europe	Siberia
Time period				
1- 10 August 2005	+60 m	+120 m	-90 m	+ 120 m
11-20 August 2005	-120	+120	+60 - 60	+90
21-31 August 2005	+200	-200	+100	+150 -150

Table 2. The daily mean of the northern hemisphere geopotential height anomalies at the 500 mb level through the period of 22-31 August 2005.

Region	North America	North Atlantic	Europe	Siberia
Duration Time				North South
22 August 2005	+150 m	-150 m	+150 m	+150 -150 m
23 August 2005	+150	-200	+150	-150 -150
24 August 2005	+200	-250	-50	+200 -150
25 August 2005	+175	-250	-50	+250 -200
26 August 2005	+200	-200	+50	+200 -200
27 August 2005	+175	-250	+100	+250 -200
28 August 2005	+225	-200	+100	+250 -150
29 August 2005	+250	-175	+150	+250 -175
30 August 2005	+250	-250	+150	+250 -150
31 August 2005	+250	-175	+150	+250 -150

Table 3. The daily mean of anomalies of the meridional wind, zonal wind, and precipitation in the tropical Atlantic and America through the period of 22-31 August 2005.

Anomalies Duration Time	Anomalies of meridional wind (m/sec)		Anomalies of Tropical Atlantic precipitation (mm/day)	Anomalies of zonal wind (m/sec)	
	Eastern America	North Atlantic		Eastern America	North Atlantic
22 August 2005	-8	+8	+15	+6	-6
23 August 2005	-4	+10	+15	-6	+8
24 August 2005	-4	+10	+25	-8	+10
25 August 2005	-8	+8	+30	-8	+10
26 August 2005	-10	+10	+50	-6	+18
27 August 2005	-15	+18	+60	-15	+18
28 August 2005	-15	+15	+60	-15	+15
29 August 2005	-15	+21	+90	-10	+15
30 August 2005	-10	+10	+55	-6	+8
31 August 2005	-8	+10	+50	+10	+10

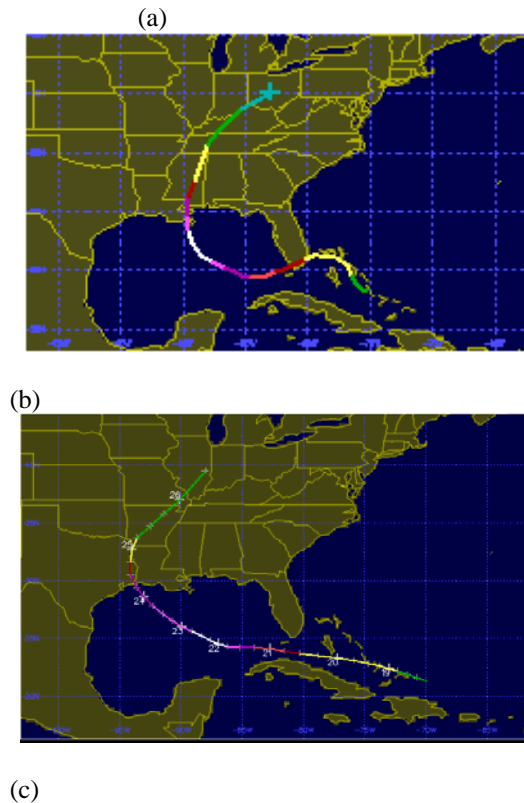
Table 4. The correlation coefficient matrix of the anomalies in the northern hemisphere geopotential height and the anomalies in meridional winds, zonal winds and precipitation in the tropical Atlantic region during the period of Hurricane Katrina.

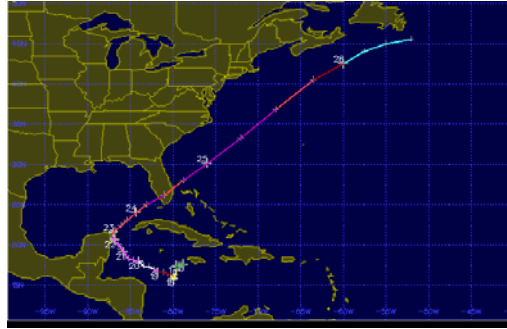
Correlation coefficient	Anomalies in the meteorological elements in regions of Tropical Atlantic (TA) and America					
	Anomalies in geopotential height of region	Meridional wind over TA and eastern America	Meridional wind over north America	Precipitation in the TA and eastern America	Zonal wind over TA and eastern America	Zonal wind over north America
	North America	-0.40	0.36	0.75*	0.01	0.38
	North Atlantic	-0.06	0.05	0.03	0.48	-0.39
	Europe	-0.30	0.29	0.28	0.30	-0.02
	North Siberia	-0.60*	0.28	0.60*	-0.01	0.03
	South Siberia	0.34	-0.19	-0.22	0.49	-0.53

(*): means of value with significant level > 95 %

3.2. Comparative study of Hurricanes Katrina, Rita, Wilma, and Andrew and the Blocking System in the Northern Atmosphere

To investigate the above described role of blocking systems over the northern hemisphere on hurricane Katrina, a comparative study among four large hurricanes in the tropical Atlantic region that made landfall, Katrina, Rita, Wilma, and Andrew, was performed. Figure 7 and Table 5 show the tracks, landfall locations, and characteristics of the hurricanes. In addition, the blocking systems over the northern hemisphere were characterized by studying the anomalies of the geopotential height at the 500 hpa level for the periods of each hurricane (Figure 8 and Table 5). The results of this comparative study revealed that during each of the five hurricanes there was a dominant diffluent block over Siberia. These blocks were simultaneously associated with strong westerly air currents over North America. Meanwhile, the northern Atlantic had extreme negative anomalies in the geopotential height values. Comparison of the anomalies at the 500 hpa level shows nearly similar values for hurricanes Katrina and Rita, as is clear from Table 5. For hurricane Wilma, the blocking system was over all of Europe, including Siberia, and the splitting of the westerly air currents occurred over the northeastern coast of the North America, with +200 m anomalies in the 500 hpa geopotential height level. For hurricane Andrew, the splitting of the westerlies occurred over the North Atlantic region, with an extreme in the westerly air current over North America with a mean value of +80 m at the 500 hpa level. Siberia had a diffluent block, with mean values of +100 and -90 m over northern and southern Siberia, respectively. The main feature through the period of hurricane Andrew was an extreme negative anomaly of geopotential height of -200 m over the northern Atlantic region, as is shown in Figure 8 and Table 5.





(d)

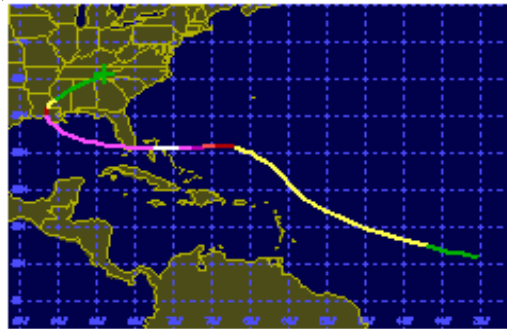


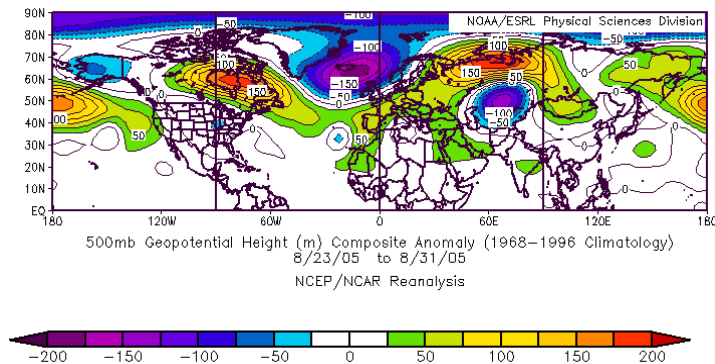
Figure 7: The tracks of the Atlantic hurricanes Katrina, Rita, Wilma, and Andrew.

Table (5): Characteristics of hurricanes Katrina, Rita, Wilma, and Andrew, and the blocking systems over the northern Hemisphere through the duration of each hurricane.

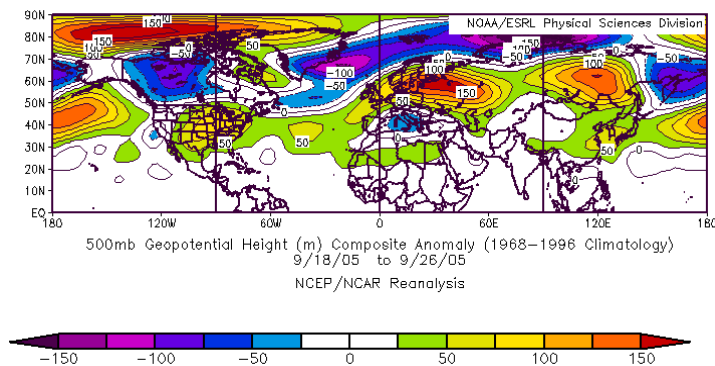
Characteristics of Atlantic Hurricanes and northern blockings	Hurricane characteristics				Blocking over the northern hemisphere during the hurricane period		
	Duration	Category	Maximum wind speed (Knot)	Landfall region	No. of days of blocking over the northern hemisphere	Maximum mean positive anomalies in 500 mb level at northern hemisphere Value and location	Minimum mean negative anomalies in 500 mb level at northern hemisphere Value and location
Name of Hurricane							
Katrina	23-31 AUG 2005	5	150	Louisiana	9 days	+200 (m) over North America, +75 (m) over Europe, +200 (m) over north	-200 (m) over North Atlantic, -150 (m) over south Siberia

						Siberia	
Rita	18-26 AUG 2005	5	155	Texas/ Louisiana	8 days (19- 26)	+150 (m) over North America, +100 (m) over Europe, +150 (m) over north Siberia	-125(m) over North Atlantic, -50 (m) over south Siberia
Wilma	15-26 OCT 2005	5	160	Florida	9 days (18-26)	+200 (m) over North America, + 75 (m) over Europe, +100 (m) over north Siberia	-150 (m) over North Atlantic, -50 (m) over south Siberia
Andrew	16-28 AUG 1992	5	150	Florida, Texas, Louisiana	7 days (22-28)	+80 (m) over North America, +100 (m) over Europe, +100 (m) over north Siberia	-200 (m) over North Atlantic, -90 (m) over south Siberia

(a)



(b)



(c)

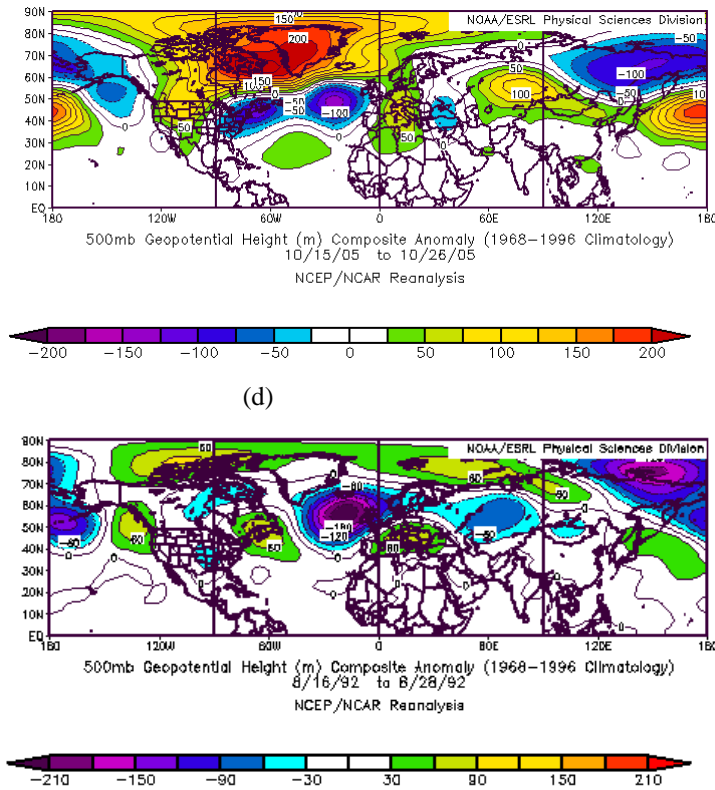


Figure 8: The distribution of the 500 mb geopotential height (m) composites anomaly in the northern hemisphere. (a) for Hurricane Katrina 23-31 Aug., 2005. (b) for Hurricane Rita 18-26 Sep., 2005. (c) for Hurricane Wilma 15-26 Oct., 2005, and (d) for Hurricane Andrew 16-28 Aug., 1992.

5. Discussion and Conclusion

The diffluent block system that persisted over Siberia prevented the large westerly air currents aloft that existed over North America from crossing the Atlantic Ocean toward Europe. The air current persisted over North America. This persistence of the two pressure systems over Siberia and North America generated a splitting of the westerly air currents over the Atlantic Ocean into two distinct branches. The first branch moved northward towards the northern Atlantic Ocean, while the second went southward and generated abnormal northeast to east air currents over the tropical Atlantic region. The Gulf of Mexico was thus under the influence of two abnormally strong winds. First, there existed strong east-northeast winds in the tropical Atlantic. Second, there were strong south winds in the southeastern part of North America. These two winds put the Gulf of Mexico under the torque force of winds, which accelerated the circulation of hurricane Katrina, controlling its track through the Gulf of Mexico and changing the track from south to the north toward land, leading to landfall in Gulfport. The results revealed that there are significant correlations between the anomalies in the geopotential height at the 500 mb level over North America and Siberia, and the precipitation rates in the tropical Atlantic and eastern North America. In addition, there is a significant correlation between the anomalies in the geopotential height at the 500 mb level over northern Siberia and the anomalies in the meridional winds over the tropical Atlantic region and eastern part of North America. One can conclude that the blocking systems over the northern hemisphere through the period of 22-31 August 2005 controlled the power and track of hurricane Katrina.

A comparative study of four large hurricanes in the tropical Atlantic region, which all made landfall (Katrina, Rita, Wilma and Andrew), was performed. All four hurricanes caused widespread damage. The results from this study show that during each hurricane, a diffluent block existed over Siberia, accompanied by strong westerly air currents aloft above North America. The splitting of the westerly air currents that

occurred over the northern Atlantic region was dominant. From the present study, one can conclude that the strong westerly air current aloft over North America *must have been prevented* from crossing the Atlantic ocean and were blocked by the diffluent block over Europe or Siberia, which controlled and forced the tracks of the tropical Atlantic hurricanes towards landfall in the southeastern USA by adding abnormal northeast–east air currents to the tropical Atlantic region.

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Anthropogenic Impacts on Protected Area of Burundi. Case Study of Ruvubu National Park

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Abstract: Forty six plots have been conducted in Ruvubu national park along the Ruvubu River in Karuzi province to determine the effects of anthropogenic impacts on the forest. Thirty six of these quadrats were laid out in steep gully hill along the south west of the park. Ten plots were outside the protected area. The Ruvubu National Park vegetation includes a complex of forest and woodland, savannah shrub, grasslands and wetlands. Overall, forest area in the Ruvubu National Park significantly impacted by three gap-forming disturbances: logging (80%), tree harvesting (13.3%), and cultivation (6.3%). Forest disturbance was greater outside the reserve (48.3%) than inside (12.2%) reiterating the significant role played by this protected area in habitat and species conservation. Two species diversity indices were calculated: Shannon-wiener's, and Evenness index E. The results revealed that Shannon's index and Evenness were the best to explain the observed differences in the structure of the forest subjected to uneven levels of disturbance. [The Journal of American Science. 2008; 4(2):26-33]. (ISSN 1545-1003).

Keywords: Anthropogenic Impact, Protected Area, Ruvubu National Park, Burundi

1. Introduction

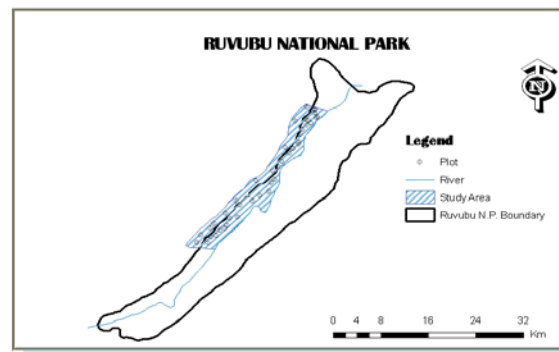
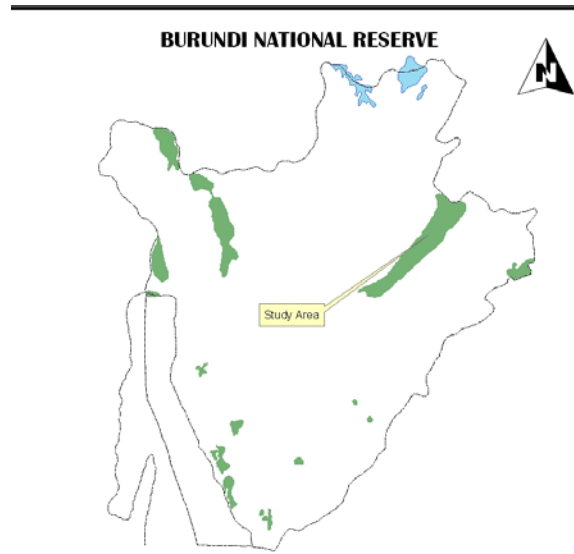
In recent years ecologists have turned their attention towards the loss of biodiversity, particularly in tropical forests around the equator where these hotspots are concentrated (Myers et al. 2000; Beck et al. 2002). Deforestation of tropical forests not only jeopardizes biological diversity but also climate systems of the world (Myers 1989; Schwartzman et al. 2000). In addition to high species diversity and endemism, tropical forests are also home to rural communities in need of economic sustainability. Conservation of tropical forest is thus one of the greatest human challenges involving delicate balance between complex-fragile ecosystems, and impoverished populations. Consequently, shifting cultivation remains the biggest threat to tropical forests (Myers 1987) and has exacerbated the natural fragmentation of landscapes affecting whole ecosystems and biota (Brender et al. 1998). Natural ecosystems in Burundi include the forests, savannas, woodlands, lowland prairies, and marshes and other aquatic settings. Burundi has 14 protected areas with a total surface area of approximately 127,662.85ha, 4.6% of the total country (MINATTE, 2000). Burundi's protected areas include several vegetation types, including some that practically do not exist outside of these defended areas. The biggest causes of biodiversity degradation are agricultural land-clearing and other poorly adapted farming methods. The Ruvubu forest park, which constitutes the study area of this paper, is a protected area which lies in the sub-humid agro-ecological zone with abimodal rainfall, the long rains from late February to May /June followed by short rains. This ecosystem remains, however, under severe threats due to many unsustainable practices maintained by the local people. These forests have been subjected to increasing destruction of forest cover due to clear cutting, burning and slashing mainly for agriculture as well as forest deterioration due to harvesting and utilization of different forest products (Deckers 1994; Medley 1993). Habitat loss, forest fires, logging, hunting for bush meat, war and the capture of live infants for sale have all contributed to this decline (Marshall et al. 2000). We examined the impacts of human activities on forest patches in and out of the Ruvubu forest park. Herein, we discuss the implication of these impacts on conservation and management of the forest ecosystems

2. Materials And Methods

2.1. Study Area

Ruvubu National Reserve is 193 square miles (500 km²) in size and covers a strip of land from one to six miles (1.5-10 km) wide along both sides of the Ruvubu River in eastern Burundi. It was freed from human inhabitants and returned to complete wild life. Wildlife in the Ruvubu basin and Pare National

de la Ruvubu includes hippo, crocs, buffalo, leopard, antelope, monkeys and some lion. More than 425 bird species have been recorded.



2.2 Data Collection

The selection of sampling points for the study areas were based on gradient-directed transects "Gradsect". This sampling approach used intensively at Chino State park of California Department of Parks and Recreation Inventory, Monitoring and Assessment Program (IMAP, 1996), and in conservation site selection in Australia (Austin and Heyliger, 1989; 1991). At each plot of the sampled location, a transect quadrat of 30m x 30m was completed. All information concerning the biological diversity and of the park were collected and evaluated for further use. The species diversity of all plot sites investigated were analyzed and compared with each other. The simplest method to determine species diversity is to count the number of species in the community (the species richness) (McIntosh, 1967). Species diversity is not only a measure for the number of species; diversity is also expressed in Evenness. The mean species percent cover

was calculated for the different disturbance groups, and diversity was quantified by mean of trees indices: Species richness(S) (Magurran, 1988), Shannon's diversity index (H) (Shannon, 1949) and Simpson's

diversity index (D) (Simpson, 1949). Shannon's index is calculated as follows: $H = \sum_{i=1}^s p_i \log p_i$ (1)

Where p_i is the relative abundance of species i . Simpson's(D) ,a diversity index heavily weighted towards the most abundant species in the sample while being less sensitive to species richness ,is calculated

$$as: D = \sum_{i=1}^s p_i^2, \sum_{i=1}^s \frac{n_i(n_i-1)}{N(N-1)} ; \quad (2)$$

Where n_i is the number of individuals in the i^{th} species and N the total number of individuals. As D increases, diversity decreases and therefore Simpson's index is usually expressed as 1-D or 1/

$$D, \quad D = \sum_{i=2}^s p_i^2 \quad (3)$$

In this study, the former expression (1-D or 1/ D) will be used.

Two indices of Evenness were used: Pielou's (J) (Pielou, 1969, 1975) and Simpson's (E)

$$J = \frac{H(s)}{H_{(max.)}} , \quad (4)$$

Where H(s) = the Shannon-Wiener information H (max.) = the theoretical maximum value for H(s) if all species in the sample were equally abundant. The Simpson's Evenness index (E) is calculated as the relation between the value of the Simpson's diversity index for the sampled site D and the maximum possible value of the index for given species number and sample site D Max (Pett,1974) ;then:

$$E = \frac{D}{D_{(max)}} , D_{(max)} = \frac{S-1}{S} \frac{N}{N-1} \quad (5)$$

Where S is the number of species and N the number of individuals.

The indices were calculated for all plants, Growth forms (trees, shrub, and herbs) and vertical layers with each plot as previously reported (Mac Arthur, 1965).

2.2 Human impacts Classification

Human activities were categorized as follows:

(1) Resource utilization is defined as human practices that do not necessarily result in partial/complete forest cover removal but resulted into deterioration of forest stature. These activities included:

Tree harvesting, which included cutting plant parts for various human utilizations such as thatching, wine tapping, constructions of animal traps and sometimes firewood collection. Thatching and wine tapping involved the chopping off the crowns of trees and tapping of the sap, respectively. Animal trapping involved the use of snares. Firewood collection involved gathering dried twigs and to a lesser extent cutting young stems and branches. Logging, which includes cutting trees for construction of canoes, furniture, building materials and charcoal burning, Charcoal burning involved burning of felled logs under earth mounds from various tree species.

(2) Land use practices are defined as human activities that resulted to partial or complete removal of forest canopy cover. These were identified as follows:

Cultivation entailed the complete or partial clearances of areas of forest for agriculture through slash and burn techniques, which affected all species. This practice sometimes also causes fragmentation of the affected forest patches.

2.3 Forest Classification

Data collected from human was collated and used to provide overall assessment of the status of forests surveyed. Disturbance levels were categorized as detailed by Muoria et al. (2002) from level 1 to 4.

3. Results And Discussion

3.1 Forest status

The Table 1 describes the main human activities observed in the forty-six quadrats sampled. In general, Logging was observed in 36 plots and accounted for 80% of human activities, tree harvesting in 6 quadrats or 13.3%, and cultivation in 3 plots (6.6%). Furniture was constructed from *Spyrostachys venenifera*, while building materials were obtained largely from *Phoenix reclinata*. From the observation, cultivation had the most devastating effects on forest cover due to partial or complete vegetation clearance. The most affected species due to tree harvesting were *Borassus aethiopiun*, *Phoenix reclinata* and *Hyphaene compressa*. While the most preferred tree species for construction of canoes and beehives were *Diospyros kabuyeana*, *Ficus sycomorus*, *Mimusops fruticosa* and *Mangifera indica*. Thirty-six plots impacted by logging were those from inside the park (numbers 11,..., 36). The remaining plots affected by other factors (harvesting, cultivation) were found outside the Park. Ten forest patches severely impacted by cultivation alone were those plotted outside the protected area (no. 1,2,3,4,5,6,7,8,9,10),. Three plots, (numbers 42, 45 and 46, respectively) were heavily impacted by human activities. Six forest transects affected by both tree harvesting and cultivation were located (no. 3, 4, 5, 6, 7, 8, 9) near the access facilities. Along the river channel, only one forest plot (no. 35), was affected by tree harvesting.

Four other forest patches affected by logging are near the current river channel (Table 1). They include all plot surveyed in the protected area (Table 1). Forests impacted by all kind of disturbance factors include all plots with the difference degree of severity.

Out of the forty-six forest plots evaluated, 12 plots had little or no disturbance while 12 plots were heavily disturbed. Of the heavily impacted forests, six were in the reserve and six outside the protected area (Table 2). Seventeen plots were found moderately impacted by human activities; among them fifteen are from the protected area while two plots are from outside (Table 2). Only five out of all have obvious impacts of human without being totally cleared. They are partly from both outside and in the protected forest (Table

Table 1. Frequencies and proportional occurrence of categorized human activities.

Activities	Frequency	%
Logging	36	80
Tree harvesting	6	13.3
Cultivation	3	6.6
total	45	100

Table 2 Severity levels of disturbance on the forest using a scale of 1-4.

Destruction levels	Forest inside the park			Forest outside of the park		
	Numbers of plots	Area	%	Numbers	Area	%
1	12	108	32.3	0	0	0
2	15	135	40.4	2	16	19.7
3	3	27	8	2	16	19.7
4	6	54	16.1	6	54	60
Total	36	334	100	10	90	100

Scale 1: little or no destruction; scale 2: moderate levels of destruction; scale 3: extensive human destruction with no section of forest completely cleared; scale 4: highest levels of destruction with sections of the forest completely cleared.

3.2 Human Activities and Natural Impacts on the Riverine Forests along the Ruvubu River.

Our study has shown that through shifting cultivation, logging and other human activities are still impacting the Ruvubu National park even if it is placed under protection laws and lead to loss of biodiversity in the forest. Anthropogenic activities out the forests are actively practiced in the form of slash-burn agriculture, selective logging and several other deleterious uses of forests (Table 1). Shifting cultivation combined with some natural impacts contributed to the alarming loss of endemic and threatened species in the forest. The most impacted forest, portion laid out from the edge of the protected area south to the north (no. 1 to 10), was affected by both cultivation and logging with high level of destruction (table 2) and affects 86.6% of the total area (table 1) the significant role played by this protected area in habitat and species. The impact of tree harvesting and natural impacts on the forests inside the Ruvubu National park is enormous. Changes caused by both tree harvesting and natural impacts outside the Ruvubu National park forests do not necessary immediately remove forest cover; instead they are more likely to cause progressive degradation of forest structure and biodiversity. In the long-term, this progressive degradation leads to partial or complete loss of forest cover. One important aspect that was not evaluated during this study was the loss of mature forest due to bank erosion. This type of evaluation would necessitate long-term monitoring of these potential sites, which was beyond the scope of this research study. Future studies should incorporate the impact of bank erosion and evaluating its role as a natural impact on the forests. As a whole, the combinative impact of cultivation and natural dieback or cultivation has resulted in the highest percentage forest area loss in the Ruvubu National park. Both human and natural impacts are responsible for changes in forest cover and forest stature. As this study has indicated, areas that have experienced significant area loss due to the Ruvubu National park dynamism could be significantly related to changes in human activities, which further complicate current and potential conservation and management strategies in and out of the reserve. Human exploitation of forest resources can involve rapid, non-sustainable harvesting of particular species (Gentry and Va'squez 1988), while Natural impacts(flooding ,dieback) can result in a progressive degradation of forest structure and biodiversity that leaves behind standing but biologically and economically depleted forests. The riverine habitats on the Ruvubu National park are highly vulnerable to perturbations due to the Ruvubu National park dynamism and the continual human overexploitation.

The riverine forests within the protected area represent 84% of the forest ecosystem and may thus be inadequate to provide resources to stem the current decline in endangered primate populations. However, the importance of the unprotected forest patches situated outside the reserve for the survival of both endangered species cannot be overemphasized. The survival of these species depends on the future management and conservation of the majority of forest patches that are situated out of the reserve. The fact

that the greatest area of forest loss was outside the reserve implies the immediate need to initiate conservation programs outside the protected area. Forest fragmentation not only isolates floral and faunal population but it also impedes gene flow between forest patches (Marsh et al. 1987). This study demonstrates that the effects of natural impacts are just as important, and therefore, future studies should not only examine the long-term effects natural impacts on the endangered species, but also study the combinative effects of both natural and human impacts on these species.

Table 3 Shannon -Wiener index (Hs) and Evenness (E) for twenty pairs plots.

Plot	Latitude	Longitude	Hs	E
1	-3.213	30.261	3.728	0.889
2	-3.2	30.267	3.08	0.854
3	-3.189	30.282	3.231	0.857
4	-3.181	30.287	3.23	0.859
5	-3.164	30.305	3.45	0.824
6	-3.144	30.321	3.24	0.834
7	-3.11	30.351	3.45	0.768
8	-3.0555	30.395	3.67	0.823
9	-3.012	30.41	3.57	0.813
10	-2.989	30.427	3.339	0.834
11	-3.212	30.278	3.267	0.822
12	-3.203	30.283	3.278	0.824
13	-3.201	30.291	3.275	0.825
14	-3.192	30.296	3.24	0.828
15	-3.189	30.303	3.53	0.854
16	-3.184	30.307	3.78	0.8957
17	-3.173	30.311	3.37	0.823
18	-3.171	30.323	3.89	0.831
19	-3.15	30.326	3.78	0.832
20	-3.144	30.345	3.78	0.849

3.3 Anthropogenic Impacts on the Species Diversity

In this paper ,we have also conducted a comparison of species composition between the different plots surveyed .The aim was to understand the composition of the forest by taking example of 20 plots .We analyzed if and in which way the tree structure of Ruvubu National park was influenced by different human activities like logging, harvesting tree and cultivation. Species within habitat diversity was measured with the Shannon-Wiener function and evenness .Table 3 present the analysis values for 20 plots studied .High diversity was recorded in the disturbed forest (outside the protected area) The higher the disturbance and the younger a forest site is, the more divers is the habitat (Scully 2001).Undisturbed or little disturbed inside the protected area of the forest have the lowest measured diversity. The evenness values are higher in than in more disturbed forest of the park. in these plots more dominant species occur beside only few rare species . The more equal a distribution of species in a given habitat and the higher an evenness value, the more species with similar abundance occur. Thus, the older secondary forest sites have lower evenness values dues to the occurrence of some dominant species, like trees in upper canopy, and a few species in low coverage or abundances in the understory vegetation. The Shannon-Wiener index can also be expressed in units of species number. In the following diagram the measured values were applied against a disturbance gradient (Figure 4). Human disturbance like selective logging or cultivation of the park seems to have an influence on the plant species diversity. After disturbance, a habitat is more heterogeneous because of small, sunny gaps beside dense forest, different microclimate conditions in a near distance, etc. These heterogeneous environments offer diverse possibilities for high amount of different species. The

theories that moderate disturbances promote species diversity are also supported by several authors and by the present study (Gentry 1982, Collins et al. 1995, Hiura 1995, Laurence et al. 2001). In sum, it can be stated that the Simpson's diversity and evenness indices are the best measurement to determine disturbance levels in forest functioning ecosystems as previously suggested (Franklin et al. 2002).

4. Conclusions

The research findings allow us to summarize the main points as follows: The pattern of forest disturbance in Ruvubu National park is dominated by logging, cultivation and tree harvesting. Comparison between all gap-forming disturbances in the forest, cultivation is the most source of disturbance in terms of the area affected. Research results also demonstrate that the tree species indices (Shannon-Wiener's H', Simpson's index d' and Evenness) of forest decrease in the order: outer to inner the forest. It has shown also that there are variations in tree species diversity between different plots of the same location, especially when taken from the ecotone or near the edges of forest.

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Feeding behaviour of wild Asian Elephants (*Elephas maximus*) in the Rajaji National Park

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Abstract: The Asian elephant's (*Elephas maximus*) feeding behaviour with food preferences was studied in Rajaji National Park area between 1999-2006. The major objective of the present study is to document the fodder plant species and their seasonal consumption by elephants. Though elephants consume a variety of plant species in the study area, but their diet mainly consisted of fifty (50) plant species, which are available to them alternately round the year. Alteration between a predominantly browse diet throughout the year with a grass diet during the early dry season was related to the seasonally changing mineral content of grasses. Consumption of tree species (74%) was highest as compared to grasses (14%) and shrubs (8%) but their diet was mainly dependent on availability of seasonal food round the year and on their migration. Elephants extensively feed on *Mallotus philippinensis*, *Acacia catechu*, *Lagerstroemia parviflora*, *Ehretia laevis*, *Dalbergia sissoo*, *Tectona grandis*, *Zizyphus mauritiana*, *Aegle marmelos* and *Ficus bengalensis* besides, elephants also utilized various grasses and shrubs as their food, which mainly included *Dendrocalamus strictus*, *Helicteres isora*, *Saccharum munja*, *Saccharum spontaneum*, *Cynodon dactylon*, *Desmostachya bipinnata* and *Neyraudia arundinacea*. Elephants sometimes spent long time to feed on some particular plant species like *Dendrocalamus strictus*, *Mallotus philippinensis* and *Tectona grandis*. Eastern populations of elephants were subjected to feed extremely on *Tectona grandis* and *Holopternites* spp. whereas currently south-western populations of elephants were not utilizing these species as their food. Crop raiding, which was sporadic during the wet season, gradually increased with more area being cultivated with the onset of monsoon. We propose that this is the first documented study, which has developed a database about the fodder plant species for Asian elephant's survival in north-west India. [The Journal of American Science. 2008;4(2):34-48]. (ISSN 1545-1003).

Keywords: Asian elephant, *Elephas maximus*, feeding behaviour, Rajaji National Park.

Introduction

The Shivalik foothills are one of the world's most spectacular landscapes, encompassing the tall grasslands and the *Shorea robusta* (Sal) forests. This entire belt is a natural home to Asian elephants (*Elephas maximus*) besides many other wild animals like *Panthera tigris* (tiger), *Panthera pardus* (leopard), *Melursus ursinus* (Sloth bear), *Hyaena hyaena* (Hyaena), *Muntiacus muntjak* (Barking deer), *Axis axis* (Spotted deer), *Cervus unicolor* (Sambhar), *Sus scrofa* (Wild boar), *Ophiophagus hannah* (King cobra) etc. This protected area is the western most limit of Asian elephant, tiger and king cobra. The Shivalik landscape is one of the last few places in the world where elephants exist and the region offers an urgent need for conservation. This protected area in India's lesser Himalayan region falls under sub tropical moist deciduous forest type with extensive stands of *Shorea robusta* (Sal), *Mallotus philippinensis* (Rohini), *Acacia catechu* (Khair), *Adina cordifolia* (Haldu), *Terminalia bellirica* (Bahera), *Ficus bengalensis* (Bar), *Dalbergia sissoo* (Shisham) etc. in its premises besides many other important fodder plant species. From conservation point of view it appears to be India's one of the most successful national park and its management has helped to boost the population of Asian elephant in their natural habitat (Figure 1).

During the recent past extensive lopping and collection of fuelwood by Gujjar (nomadic community) and local people has restricted the regeneration potential of many important fodder plant species. Besides, elephant caused damages has also acted as a barrier to some extent in management related practices. The human population around the Rajaji National Park has doubled during past one decade and

rapid urbanization and industrialization has resulted in the loss of many forestlands to townships and thereby increasing the major problem during the recent past.

Since Independence, forest were cleared and felled and brought under the plough on a large scale. Construction work along with developmental activities like establishment of hydro-electric power plants, irrigation canals and national highways entailed deforestation of large tracts and colonization brought in its wake have resulted in a significant shrinkage in the habitat of wild animals (Singh, 1969). Presently most of the elephant habitats are destructed by various developmental activities or for human needs. There has been a rise in the competition among the same species for the food, shelter and other basic requirements. The status of the elephant in the adjoining countries is equally poor. Nepal, which has the lowest country population, has lost over 80% of its elephant habitat on account of human settlements. Bangladesh, Myanmar, Cambodia, Vietnam, Laos and Sri Lanka are also losing rapidly the natural forest cover, specially the elephant habitats. In Thailand, in spite of the elephant having been a protected species since the 18th century, over exploitation of the habitat and the pressure of human population has made the species highly vulnerable (Daniel, 1996).

The most charismatic among the wild animals of south-east Asia is probably the elephant in India, but somehow this giant Proboscidian is only restricted to only a few of the protected areas. Rapid developmental activities along with the encroachment into the deeper forest regime have made them to survive less, basically due to loss of their natural habitats. On account of their rapidly declining natural habitat and shrinking of migratory routes and feeding grounds the elephant population often scumb to various modes of unnatural deaths like train accidents, electrocution deaths and road accidents, and as a result, their population is falling rapidly. In this situation there is a need for some applied action oriented research studies, which may provide database about ground facts, that will be useful in achieving the goal of biological diversity conservation especially in conserving wildlife species that are categorized under endangered category. The major objective of the present study was to document the fodder plant species and their seasonal consumption by elephants. The study is a part of our long term study on the behavioural biology of Asian elephant in sub tropical moist deciduous forests of India.

Methods

Study area

Rajaji National Park [29° 15' to 30° 31' North Latitude, 77° 52' to 78° 22' East Longitude] is spread over an area of 820.42 Km² in and around the Shivalik foothills, which lies in the lesser Himalayas and the upper Gangetic plains (Figure 2). Spread across Hardwar, Dehradun and Pauri districts of Uttarakhand state, Rajaji National Park (RNP) has been designated as a reserved area for the "Project Elephant" by the Ministry of Environment and Forests, Government of India with the major aim of maintaining the viable population of Asian elephants in their natural habitat. The Shivalik foothills offer the most prominent geomorphic features of this tract. The river Ganges cuts across these hills at Hardwar. The Chilla forest area of the RNP lies to the east of the river Ganges and is attached with the Garhwal Forest Division. The study is ongoing in Hardwar (District-Hardwar), Chilla (District-Pauri) and Motichur (District-Dehradun) forest ranges of the RNP. The altitude lies between 302-1000 m asl. The study site falls in sub-tropical moist deciduous forest type.

Data collection

For studying the feeding behaviour of elephants, the study areas were surveyed in depth for about eight years. All plants on which elephants were observed to feed in the study area were identified either through the flora dictionary or by the help of subject experts (herbarium identification). Some plant species were well-known to us. The majority of plants were collected after observing an elephant feeding on a plant then waiting until the animal had moved away. Besides, elephant's traditional movement tracks along with feeding grounds were searched and observed directly. Different forest blocks of concerned forest ranges were chosen one after another sequentially and searched for elephants for about 10 – 12 hrs. (depending upon weather conditions) in a single day. The observations started at early hours in the morning being the best time to search and observe the elephant in open areas and four hours in the afternoon i.e. before the sunset. Field binocular was also used for observing their feeding behaviour without disturbing the animal

from an adequate and safe distance. The daily record was based on direct sighting of animal's feeding, indirect evidences like feeding sign, footprints impression time and fresh dung piles. The direct sightings were noted in duly prepared proformas, recording the group composition and also the place of sighting, time and vegetation composition. Besides, villagers of adjoining areas, Gujjars (where available), staff of forest department, the researchers from various scientific institutions and non-government organizations and other individuals working in this area, were also interviewed.

Identification of the elephants is important to verify their movement as in the same area there is a possibility that the same group was observed in the different forest beats. Therefore, distinctive features, with certain identification marks of individual elephants were noted like; shape of the ears, tusk size and shape, scars and tubercles on the body, tail length, total number of individuals (all ages separately), body mass and nature of group or solitary bull.

Results

Generally elephants fed in the early hours of the morning and most markedly in the evenings, just before dark. They were observed to feed in mid-day hours in winter but in summer, they rested during midday. It was observed during the study period that sometimes elephants were continuously feeding throughout the night. In summer, they spent their nights in open forest areas and when the day advanced they move towards the denser forest. In evening when the sun begins to set they again came out of the thick forest cover into the open forest areas.

In RNP elephants fed on the tree species like *Mallotus philippinensis* (Rohini), *Acacia catechu* (Khair), *Dalbergia sissoo* (Shisham), *Tectona grandis* (Teak), *Zizyphus mauritiana* (Ber), *Aegle marmelos* (Bel), *Ficus bengalensis* (Bar), *Ficus glomerata* (Gular), *Grewia oppositifolia* (Bhimal), *Bombax ceiba* (Semal), *Lannea grandis* (Jhingan), *Bauhinia variegata* (Kachnar), *Lagerstroemia parviflora* (Dhauri), *Kydia calycina* (Pula), *Syzygium cumini* (Jamun), *Flacourtia indica* (Kandai) and *Ehretia laevis* (Chamror). Besides elephants also used various grasses and shrubs as their food resources, which included *Dendrocalamus strictus* (Bamboo), *Helicteres isora* (Kapasi), *Saccharum munja* (Pula), *Saccharum spontaneum* (Kans), *Cynodon dactylon* (Doob Grass), *Eulaliopsis binata* (Bhabhar Grass), *Tinospora malabarica* (Giloe) and *Neyraudia arundinacea* (Bichhloo Grass).

A total of 50 plants species were recorded, which were observed to be favourite fodder species for elephants (Table 1). This list has been compiled from the identification of the leaves and fruits directly or taken from those plants that had signs of elephant feeding, and are based on the basis of their vernacular / local names. At the same time data was also collected and documented based on month wise utilization of fodder resources by elephants, which was based on direct observations and indirect evidences of feeding signs observed during the study period (Table 2).

In few of the plant species elephants utilized both leaves and twigs as their fodder for example when they were feeding on species like *Dalbergia sissoo*, *Acacia catechu*, *Bombax ceiba*, *Aegle marmelos*, *Ficus bengalensis* and *Ougeinia oojeinensis*, they ate different parts of the plant according to various seasons. It was observed from the present investigation that during January to March elephants mainly utilized the bark of different trees (*Shorea robusta*, *Bauhinia variegata*, *Mitragyna parvifolia*, *Schleichera oleosa*, *Lagerstroemia parviflora*, *Cordia obliqua*, *Tectona grandis*, *Holophramitis* spp. and *Bombax ceiba*) as their food. Elephants preferred to feed extensively on the bark and twigs of *Tectona grandis* at the onset of summer whereas they were observed to eat bark of *Bombax ceiba* tree during very hot season. Barks of the trees were mostly removed with the help of trunk but sometimes were also scrapped by using the tusks in case of bull elephant. Bulls have more options for feeding purpose as compared to cow elephants as sometimes cows could not remove the young and compact bark of trees whereas bulls are very able to remove such barks easily with the help of their tusks.

Fruits of *Aegle marmelos*, *Flacourtia indica*, *Ehretia laevis* and *Zizyphus mauritiana* were consumed by elephants. They often uprooted the plant with the help of the trunk and sometimes with the help of forefoot. Succulent grass species such as *Saccharum munja* and *Saccharum spontaneum* were favoured, although these are not the perennial food resources in the park area. *Tectona grandis* and *Holophramitis* spp. are also important fodder species, which were directly linked with elephant foraging as few of the area comprises of extensive stands of both of these species and currently elephants are utilizing

these food resources in some particular months of the year (from December to June). Direct observations indicated that these species are preferable food item for elephants and it was noticeable that elephants are feeding extensively on these species since last 5-6 years whereas before 2002 elephants were not reported to feed on these species (Figure 3). Only bark of these trees is being utilized by elephants they spent even whole of their day to feed on these species. Elephant induced damages to these species is quite large. Both of these species were planted in few forest pockets sometimes 20 years back to get rid of open damaged forests besides the fact that the regeneration potential of these species is very fast. Extensive feeding was observed on these food resources by elephant in eastern part of river Ganges whereas currently south-western population of elephants were not utilizing these species as their food. Although these plants are not the natural food but now as per the results of our observations these fodder species can be categorized under primary food.

Cordia obliqua, *Holarrhena antidysenterica* and *Mitragyna parvifolia* were also eaten by elephants occasionally. Generally bark and soft twigs (without leaves) were consumed as food especially during dry periods (April-June). We have described about these important fodder species, which are completely seasonal for the first time and all of these new findings have wider implications in conservation of Asian elephants through habitat improvement and management approaches.

Ranipur, Ravli and Chirak forest beats of the Hardwar forest range are famous for *Dendrocalamus strictus* (Bamboo) and due to the presence of huge amount of bamboo patches elephants have utilized these forest pockets throughout the year before 2002. Since last 3-4 years the regeneration potential of the bamboo is decreasing continuously. Besides, over feeding on bamboo bushes by elephants has led to destruction of this fodder species. Forest fire also restricts the frequent regeneration of bamboo in this area. Besides, few of the villagers also uproot whole of the plant body to fulfill their energy requirements. Our earlier observations review that the declining rate of elephant's population in some particular areas was mainly due to the impact of scarcity of natural water and falling rate of the status of fodder species in the area.

Elephants sometimes spent long time to feed on some particular plant species like *Dendrocalamus strictus* (Bamboo), *Mallotus philippinensis* (Rohini), *Cynodon dactylon* (Doob grass), *Ficus religiosa* (Pipal), *Saccharum spontaneum* (Kans) and *Saccharum munja* (Sarkanda). The consumption of tree species was highest, followed by few important shrubs and grasses.

Study revealed that the total amount of plant matter removed by the elephants was not fully consumed. In fact a relatively large part was dropped to the ground and left as such, which was sometimes utilized by other herbivores thus representing associational behaviour. The elephants in RNP fed extensively on the mixed vegetation including trees, grasses and shrubs. Although the study area has dominant plant species like *Shorea robusta*, *Mallotus philippinensis*, *Acacia catechu*, *Dalbergia sissoo*, *Terminalia tomentosa*, *Syzygium cumini*, *Ehretia laevis*, *Lagerstroemia parviflora*, *Holarrhena antidysenterica*, *Helicteres isora* and *Lannea coromandelica* besides, few species of *Ficus* and *Zizyphus* are available. The most preferred food item in this area was *Dendrocalamus strictus* (Bamboo) and *Mallotus philippinensis* (Rohini) but elephants used different food resources round the year as per their availability.

Elephants and woody vegetation

We also recorded the damages caused by the elephants in few forest ranges of the park. Elephants sometimes broke entire favourite plants like *Dendrocalamus strictus*, *Aegle marmelos*, *Dalbergia sissoo*, *Tectona grandis*, *Schleichera oleosa*, *Mallotus philippinensis*, *Grewia oppositifolia*, *Garuga pinnata* and *Ehretia laevis* besides, they also peeled off the bark of few plant species like *Bombax ceiba*, *Ficus bengalensis*, *Bauhinia variegata*, *Mitragyna parvifolia*, *Tectona grandis* and *Lagerstroemia parviflora*. The percentage frequencies of the five categories (twig breaking, bark peeling, branch breaking, stem twisting and pushing over) of damages inflicted on the woody vegetation by elephants were observed on several occasions (Figure 4). Out of five categories of damages, twig breaking (40%), and bark peeling (25%) were accounted for highest damage followed by branch breaking (18%), stem twisting (11%) and pushing over (6%). The elephants preferred to feed on soft twigs after removing the leafy portion from it.

Elephant is a wasteful feeder, judging by the amount of vegetation that is not eaten. The pushing over trees enables the animal to have access to the higher branches, which are out of range of its trunk.

Nevertheless, it represents a wasteful mode of feeding. Perhaps, the non-eaten vegetation could form a secondary food source to other herbivores in such cases thus; it is not a waste after all. An abundance of alternative food items (regenerating trees) is perhaps the reason for the low incidence (6%) of pushing over.

Time-activity budget

Generally elephants became active well before dawn and start their morning activities in the vicinity of the area where they spent night. During hot hours of the day various members of the group retired in available shade, whereas in the wet season they spent more time in feeding related activities. In the afternoon, begin their evening activities, which were quite similar to the morning activities. Evening hour was the time for drinking and bathing especially during summers. The feeding activity during summer was observed to be more in early morning hours and late hours in the afternoon and the mid-day is the time for rest, whereas in winter, feeding activity is near about constant but it is maximum in late evening hours. During the monsoon period, the moving and resting activity generally fluctuate because of slight restriction in movements. Resting during the monsoon largely depends on heavy rains while moving long distances, as at the onset of monsoon elephants show their long-term migration towards upper slopes in some of the areas.

Resting follows the standing of elephants in any shaded area especially in sparse cool shaded trees like *Ficus bengalensis*, *Adina cordiafolia* and *Butea tetrasperma*. Animal spends more time in resting during summer because the mid-day period is too hot and elephants may not tolerate high temperature and direct sun light for a very long time. Whereas during the winter they used open areas for standing and taking the sunbath while feeding activity was also ongoing. In summer season percentage of movement found more due to lack of fodder species and shrinkage of natural water sources. At that time animals have to travel more in search of food and water, while in winter and monsoon there is abundance of fodder species and water within the park area and during that time elephants do not perform very long distances.

The time-activity budget of different seasons during 12 hours of the day (feeding, moving, resting and others) of elephants was observed for two years during the course of this long-term study (Figure 5). Feeding during the winter (11.1 hours), accounted for the highest duration followed by feeding during the summer (10.5 hours) and monsoon (9.1 hours). Movement activity accounted for 1.4 hours (winter), 1.5 hours (summer) and 1.3 hours (monsoon). Fluctuations were observed in resting activity as this largely depended upon season (.4 hours in winter, 2.5 hours in summer and 1.4 hours in monsoon). Apart from this other activities like drinking, bathing, playing etc. accounted for 2.05 hours in winter, 0.4 hours in summer and 3.1 hours in monsoon.

Table 1. Elephant food plant species, their life form and the plant parts eaten in the Rajaji National Park

S. No.	Botanical Name	Vernacular / Local Name	Life forms *	Parts eaten #
1.	<i>Acacia catechu</i> (Linn.) Willd.	Khair	T	l, t, b
2.	<i>Acacia arabica</i> (Lamk.) Willd.	Babool	T	l, t, b
3.	<i>Aegle marmelos</i> Correa.	Bel	T	l, t, f
4.	<i>Albizia lebbek</i> Benth.	Kala siris	T	l, t
5.	<i>Albizia procera</i> , Benth.	Safed siris	T	l, t
6.	<i>Bauhinia variegata</i> Linn.	Kachnar	T	l, t, b
7.	<i>Bauhinia vahlii</i> , Wight. & Arn.	Maljhan	C	l, t
8.	<i>Bauhinia malabarica</i> , Roxb.	Khatua / Amla	T	l, t
9.	<i>Bombax ceiba</i> (Linn.) DC.	Semal	T	b
10.	<i>Bridelia retusa</i> , (L.) Spr.	Ekdana	T	t
11.	<i>Cordia obliqua</i> , Wild	Lassora	T	t
12.	<i>Cynodon dactylon</i> (Linn.) Pers.	Doob Grass	G	l, r
13.	<i>Dalbergia sissoo</i> Roxb.	Shisham	T	l, t, b

14.	<i>Dendrocalamus strictus</i> (Roxb.) Nees.	Bans / Bamboo	S	l, t
15.	<i>Desmostachya bipinnata</i> , (L.) Stapf.	Dav / Kush	G	l, r
16.	<i>Ehretia laevis</i> (Linn.) Roxb.	Chamror	T	l, t
17.	<i>Embelica officinalis</i> Gaertn.	Amla	T	l, t
18.	<i>Eulaliopsis binata</i> (Retz.) C.E. Hubbard.	Bhabhar Ghas	G	l, r
-	[Syn. <i>Ischaemum angustifolium</i> Hack.]	-	-	-
19.	<i>Ficus bengalensis</i> Linn.	Bargad / Bar	T	l, t, b
20.	<i>Ficus glomerata</i> Roxb.	Gular	T	l, t, b, f
21.	<i>Ficus religiosa</i> Linn.	Pipal	T	l, t
22.	<i>Ficus rumphii</i> , Bl.	Pilkhan	T	l, t
23.	<i>Ficus infectoria</i> , Roxb.	Khabar	T	l, t
24.	<i>Flacourtia indica</i> (Burm. F) Merr.	Kandai	T	t, b
25.	<i>Garuga pinnata</i> Roxb.	Kharpat	T	l, t
26.	<i>Grewia oppositifolia</i> , Roxb.	Bhimal	T	l, t
27.	<i>Grewia elastica</i> , Royle	Dhaman	T	t
28.	<i>Helicteres isora</i> Linn.	Kapasi	S	l, t
29.	<i>Holarrhena antidysenterica</i> , Wall.	Kura	T	t
30.	<i>Holophranitis</i> spp. ¹	Kut sagaun	T	l, t, b
31.	<i>Kydia calycina</i> , Roxb.	Pula	T	t
32.	<i>Lagerstroemia parviflora</i> , Roxb.	Dhauri	T	l, t
33.	<i>Lannea coromandelica</i> , (Houtt.) Merr.	Jhinghan	T	l, t
34.	<i>Mallotus philippinensis</i> Muell. Arg.	Rohini	T	t
35.	<i>Mitragyna parvifolia</i> , Korth.	Phaldu / Kaem	T	b
36.	<i>Neyraudia arundinacea</i> , (L.) Hen.	Bichhloo	G	l, r
37.	<i>Ougeinia oojeinensis</i> , (Roxb.) Hochr.	Saandan	T	l, t
38.	<i>Pithecellobium dulce</i> Benth.	Jangal Jalebi	T	l, t
39.	<i>Randia dumetorium</i> , Lamk.	Mainphal	T	t
40.	<i>Saccharum munja</i> Roxb.	Phoos / Sarkanda	G	l, r
41.	<i>Saccharum spontaneum</i> Linn.	Kans	G	l, r
42.	<i>Schleichera oleosa</i> , Willd.	Kusum	T	l, t
43.	<i>Shorea robusta</i> Gaertn.f.	Sal	T	b
44.	<i>Syzygium cumini</i> (Linn.) Skeels.	Jamun	T	l, t, b
45.	<i>Terminalia tomentosa</i> , W.&A.	Sain	T	b
46.	<i>Tectona grandis</i> , L. f. ¹	Sagaun/Teak	T	t, b
47.	<i>Tinospora malabarica</i> , Miers.	Giloe / Gurch	C	l, t
48.	<i>Thysanolaena agrostis</i> , Nees.	Hathi ghas / Pirlu	G	l, r
49.	<i>Zizyphus mauritiana</i> , Lam.	Ber / Beri	S	l, t
50.	<i>Zizyphus xylophyra</i> , (Retz.) Willd.	Bhander	S	l, t

* T – Tree, S – Shrub, G – Grass, C - Climber

l – leaves, t – twigs (twigs are generally eaten by removing the leafy portion from it), b – bark, r – root, f – fruits

¹ Present mainly in adjoining areas of forest rest houses and field sub-stations.

Table 2. Month - wise utilization of fodder resources by elephants in the Rajaji National Park area

Botanical Name	Verna cular Name	Winter					Summer				Rainy		
		Oct.	Nov.	Dec.	Jan,	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.
<i>Acacia catechu</i> (Linn.) Willd.	Khair	-	✓	✓	-	✓	✓	✓	✓	-	✓	-	-
<i>Acacia arabica</i> (Lamk.) Willd.	Babool	✓	-	-	-	-	-	-	-	-	-	-	✓
<i>Aegle marmelos</i> Correa.	Bel	✓	-	-	-	✓	✓	✓	✓	✓	-	-	✓
<i>Albizia lebbek</i> Benth.	Kala siris	-	-	-	-	-	✓	✓	✓	-	-	-	-
<i>Albizia procera</i> , Benth.	Safed siris	-	-	-	-	✓	✓	✓	-	-	-	-	-
<i>Bauhinia variegata</i> Linn.	Kachnar	-	-	-	-	✓	✓	✓	✓	-	-	-	-
<i>Bauhinia vahlii</i> , Wight. & Arn.	Maljhan	-	-	✓	✓	-	✓	✓	✓	-	-	-	-
<i>Bauhinia malabarica</i> , Roxb.	Khatua	-	-	-	-	✓	✓	✓	-	-	-	✓	✓
<i>Bombax ceiba</i> (Linn.) DC.	Semal	✓	-	-	-	-	✓	✓	✓	✓	-	-	✓
<i>Bridelia retusa</i> , (L.) Spr.	Ekdana	-	✓	-	-	-	-	-	-	-	-	-	-
<i>Cordia obliqua</i> , Wild	Lassora	-	-	-	-	-	-	-	✓	-	-	-	-
<i>Cynodon dactylon</i> (Linn.) Pers.	Doob ghas	-	-	-	✓	✓	✓	✓	-	-	-	-	✓
<i>Dalbergia sissoo</i> Roxb.	Shisham	✓	-	-	-	✓	✓	✓	✓	✓	✓	-	✓
<i>Dendrocalamus strictus</i> (Roxb.) Nees.	Bans	✓	✓	-	-	✓	✓	-	-	-	✓	✓	✓
<i>Desmostachya bipinnata</i> , (L.) Stapf.	Dav	✓	✓	-	-	-	✓	✓	✓	-	-	✓	✓
<i>Ehretia laevis</i> (Linn.) Roxb.	Chamror	-	✓	✓	✓	✓	✓	-	-	-	-	-	-
<i>Embelica officinalis</i> Gaertn.	Amla	-	-	-	✓	✓	✓	✓	-	-	-	-	-
<i>Eulaliopsis binata</i> (Retz.) C.E.	Bhabhar	✓	✓	-	-	-	✓	-	-	-	-	✓	✓
<i>Ficus bengalensis</i> Linn.	Bargad	-	-	-	-	✓	✓	✓	✓	✓	✓	✓	✓
<i>Ficus glomerata</i> Roxb.	Gular	✓	-	-	-	-	✓	✓	✓	-	-	-	-

<i>Ficus religiosa</i> Linn.	Pipal	-	✓	✓	-	-	✓	✓	✓	✓	✓	-	-
<i>Ficus rumphii</i> , Bl.	Pilkhan	-	-	✓	-	-	✓	✓	-	-	-	-	-
<i>Ficus infectoria</i> , Roxb.	Khabar	-	-	✓	-	✓	✓	-	-	✓	✓	✓	-
<i>Flacourtia indica</i> (Burm. F) Merr.	Kandai							✓	✓	✓			
<i>Garuga pinnata</i> Roxb.	Kharpat	-	✓	✓	-	✓	✓	-	-	-	-	✓	✓
<i>Grewia oppositifolia</i> , Roxb.	Bhimal	-	✓	-	✓	✓	✓	-	-	-	-	-	-
<i>Grewia elastica</i> , Royle	Dhaman	-	✓	-	✓	✓	-	-	-	-	-	-	-
<i>Helicteres isora</i> Linn.	Kapasi	✓	-	-	-	-	-	-	✓	✓	✓	✓	✓
<i>Holarrhena antidyenterica</i> , Wall.	Kura	-	-	-	-	✓	✓	-	-	-	-	-	-
<i>Holophramitis</i> spp. ¹	KutSagau n	-	-	-	✓	✓	✓	✓	✓	-	-	-	-
<i>Kydia calycina</i> , Roxb.	Pula	-	-	-	✓	✓	✓	✓	-	-	-	-	-
<i>Lagerstroemia parviflora</i> , Roxb.	Dhuri	-	-	✓	✓	✓	✓	✓	✓	-	-	-	-
<i>Lansea coromandelica</i> , (Houtt.) Merr.	Jhingan	✓	✓	-	-	✓	✓	✓	-	-	-	✓	✓
<i>Mallotus philippinensis</i> Muell. Arg.	Rohini	-	-	-	✓	✓	✓	✓	✓	✓	✓	-	-
<i>Mitragyna parvifolia</i> , Korth.	Phaldu	-	-	-	✓	✓	-	-	-	-	-	-	-
<i>Neyraudia arundinacea</i> , (L.) Hen.	Bichhloo	✓	-	-	-	-	-	-	-	-	✓	✓	✓
<i>Ougeinia oojeinensis</i> , (Roxb.) Hochr.	Saandan	✓	✓	✓	✓	✓	-	-	-	-	-	✓	✓
<i>Pithecellobium dulce</i> Benth.	Jangal jelebi	-	✓	-	✓	✓	-	-	-	-	-	-	-
<i>Randia dumetorium</i> , Lamk.	Mainphal	-	-	✓	✓	-	-	-	✓	-	-	-	-
<i>Saccharum munja</i> Roxb.	Phoos	✓	✓	✓	✓	✓	✓	-	-	-	-	-	-
<i>Saccharum spontaneum</i> Linn.	Kans	-	-	✓	✓	✓	✓	✓	-	-	-	-	-
<i>Schleichera oleosa</i> , Willd.	Kusum	✓	-	-	-	✓	✓	-	-	-	✓	-	✓
<i>Shorea robusta</i> Gaertn.f.	Sal	-	-	✓	✓	✓	✓	-	-	-	-	✓	-

<i>Syzygium cumini</i> (Linn.) Skeels.	Jamun	-	-	-	✓	✓	-	-	✓	✓	✓	-	-
<i>Terminalia tomentosa</i> , W.&A.	Sain	-	-	-	-	-	✓	-	-	-	-	✓	-
<i>Tectona grandis</i> , L. f. ¹	Sagaun	-	-	-	✓	✓	✓	✓	-	-	-	-	-
<i>Tinospora malabarica</i> , Miers.	Giloe	✓	✓	✓	-	-	✓	✓	✓	✓	-	-	-
<i>Thysanolaena agrostis</i> , Nees.	Hathi ghas	-	✓	✓	✓	✓	✓	-	-	-	-	-	-
<i>Zizyphus mauritiana</i> , Lam.	Ber	✓	✓	✓	✓	✓	-	-	-	-	-	-	-
<i>Zizyphus xylophyra</i> , (Retz.) Willd.	Beri	-	✓	✓	✓	✓	-	-	-	-	-	-	-

✓ When elephants were observed to feed on above mentioned fodder resources [based on direct and indirect observations].

¹ Plantation present mainly in buffer / outer areas of forest.



Figure 1. Baby elephants with their mother in Rajaji.

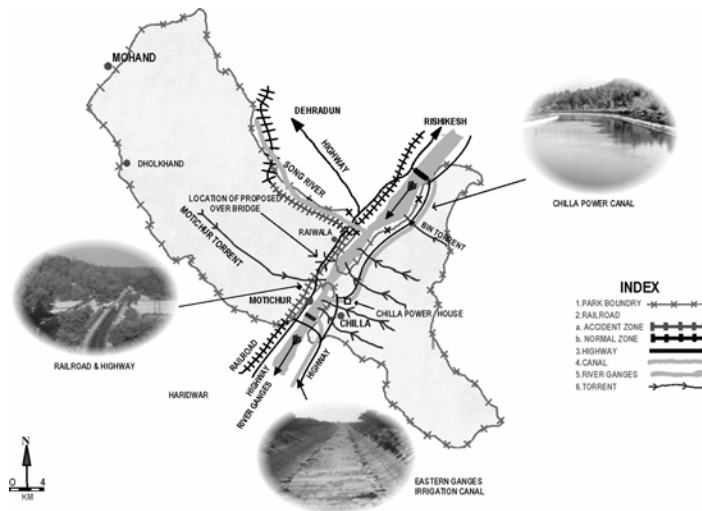


Figure 2. Map of the study area.



Figure 3. A cow elephant feeding on *Tectona grandis* in Rajaji National Park.

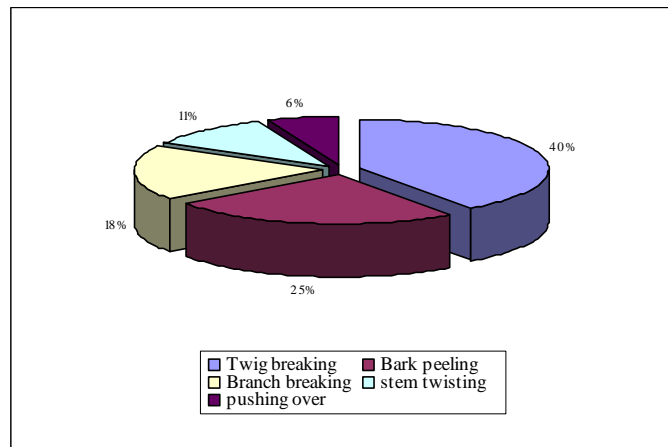


Figure 4. Relative frequencies of the various elephant-induced damages observed in the woody vegetation (n = 108 days).

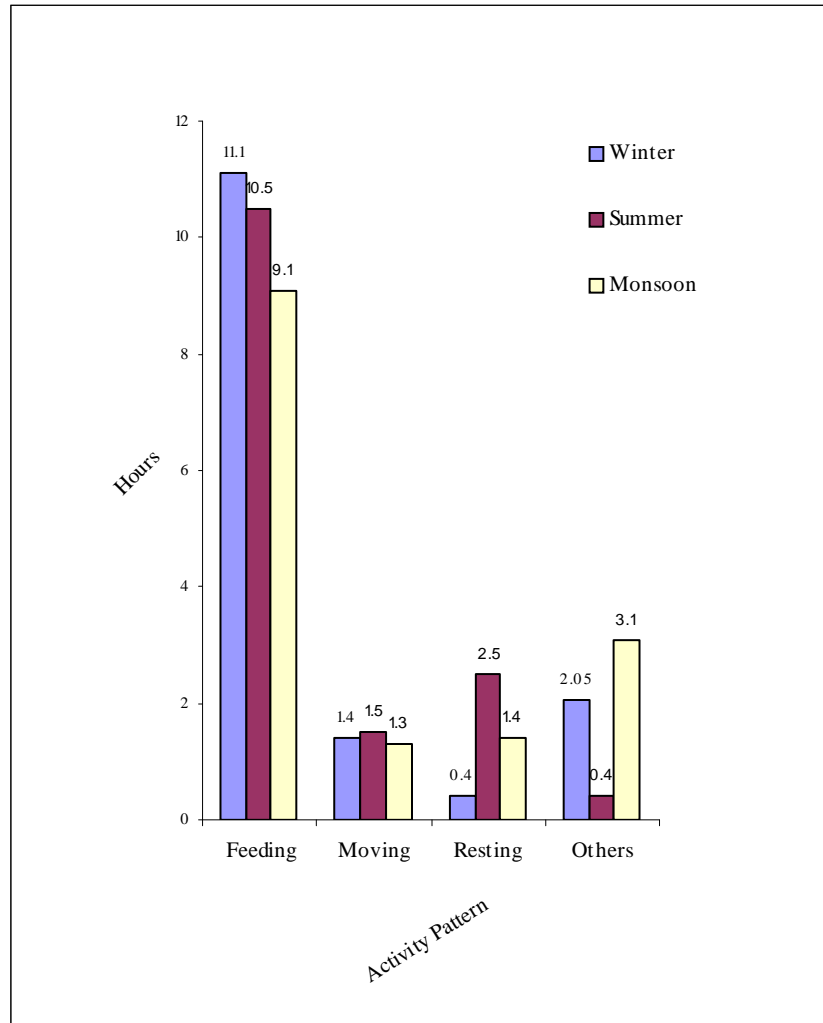


Figure 5. Comparative time-activity budget for different activities in different seasons

Discussion

The study enumerated 50 plant species in the diet of elephants in RNP. The bulk of the diet in number of species and quantities eaten came from twigs, bark, fruits and leaves. Though study area comprises of 128 tree species, 63 shrub and herb species, 33 climber species, 1 bamboo species and 37 grass species but out of total recorded fodder plant species (50), trees represented 74% of the species that elephants fed followed by 14% (grass species), 8% (shrub species) and 4% (climber species).

Elephants are known to feed on a wide variety of plant species. Research on forest elephant feeding ecology in Nouabale – Ndoki National Park in northern Congo has shown that elephants have a general diet comprising more than 350 species (Blake, 2002). A preliminary study on elephant's habitat in the RNP area has pointed out that 30 plant species were present in this area, which are being utilized by

elephants (Williams, 2002). A study on Asian elephant's foraging behaviour in southern India pointed out that elephants consumed at least 112 plant species and 85% of their diet consisted of only 25 species (Sukumar, 1990). During the dry season, 18 species of flowering plants were found to be eaten by the elephants in the Manas National Park (Lahkar et al., 2007). Another study on the conservation of Asian elephant in Bangladesh indicated that 143 plant species were present in Chunati Wildlife Sanctuary, out of which only 17 species were utilized by elephants that represents only 12% of the total local plant species (UCN Report). Similarly, a study on the diet and foraging ecology of the Asian elephant was conducted in the Shangyong National Natural Reserve, Xishuangbanna, China and pointed out that 106 plant species were eaten by elephants as their food (Chen et al., 2006).

RNP area falls under sub-tropical moist deciduous forest type and hence one can assume that in RNP, elephants eat seasonal food resources to provide the necessary range of nutrients. There is a distinct difference in the quantity and number of fruit species eaten seasonally by elephants and this influences elephant's feeding behaviour. As fruits abundance (*Aegle marmelos*, *Zizyphus mauritiana*, *Syzygium cumini* and *Ehretia laevis*) increases during various seasons, therefore, elephants consumed the available fruits round the year. During this period, they are probably less attracted to other sources of food.

Elephants use the soft twigs of the trees by removing the leafy portion from it; bark from woody plant is often ripped off for feeding purpose. Since bark is rich in calcium, elephants would select this resource, which is sometimes essential for the favourable growth of skeleton and for the tusks in males. A study was also conducted on the debarking behaviour of elephants in southern India, which shows that maximum number of debarked trees was from dry deciduous forests (Vanaraj, 2001). Comparatively captive elephants were observed having better health and grow faster than wild elephants, owing to better plane of nutrition and decreased parasitism. In the wild, a mature elephant will spend as many as 18 hours per day feeding, consuming as much as 280 kilograms of food. Obviously the food consumed in the wild is low in nutrients and high in fiber (John and Subramanian, 1991).

Present study revealed that tree species consist of major food for elephants and their diet is dependent on their migration and movement related activities. Study further indicated that few of the fodder species are common throughout the year while few are only available to elephants in particular season of the year. Present investigation suggested that widely distributed species were utilized throughout the year whereas altitude-wise distributed species were utilized in particular months during seasonal movement. For example elephants generally feed on *Saccharum munja* and *Saccharum spontaneum* when their movement is towards lower areas and *Neyraudia arundinacea* is utilized especially during monsoon season when their movement is towards upper slopes i.e. in Rawasan and Pulani forest beat. *Mallotus philippinensis* is commonly eaten from the onset of summer whereas elephants start feeding on *Dalbergia sissoo* from February onwards. Elephants were observed most markedly to feed on *Dendrocalamus strictus* from July to December whereas *Ficus* species were most favourable food item and elephants utilize these resources in most of the months in a year. Elephants feed extensively on large number of food resources during March, April and May months when they are performing their longer movements within and outside (adjoining reserve forests) from the park area basically in search of water. Availability of fresh water further ensures the presence of elephants in any particular area.

During the past six years, we have made extensive studies on different fodder resources and observed that only few populations of the elephants were observed to feed on *Tectona grandis* and *Holopternites* spp. species, whereas in few of the areas elephants are not utilizing both of these species as their food. Eastern part of the RNP area comprises of Chilla and Gohri forest range whereas south-western portion consists of Kansro, Motichur, Hardwar, Beribara and Dholkhand forest ranges. There is complete isolation between western and eastern components of an internal ecological unit mainly because of presence of Army dump, various villages, shrines, Ganga canal, hydro-electric power plant, national highways and railway track in between these two forest zones. Ongoing anthropogenic activities inside and peripheral to the protected forests are another major obstacle in these corridors.

The motor roads, which are adjacent to the forests like Hardwar-Dehradun National Highway, BHEL roads etc. have heavy traffic pressure. As per a preliminary study, the average number of vehicles passing on Dehradun-Hardwar road per day is 7,929 and all the wild animals, including elephants, are not in a position to cross this track at any time due to the presence of heavy traffic (Singh and Sharma, 2001).

Same situation is with other corridors present adjacent to the RNP area. Kotdwar – Lansdowne road runs parallel to the river Kho and crosses the Rajaji-Corbett corridor, the major movement track of northwestern elephant population between the Yamuna and river Sharda. This road serves as the major transport link between Pauri town and Kotdwar area. The presence of traffic on the road, construction of steep retaining walls and the presence of human population along the entire corridor area have almost restricted the migration of elephants (Johnsingh and Williams, 1999).

Crop raiding by elephants is a common phenomenon in adjoining areas of the RNP. Indigenous villages are situated around various forest ranges of the park and grow many potential cash crops to enrich their economy. The major cash crops are *Saccharum officinarum* (Sugarcane), *Oryza sativa* (Paddy), *Triticum* spp. (Wheat) and *Zea mays* (Maize) and few cultivators also grow fruit yielding species in their fields like *Musa paradisiaca* (Banana) and *Mangifera indica* (Mango). Elephants traditionally often leave the forest to feed in nearby villages, usually during nights. Even before 1998 elephants were reported to be raiding fields but their outside movement was more common from 2001 (Joshi et al., 2001). Currently the raids have become more frequent and the number of complaints by farmers has increased.

Elephant's movement towards outside areas is more common between November to February. During monsoon period only few elephants are found in these areas, which are mainly loners. Depredation of sugarcane took place throughout the year but was highest between November to February. The raiding group size also differs as per different seasons. During November to February group size is larger between 2 - 14 elephants than in the other seasons. Crop depredation pattern and season coincide with human deaths in the area. The peak depredation period is between the months of October to March during which time human deaths by elephants are also very high. Movement of elephants was noted outside of the park area generally in the late evening hours and in the night, but occasionally, it was also reported after mid-day. According to a long term preliminary study in southern India raiding was at peak during October mainly by bulls and to a lesser extent by herds (Sukumar, 1989). Stray behaviour among elephants has been more common from last two years as compared to previous years as they are making their tendency to feed on the cultivated crops (Joshi and Joshi, 2001).

It has been observed that the elephants enter in villages after sunset and re-enter the park area before dawn. In few of the places their village leaving time is 2 to 3 hours after dawn. In many places a same herd was reported continuously for about 14-15 days. A surprising thing was that only identified bull elephants and groups were reported to move outside of the conservation area. A study pointed out that most of the incidents of raiding were found to be in late evening hours or during night period (Nair, 1990) whereas another study indicated that raids by elephants were the results of either solitary individual (adult males) or small groups (Santiapillai and Suprahman, 1986).

Conclusion

The feeding habits of elephants show a great variance with respect to the seasons, availability of natural water and traditional movements. Elephants of RNP are well adapted to feed on seasonal fodder species present round the year in this hilly track, which lies in Shivalik foothills. The RNP has been intensively logged during the past few years as the result of which many of the original sub-tropical moist deciduous forest cover has disappeared and what are left today are large areas of annual or evergreen trees and extensive stands of Sal (*Shorea robusta*). From the point of elephant conservation, RNP is a rich habitat but is under biotic pressure mainly due to the traditional lopping of trees by Gujjars in few of the forest ranges (where they still exist) as Gujjar rehabilitation programme is still ongoing in the RNP area. Therefore, in few of the areas elephant are utilizing all the forest compartments frequently to fulfill their routine requirements. Other major factors are agricultural expansion peripheral to the different forest ranges and increasing number of anthropogenic and developmental activities. Study indicated that 50 plant species were consumed by elephants as their food but it has been suggested that different populations of elephants in the same forest stretch use some separate and specific fodder species (as having different geological conditions) hence more information is needed on their feeding biology to properly develop management strategies for their conservation. The RNP is one of the finest of the few remaining examples of the exceedingly diverse and productive lesser Himalayan eco-systems. Therefore, management practices are needed to conserve the elephants' habitat for their long-term survival.

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Comparison Of Dac-Elisa And Dot-Blot-Elisa For The Detection Of Cucumber Mosaic And Banana Streak Viruses Infecting Banana

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ABSTRACT

This paper presents the details of direct antigen coating (DAC) - ELISA and Dot-blot-ELISA for the detection of banana streak badnavirus (BSV) and cucumber mosaic cucumovirus (CMV) in banana leaf and pseudostem tissues. Suckers were collected from banana plants infected with BSV and CMV. The plants were indexed for presence of viruses by DAC-ELISA. The DAC form of indirect ELISA was adopted to detect viruses in plants. From the studies, it is observed that BSV and CMV induced similar interveinal chlorotic streaks of varied sizes in banana. In DAC-ELISA, BSV was detected up to 10^{-2} dilutions of tissue extracts but it was detected by Dot-blot-ELISA up to 10^{-3} dilution. Further, it is observed that among ten field collected samples, none were positive for CMV in DAC-ELISA, but three samples reacted positively in Dot-blot-ELISA. Out of ten field samples tested for BSV, only one weakly reacted. However, in Dot-blot-ELISA, none were found positive for BSV. Out of ten samples, none were found mixedly infected. Above findings indicate that Dot-blot-ELISA is relatively more sensitive for the detection of BSV and CMV in banana. [The Journal of American Science. 2008;4(2):49-57]. (ISSN 1545-1003).

Keywords: Banana streak virus, Cucumber mosaic virus, DAC-ELISA, Dot-blot-ELISA

1. Introduction

Banana is one of the world's most important tropical fruit crop. It is grown both as a staple food and a major cash crop. It is propagated vegetatively through suckers. Asia accounts nearly 40% of world banana production. Bananas grown in South India can be broadly grouped into three types like deserts, culinary and dual purpose varieties. Successful cultivation of banana is varied, because it is influenced by abiotic and biotic factors. Among the biotic factors, several insect and nematode pests and fungal, bacterial and viral pathogens are known to limit the growth and fruit yield of banana (Jeger et al., 1995). Banana is a humid tropic plant coming up with a temperature range of 10°C to 40°C and average of 23°C . Four viruses known to naturally infect banana widely in different countries are BBTv, CMV, BBrMV and BSV ([Jeger et al., 1995). Table 1 shows the details of virus diseases of banana.

Table 1. Virus diseases of banana

Sl. No.	Disease	Causal virus
1	Bunchy top	Banana Bunchy top virus (BBTV)
2	Infectious chlorosis	Cucumber mosaic virus (CMV)
3	Bract mosaic	Banana bract mosaic virus (BBrMV)
4	Streak	Banana streak virus (BSV)
5	Mosaic	Tobacco mosaic virus (TMV)
6	Die-back	Nepovirus
7		Potexvirus
8	Abaca mosaic	Abaca mosaic virus (AbaMV)

So far TMV was reported to infect banana only in India (Singh, 1988). Abaca mosaic virus, nepovirus and potexvirus infecting banana in few countries have less significance (Brunt et al., 1996, Anonyms, 1997). BSV causing streak disease of banana has been reported from several countries. Streak symptoms of BSV

infection of *Musa* spp. are sometimes similar to those caused by CMV and the two diseases have been confused (Lockhart et al., 1992). Causal virions are nonenveloped, bacilliform belongs to badnavirus. It is transmitted by the citrus mealy bug (*Planococcus citri*) and through suckers (Jones and Lockhart, 1993).

Harper et al., (1999) developed a PCR based strategy to detect episomal banana streak badnavirus (BSV) in banana and plantain plants that carry integrated BSV sequences. Antisera used in immuno capture polymerase chain reaction (IC-PCR) are capable of binding a large number of BSV serotypes. They found that IC-PCR is suitable for the large scale screening of *Musa* for episomal BSV which is necessary for germplasm movement. Geering et al., (2000) cloned and sequenced part of the genomes of four isolates of BSV and compared with those of other badnaviruses. Immunocapture polymerase chain reaction assays were developed allowing specific detection and differentiation of the four isolates of BSV. Helliott et al., (2003) reported that the anti retroviral and anti hepadnavirus molecules, adefovir, tenofovir and 9-(2-phosphonomethoxyethyl)-2, 6-diamino-purine (PMEDAP), efficiently eradicate the episomal form of BSV from banana plants. Harper et al., (2004) isolated BSV from infected plants sampled across the Uganda *Musa* growing area and the isolates were analysed using molecular and serological techniques. Their analyses showed that BSV is very highly variable in Uganda. Provost et al., (2006) developed a multiplex immunocapture PCR (M-IC-PCR) for the detection of BSV. *Musa* sequence tagged microsatellite site (STMS) primers were selected and used in combination with BSV species specific primers in order to monitor possible contamination by *Musa* genomic DNA, using multiplex PCR. Teycheney et al., (2007) adapted an existing polyvalent degenerate oligonucleotide RT-PCR (PDO-RT-PCR) assay to the detection of banana mild mosaic virus (BanMMV) and banana virus X, two flexiviridae infecting *Musa* spp. PDO inosine containing primers were found to be well suited to the detection of BanMMV, despite its high molecular diversity, but not to that of the highly conserved BVX, for which species-specific primers were designed.

There is a need to detect these viruses for the selection of virus free planting material. Planting of virus free seed or other propagation material is a prime practice for effective disease control. Dot-blot-ELISA using nitrocellulose or nylon membrane as support has been used for the detection of potato viruses initially. Subsequently, this technique has been applied for the detection of several viruses in both plant tissues and insect vectors. It was reported that Dot-blot-ELISA is a relatively more sensitive and economical in using the different reagents when compared to conventional ELISA performed in plastic plates. Further, the test sample extracts can be blotted on the membrane at the field level and send them to laboratories for further processing. This indicates a wide potential application of the technique for the large scale detection of viruses.

In the present study, an attempt was made to compare the DAC-ELISA and Dot-blot-ELISA for the detection of BSV in banana leaf and pseudostem tissues using heterologous RTBV-polyclonal antiserum.

2. Materials and Methods

Suckers collected from banana plants infected with BSV and CMV from West Godavari district of Andhra Pradesh state, India were propagated in the garden of Virology Department, S.V. University, Tirupati. The plants were indexed for presence of viruses by DAC-ELISA. The direct antigen coating (DAC) form of indirect ELISA described by Hobbs et al., (1987) and Mowat and Dawson (1987) was adopted to detect viruses in plants.

Preparation of reagents:

- (a) Phosphate buffered saline (PBS), pH 7.4:
- | | |
|---|--------------|
| NaCl | = 8.0 g |
| Na ₂ HPO ₄ ·2H ₂ O | = 1.44 g |
| KH ₂ PO ₄ | = 0.2 g |
| KCl | = 0.2 g |
| Distilled water | = 1000.00 ml |
- (b) Phosphate buffered saline – tween – 20 (PBS-T), pH 7.4:

- 0.5 ml of Tween-20 was added to 1000 ml of PBS
- (c) PBS – TPO:
Polyvinyl pyrrolidone (MW 40000) = 2 g
Ovalbumin = 0.2 g
PBS – T = 100.0 ml
- (d) Coating buffer, pH 9.6:
Na₂CO₃ = 1.59 g
NaHCO₃ = 2.93 g
DIECA = 2.25 g
Distilled water to 1000 ml
- (e) Diethanolamine substrate buffer, pH 9.8:
Diethanolamine = 97.0 ml
Distilled water = 800.0 ml
pH adjusted to 9.8 with IN HCl (about 67 ml), made up to 1000 ml with distilled water and stored at room temperature.
- (f) Alkaline phosphates (ALP) conjugate
Goat antirabbit antibodies labelled with ALP (Genei, Bangalore) diluted (1:5000) with PBS-TPO was used
- (g) Substrate para-nitrophenylphosphate (PNP) solution
Two 5 mg tablets of PNP (Sigma) were dissolved in 20 ml of substrate buffer
- (h) Antiserum
RTBV and CMV-Banana antisera (Virology Department, S.V. University, Tirupati) were used at 1:5000 dilution in PBS-TPO respectively
- (i) Antigen Extraction Buffer
For DAC-ELISA, virus infected and healthy leaf and pseudostem tissues were extracted in carbonate buffer containing 0.01 M DIECA

Procedure:

Antigen samples prepared in carbonate buffer were added to wells of the plate and incubated for 90 min. at 37°C. The plate was washed three times with PBS-T. RTBV and CMV-Banana antisera were added to the wells. The plate was covered with a lid and incubated at 37°C for 90 min. Then the plate was washed 3 times with PBS-T with 3 min. gap between each wash. The goat antirabbit labelled with ALP diluted (1:5000) with PBS-TPO was added to wells. The plate was incubated at 37°C for 90 min. and washed with PBS-T three times as above. The enzyme substrate PNP (sigma no-104) added to wells and incubated at room temperature for 1 hour in dark for colour development. The reaction was terminated by adding 3N NaOH solution at 50 µl/well. The reactions were noted according to colour intensity. The plate was read at A_{405nm} in ELISA plate reader.

Dot-blot-ELISA

Dot-blot-ELISA was carried out according to the method described by Bantari and Goodwin (1985) and Hibi and Satio (1985).

Preparation of reagents:

- (a) Coating buffer, pH 9.6
Na₂CO₃ = 1.59 g
NaHCO₃ = 2.93 g
Dissolved in about 900 ml distilled. H₂O, adjusted pH to 9.6 and made up to 1000 ml with distilled water.
- (b) Tris – buffered saline (TBS), pH 7.5:
Tris (0.02 m) = 4.84 g
NaCl (0.15 m) = 58.8 g
Dissolved in 1900 ml distilled water, adjusted pH to 7.5 and made up the volume to 2000 ml with distilled water.

- (c) TBS-Tween
TBS = 1000 ml
Tween-20 = 0.5 ml
- (d) Blocking solution
TBS = 100 ml
Non fat dried Milk powder = 5g
- (e) Antibody buffer
TBS-T = 100 ml
Nonfat dried milk powder = 5 g
- (f) HRP labeled goat antirabbit IgG
Diluted in antibody buffer (1:5000) just before use.
- (g) Substrate buffer (0.5M sodium citrate, pH 5.2)
for HRP system:
Trisodium citrate = 735 mg
Dissolved in 30 ml distilled H₂O adjusted pH to 5.2 with IN HCl and made up to 50 ml with distilled H₂O
- (g) Substrate solution
For HRP system
Dissolved 6 mg DAB in 9 ml substrate buffer and added 1ml of 0.3% cobaltous chloride and 10 ml of 30% H₂O₂, mixed well and used it immediately
- (h) Antisera
RTBV – heterologous antiserum and CMV banana antiserum were diluted (1:5000) and (1:500) using antibody buffer respectively.
- (i) Antigens
For Dot-blot-ELISA, virus infected (BSV and CMV) and healthy leaf and pseudostem tissues were extracted in carbonate buffer containing 0.01 M DIECA, subsequent dilutions of the antigens was made in carbonate buffer.

Antigen samples with a micropipette were applied on to the nitrocellulose membrane according to labelling. The membrane was allowed for drying and then transferred to a petriplate and blocking solution added till the membrane was fully immersed. The membrane was kept constant in blocking solution for 3 hours at room temperature with intermittent shaking. The membrane was transferred from blocking solution to diluted antiserum in blocking buffer and kept at 37°C for 1 hour. The antibody solution was discarded and washed the membrane thrice with TBS-T at 5 min. interval. The goat antirabbit antibodies labelled with HRP were added to the antibody buffer and placed the membrane in it under constant shaking conditions. The conjugate solution was discarded and the membrane was washed thrice with TBS-T at 5 min. interval. The substrate solution specific to enzyme was added and kept in shaking till sufficient colour was developed. The membrane was washed with water and then it was treated with 1.05% sodium hypochlorite solution for decreasing the back ground colour.

Leaf and pseudostem samples from suspected banana (variety Robusta) plants in commercial gardens near Duvvur, Buchireddypalam Mandal, Nellore District, Andhra Pradesh, India were collected and tested for the presence of CMV and BSV by employing DAC-ELISA and Dot-blot-ELISA described above.

Results and Discussion

In sucker propagated banana plants interveinal chlorotic streaks of varied sizes were noticed on fully expanded leaves (Figure 1). The distribution of the chlorotic streaks is not uniform throughout the leaf in certain plants. As the leaves matured, the chlorotic streaks were less prominent and in certain leaves necrosis was initiated in chlorotic streaks. Overall the symptoms induced by BSV are comparatively less severe as comparative to the symptoms induced by CMV.



Figure 1. BSV infected banana leaf showing interveinal chlorotic streaks

The sucker propagated banana plants exhibited bright yellow chlorotic speckles spindle shaped streaks and sometimes continuous interveinal chlorotic streaks. Like BSV, the symptoms induced by CMV are also not uniformly distributed throughout the leaves in infected plants. As the leaves aged, the severity of the symptoms reduced. The lateral veins appear prominent in diseased leaves compared to healthy leaves. Banana (variety Robusta) leaf samples collected from Nellore district also exhibited interveinal chlorotic specks and streaks of varied sizes (Figure 2).



(a) CMV infected banana leaf showing interveinal chlorotic specks and streaks



(b) Healthy banana leaf

Figure 2. Various types of banana leaves

DAC-ELISA and Dot-blot-ELISA were performed using homologous CMV and heterologous RTBV antisera. For this, two sets were first evaluated using the BSV and CMV infected samples of banana propagated in the garden of Virology Department. In DAC-ELISA, BSV was detected up to 10^{-2} dilutions in leaf samples up to 10^{-1} dilution in pseudostem samples of infected banana using RTBV antiserum (Figure 3 and Table 2). But in Dot-blot-ELISA, BSV was detected in both leaf and pseudostem samples up to 10^{-3} dilution. However, background reaction was noticed with healthy samples in 10^{-1} dilution (Figure 4).



Figure 3. ELISA plate showing positive reactions (yellow colour) of BSV infected banana samples with RTBV antiserum

DAC-ELISA and Dot-blot-ELISA were used for the detection of CMV and BSV in banana (variety Robusta) collected from Duvvur area of Buchireddypalem of Nellore District. Among all the ten tested samples (with mild/faint-chlorotic streaks), none were found positively reacted with CMV-banana antiserum in DAC-ELISA. However, one sample reacted weakly with heterologus RTBV antiserum. Out of ten CMV negative samples, three samples were further tested by Dot-blot-ELISA. Three samples positively reacted with CMV antiserum up to 10^{-3} to 10^{-4} dilutions (Figure 5, Table 3). Light back ground reaction is also noticed in healthy samples up to 10^{-1} dilution. However one sample positive for BSV in DAC-ELISA was turned out to be negative in Dot-blot-ELISA.

Table 2. Detection of BSV in banana samples by DAC-ELISA using heterologous RTBV polyclonal antiserum

Nature of the sample	Dilution	A ₄₀₅ reading
Healthy rice leaf extract	10^{-1}	0.14
	10^{-2}	-0.07
	10^{-3}	-0.02
Infected rice leaf extract	10^{-1}	0.77
	10^{-2}	0.50
	10^{-3}	0.21
Healthy banana leaf extract	10^{-1}	0.12
	10^{-2}	-0.04
	10^{-3}	-0.04
Infected banana leaf extract	10^{-1}	0.48
	10^{-2}	0.34
	10^{-3}	0.06
Healthy banana Pseudostem extract	10^{-1}	0.12
	10^{-2}	-0.04
	10^{-3}	-0.04
Infected pseudostem extract	10^{-1}	0.44
	10^{-2}	0.22
	10^{-3}	0.041

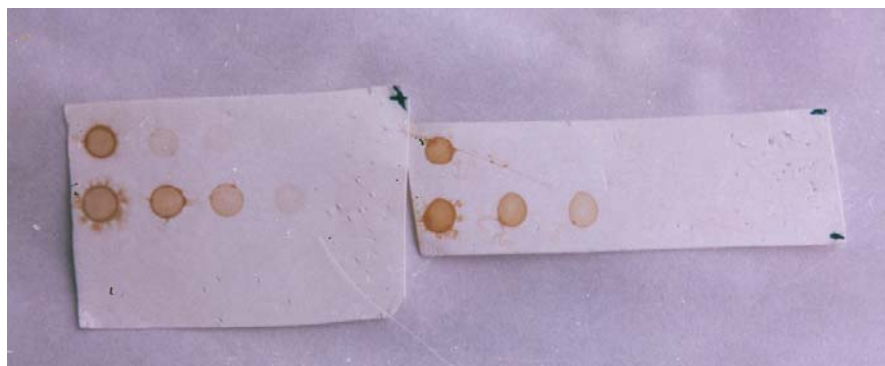


Figure 4. Detection of BSV in banana leaf and pseudostem samples using RTBV antiserum by dot-blot-ELISA

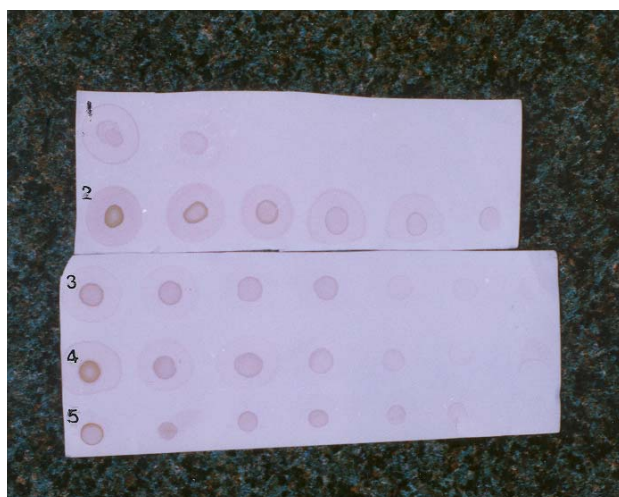


Figure 5. Detection of CMV in banana leaf samples using CMV-banana antiserum by dot-blot-ELISA

Table 3. Comparison of DAC-ELISA and dot-blot-ELISA for detection of CMV and BSV in field collected banana samples

Sample No.	Virus	DAC-ELISA	Dot-blot-ELISA
1	CMV	0.04	+
2		0.33	+
3		0.04	+
4		0.03	-
5		0.18	-
6		0.11	-
7		0.27	-
8		0.23	-
9		0.14	-
10		0.27	-
Healthy banana leaf extract		0.30	-

CMV infected banana leaf extract		3.58	+
11		0.04	-
12		0.01	-
13		0.04	-
14		0.02	-
15		0.44	-
16		0.03	-
17		0.25	-
18		0.13	-
19		0.24	-
20		0.12	-
Healthy banana leaf extract		0.26	-
BSV infected banana leaf extract		0.48	+
Healthy rice leaf extract		0.13	-
RTBV infected rice leaf extract		1.40	+

Summary and Concluding Remarks

An attempt was made to compare DAC-ELISA and Dot-blot-ELISA for the detection of BSV and CMV in banana leaf and pseudostem tissues. The significant observations are summarized below:

- BSV and CMV induced similar interveinal chlorotic streaks of varied sizes in banana and hence difficult to identify based on visual symptoms.
- In DAC-ELISA, BSV was detected up to 10^{-2} dilutions of tissue extracts but it was detected by Dot-blot-ELISA up to 10^{-3} dilution.
- Out of ten field collected samples, none were positive for CMV in DAC-ELISA, but three samples reacted positively in Dot-blot-ELISA.
- Out ten field samples tested for BSV, only one weakly reacted. However, in Dot-blot-ELISA, none were found positive for BSV.
- Out of ten samples, none were found mixedly infected.

Above findings indicate that Dot-blot-ELISA is relatively more sensitive for the detection of BSV and CMV in banana.

3/26/2008

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Research on the New Accounting Control Based on the Environment of IT

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Abstract: Purpose The information technology, such as communications technology, computer technology and network technology, is widely applied in business administration and financial management since 21st centuries. The people weren't pleased with the facilities of accounting account brought by information technology, begun to study the changes of accounting control functions from accounting to management changes under it environment, and hoped to achieve real-time control of accounting. However, the accounting real-time control also can not be regarded as a complete theoretical. **Design/methodology/approach** The paper takes the basic accounting control theory as the beginning, combines with the systems and methods of Accounting Computerization and Network Accounting under information technology, and studies the theory systems and operational mode of accounting real-time control. **Findings** This paper gives the third-class control system of and partitions the control object, content and real-time control methods. And the paper discusses the building of IT environment, the rebuilding of accounting process and the designing of control mode. The paper analyzes the new models of real-time control implementation. **Originality/value** Accountant real time control theory is the theory having the vast application prospect puts important guiding principle and means controlling into practice especially with the fact that development of IT technology, it will certainly become enterprise. . [The Journal of American Science. 2008;4(2):58-64]. (ISSN 1545-1003).

Keywords: Accounting control; Accounting process rebuild; Information technology; Real-time control

1 Introduction

Under the environment of IT, accountant's calculation work may be completed by the network, the information processing highly automates, thus accountant's calculation function is gradually desalinating, and center of gravity transfers to the management function. The integration financial control system based on the Internet makes the accounting information become the opening information system in the enterprise public information platform. The openness of the network environment causes the tradition manual operation and the internal accountant controls expire in the certain degree. Different internal users will pull and read the accounting information according to the authorized right. And the exterior user is also possibly authorized to go into the system interior and directly to pull and read the accounting information^[1]. The massive accounting information is transmits through the network communication lines, and it may be illegally intercepted, steals even tampers with. It is more possible that the network accounting information system will suffer "the virus" to invade or "the hacker" to attack^[2]. Therefore, accountant controls must carry on a bigger change, and then can meet the impact which IT brings to.

2 Accountant real-time controls and its way

2.1 Accountant real-time control

The Accountant real-time control is means that in the IT environment the finance and accounting personnel carry on the real-time contrast and the real-time analysis to the operational process of the

enterprise by the modernized technology method^[3]. It is intervention enterprise's the promotion management service through instructs, adjusts, restrains links and so on, to realize the enhancement management benefit thus and realize this ultimate objective of to achieve the value rises in value^[4]. The accountant real-time control is mainly performance to surmount the space and time, and carries on the dynamic control and the coordination^[5]. Its goal lies in the solution of problems, such as the accountant systematically records the account not in time, calculates the account not in exact, the accounting report not in time ,and so on, which are bring by the accounting information transfer lags.

The Real-time accountant control system^[6], firstly, requests all links of this organizations and agencies operation to have been the close relation with the accountant control system. Secondly, it requests all processes change of this organizations and agencies operation to have been real-time rapidly reflected into the accountant control system. Finally, it requests the result of financial inventory accounting processes to have been feed back to each kind of demand men of accounting information as soon as possible, and feed back to all levels of superintendent of the enterprise. And then it enables them to make rapidly the decision-making and the response, and improve the service or the management and enhance the achievements.

2.2. The accountant real-time control way

The accountant real-time control way specifically includes process control, Dynamic control, the cross space and time controls^[7]. The main content of accountant real-time control firstly are these economic work related with each item of accountant essential factor which Including monetary fund, Real asset, Foreign invests, Engineering project, Purchases and pays money, Finances, The sale with receives money, Cost expense, guarantee Economic works and so on, this is the basic content which accountant controls, also is the first level control content of the accountant real-time control. the second level content of the accountant real-time control is controlling to the accountant real-time control system which Including system development and maintenance, systematic security, software security, data security, operation control, input control, output control, processing control and so on. The third level content of the accountant real-time control include two aspects: multi-dimensional control to the value chain and abundantly controls to the accounting information^[8,9].

In order to make the accountant control become truly the management tool which causes the enterprise value to rise, it needs system and comprehensively studies the IT environment of real-time control, the accountant flow makes again, Accountant real-time control method, Accountant real-time control patterned. Finally it is guaranteed the goal of accountant controls will be able to realize. the real-time control frame system of Modern enterprise accountant is at least composed by four parts, mainly includes the IT environment of real-time control constructs, Accountant flow makes Again, Accountant real-time control method and Accountant real-time control pattern^[10].

3 Establishment the service data warehouse constructs the IT environment

The IT environment of real-time control usually includes network, Database and management software (including accountant software). The network provides the information transmission and the sharing foundation. The database is the gold treasure house of the memory and management data. The accounting information system (AIS) is method to carry on gathers, Processes, Report to the operational active information of the enterprise^[11]. The IT environment of the real-time control is carrier of accountant flow, accountant real-time control method, accountant real-time control pattern. Therefore, the IT environment is in the strut and foundation status of the real-time control frame system. Under the IT environment, the accounting information system should be the accounting information system of service actuation. It is composed by the service data warehouse, the service information processing rule and the internal network. It can integrate the traditional automation accountant system with the various services function information system, and realize real-time gathering and processing to the financial information and non- financial information through an integrated frame and realize gathering of information real-time, processing, and memorizing and transmission, and then satisfy the different information demand of each

kind of information users^[12].

Whether the information which the information user obtains is in-time and accurate, it is decided by whether in-time and accuracy or not the data in the service data warehouse is. And then it is the foundation data gain, but the foundation data gain is carries on in the service scene. Therefore whether in-time and accurate or not the user information is, it is directly decided by the service data gathering in first-hand of a various services department.

4 Establishment and consummation business computer interior network

Under the instruction of perfect information processing rule, the accounting information system of the service actuates can be allowed to integrate the primary data of the memory service event and to rich the service data warehouse^[13]. The information the department level accounting information system collects is for the processing information which has the department specific use or the fragment which intercepts event attribute. In the accountant system of the event actuates, the system structure is simpler and it may directly record the event attribute. The majority of events data will be depositing in primitive and uncrossing way.

It is an important precondition to establish and consummate the business computer network. And this network will cover each department of the enterprise and realize the interconnection and data sharing between various departments. Consummation enterprise interior network includes three aspects. Firstly, it must be able to guarantee the long-distance information transmission. The scope enterprise's service activity is very widespread. It would be assure the service information of the enterprise transmit in real-time to the service data warehouse of the enterprise. And it must be done to establish the security network of good long-distance transmission. Secondly, the information sharing between various departments of the enterprise would be guarantee, and various business agencies can promptly transmits the information to the service warehouse. At the same time, it also can real-time collect the essential feedback information from the service warehouse^[14]. So the effective auxiliary decision-making will carry on to the service activity. Finally, it must be able to guarantee the security of the entire information system. It includes two aspects of the internal security and outside security. The internal security is to guard against the enterprise interior personnel tamper with the information in evil intention. And the outside security is to guard against hacker's invasion.

5 Making again of the Accountant flow

If the enterprise actual flow can be effectively abstracted, it usually has three kind of main flows: the service flow, the accountant flow and the management flow. Among them, the accountant flow is the important constituent in the enterprise flow. It is the bridge which connects the service flow and the management flow. It is responsible to gather the data from the service flow^[15]. By processing it will produce the information which the enterprise needs to manage the operate activity, and then it will support the enterprise's management.

When the accountant flow has become the secret work which separates with the service flow, the business management personnel can possibly change the service flow but actually not link up with the finance and accounting personality causes more and more big barrier between the finance and accounting personnel and the business management personnel. Thus it will form the nonessential anxiety between the service process management and the finance and accounting work. And it is unable effectively to display to affect the accountant real-time control. Therefore, if the accountant real-time control question will be study, the accountant flow making again must be study. The real-time control view which instructed by the service flow making again thought must be established. It will use IT fully to organic fusion among the accountant flow, the service flow and the management flow. And it will insert the flow into the IT environment and Insert the finance and accounting personnel into the management process. It can guarantee timely, complete, accuracy and usefulness of the information gathering and processing. Under the coordination of control pattern and method, it can fully displays the dynamics and quality in Accountant real-time control. It can

realize to enhance the efficiency and the benefit the enterprise manages, and then it will guarantee goal which the value raises in value.

5.1 Accountant flow making again based on IT

5.1.1 Establish the accountant flow based on service

In new accountant flow, when the service event occurs, the service information of various business agencies is designed directly to input to the service database in maintaining the original of the primitive certificate. The information the financial department and other each department need all withdraw from the service database. Like this, it has guaranteed the homology of the financial data and the management data, and then it can make a better coordination between the accountant flow and the service flow.

5.1.2 Establishment the accountant flow favoring for the management

The financial budget and the analysis are the link which can most manifest the assistance management decision-making function of the accountant flow. Modern management requests the accountant flow feedback real-timely the actual data and the contrast of it with the budget data, and adjustment promptly the budget goal. Therefore, to establish the accountant flow which favor for the management in the new flow can realize the real-time feedback of the financial data, and it can carry on the real-time contrast and analysis between the actual data with the budget. So it guarantees the smooth implementation of the management decision-making.

The development of IT, especially the application of the data integration technology and network technology, provided the reliable guarantee for the processing complex data question. The IT has been able to bring a bigger revolution to the domain of financial inventory accounting. The accountant making again flow shows as Fig.1:

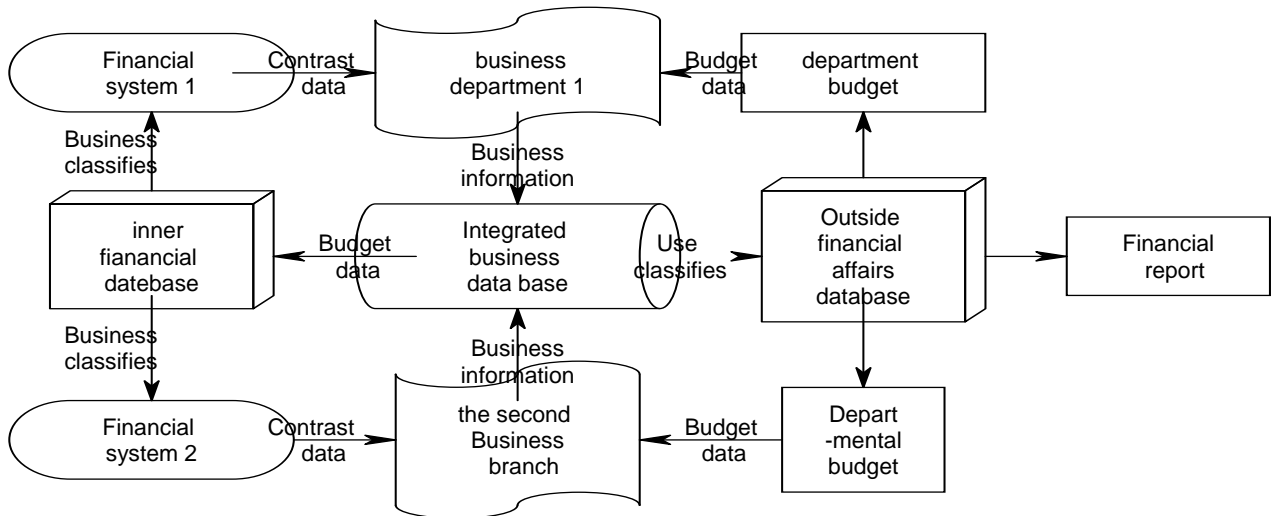


Fig.1 Rebuilding accountant flow

In new accountant flow, the business agency develops the office work by the opening departmental budget, and real-timely submits the service information to the integrated service database. The interior and exterior financial database separately withdraw the service data from the integrated service database according to their own collection rule of information, and forms the financial data based on the business agency's financial system by processing^[16]. And then it withdraws data message correlated with the service from the internal finance database, and promptly feeds back to the business agency. The business agency adjusts own service goal according to the basis real-time feedback correlation data.

5.2 The character of the new accounting flow

5.2.1 The characteristic of accounting data

Firstly, the service data is not directly input to the accounting information system, but it is input to the service integration database. Like this it has guaranteed the service data integrity. Secondly, the data in the financial database directly originates from the integrated service database and moreover has carried on the classification according to the difference between the internal use and exterior use finance data. The internal finance database is directly faces the service flow and the management flow^[17]. The financial data output from the financial database which classifies by the different business agency is strong pointed and can effectively carry on the auxiliary decision-making to the operation management.

5.2.2 The characters of the real-time monitoring

Firstly, in the new accountant flow, through the association of internal Internet, the service data may be promptly transmitted to the service database and be unified to input by the financial department. Like this it has saved the time for the data processing and the feedback. At the same time it has also saved the financial department's manpower and enhanced the efficiency of the financial department's working. Secondly, as a result of the service data prompt transmission and processing, the financial data based on business agency output from internal financial database may also real-timely transfer to the business agency and provide the basis for the policy-making adjustment of the business agency

5.2.3 Making again to the financial department structure

Because the primary data input work carries on in the business agency, the data input work of the finance and accounting personnel will be able greatly to reduce. At the same time, because the new accountant flow is based on the economic work actuation, the auxiliary policy-making function of the financial department to business agency also can full manifesting. Therefore it is the necessity to be established the corresponding financial group in view of the different business agency. The division of labor in the financial department could even more be clear. Considering the importance of the database and the data processing principle to the accountant work, besides the financial group based on business agency, but the accountant database maintenance group also will be supposed to establish, and so on.

6 Design of the Accountant real-time control pattern

Along with the IT development and the widespread application, the demand of the modern enterprise to accountant control is more and more high. The Accountant real-time control pattern is refers to the accountant controls application pattern which demand by the real-time control concrete constructs in view of the enterprise^[18]. It is carrier by the IT environment the implementation, and it is supported under accountant real-time control method and the making again in the flow. It is the safeguard mechanism which the accountant real-time control displays its control affected in the modern enterprise.

Generally says, the basic pattern of the accountant real-time control may divide into two kinds: A kind is the aileron control pattern, shows as Fig. 2, it is refers to the real-time control to the entire process of the enterprise manages moves (to Process of purchase, Stores in a storehouse, Produces, Sells, finance and so on). Its characteristic is that, Looked from the core enterprise interior, constructs the best integration flow of financial service in a line. In IT environment and the support of the accountant real-time control method and accountant personnel, to real-timely control to the thing flows and fund flows to the entire management process. And it coordinates various departments to have the foreword operation, and maximum limit reduces the stock. So it enhances the turnover of capital and guaranteed the efficiency and the benefit enhancement of the enterprise manages. Looked from the enterprise, it constructs a value chain to take the core enterprise as the main body, and coordinates the commercial relations between the enterprise. Through the information flow, and realizes the entire value chain increment.

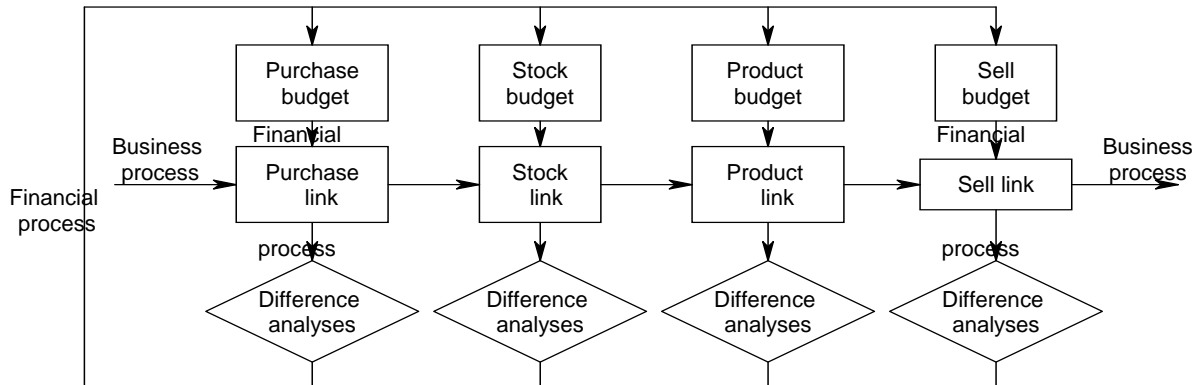


Fig.2 taileron accountants real-time control pattern

Another kind is the pitch control pattern, it refers to the real-time control which carries on to between fund movement of the enterprise group under surmounts the space and time. Its characteristic is that in IT environment and the support of accountant real-time control method and accountant personnel, will make the enterprise group member distribute in different local and city and even different nation connects in together through the information flow, and real-timely gains the fund dynamic information of entire enterprise group, and found out and keeps abreast of the overall fund situation, carries on the real-time control to settle accounts between member's fund and fund settlement of Exterior bank service and so on. It grasps the fund to flow to and the fund current capacity and the speed of flow of his regulation enterprise group. So it enhances the efficiency of fund use.

7 Conclusions

Along with the computerization accountant's production and the development as well as present network accountant's starting, the accountant's calculation function obtained full manifesting. At the same time, more and more experts devote fully to realize accountants manage function. At present the accountant real-time control is also only restricted in one idea, but not a kind of theory. The Accountant real-time control theory is the theory which has the broad application prospect. Especially along with the IT technology development, it will certainly be the important policy and the method which the enterprise implements to control. But at present it actually also has very many questions to need the further research, for example, the choosing of service data, the concrete design proposal of service database and the transformation from the service data to the accounting data and so on.

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The Truth about Global Warming

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Abstract: The real reason for global warming is the earth's orbit around the sun is decaying, in other words the earth is moving closer to the sun. This article describes The Truth about Global Warming. [The Journal of American Science. 2008;4(2):65-67]. (ISSN 1545-1003).

I'm an expert in the subject of global warming. The real reason for global warming is the earth's orbit around the sun is decaying, in other words the earth is moving closer to the sun. I've studied this phenomenon since July-1983, warning people of the coming destruction, and death. People called me crazy at first. I understand the weather was normal at that time, now the weather is beginning to support my findings. December- 2007: Houston, Texas is experiencing warm sunny winters, a record high of 81degrees. Eventually Houston's winters will completely disappear, as time goes on. Houston is the perfect place to observe global warming, what's occurring in Houston in winter will occur to the rest of the world. The sun is over the southern hemisphere now, and in the past the sun's peripheral heat and rays stayed within that part of the hemisphere, away from the equator, and the outer edges of the polar ice caps. The sun is thousands of times larger, than earth. The earth has moved, so close to the sun that it's peripheral rays, and heat has spread over the equator from the southern hemisphere to the southern part of the northern hemisphere, where Houston, Texas is located, and the sun's peripheral heat, and rays has spread over the outer edges of the south pole, and is melting the ice. The same thing will occur, when the sun reaches the northern hemisphere. The peripheral heat and rays will spread over the equator, and heat the northern part of the southern hemisphere, and melt the ice on the outer edges of the North Pole, Just ask the governments of Greenland, and Iceland. This trend will continue, until all the ice in both polar ice caps are melted, and until winter no longer exist in both hemispheres, back, and forth one polar ice cap at a time. There is enough ice in both polar ice caps to flood 90% of the existing land mass of this planet. The warmer the winters, the hotter the summers. The direct heat, and rays from the sun will intensify as the earth move closer, that's the area of the earth the sun is stationed directly over. I grew up in Houston, Texas. I remember the hotter part of the day use to be 12:00 noon, now its 5:00 o'clock in the after noon. This is more evidence of earth's orbit is decaying.

December- 2007: The thunderstorm, and floods that occurred in the States of Oregon, And Washington was suppose to by a snowstorm, after all its winter, but the atmosphere was too warm to support a snowstorm, so a thunderstorm was created instead, the flooding was extraordinary. The only different between a snowstorm and a thunderstorm is the temperature of the upper atmosphere. The position of the sun to the earth determines the temperature of the upper atmosphere. This is the type of weather that will dominate in the future, floods, and tornados during the winter months. The weather will go from one extreme to the other, from flooding to droughts in various parts of the United States, and the world. Food production will gradually come to a halt, because of the weather.

As I said in the past global warming has nothing to do with C02 gases, R-12 gases, CFC gases, a hole in the ozone, the sun going nova, nor methane gases leaking from the ocean's floor, as you will see in the future. Global warming will not be reverse by riding the atmosphere of these gases. Some of these gases have polluted the atmosphere since the industrial revolution in America, and Europe. In the early 20th century, before emission devices were installed on automobiles, and trucks the air in many cities was, so polluted it blotted out the sun, And cause respatory problems. There was no global temperature increase during this time period. Global warming is in its beginning stages, and will gradually get worse. It will not occur over night, and the winters will diminish gradually, over the decades. June-1978: I went to the mountains of Big Bear, California. It over looks the city of Los Angeles, California. The greenhouse gas emissions from automobiles, trucks, and industrial activity was, so bad a very noticeable thick haze formed

reducing visibility by 40%. There was no noticeable spike in temperature in Los Angeles, California, during that period. The reason the earth is moving closer to the sun the molten core of the planet is cooling, because its not getting enough crude oil (fuel). The oil companies drill into an oil well to extract the crude oil. These oil wells are actually self- pressurizing fuel cells, and over time the crude oil extraction process used by the oil companies releases the pressures needed to force the oil into the outer core. All oil wells (self pressurizing fuel cells) must be capped off, and the pressure within them brought back to normal, so the crude oil can be forced into the outer core. This will raise the temperature in the core, and strengthen the earth's magnetic field, and push the earth away from the sun. The higher the temperature in the core the stronger the earth's magnetic field, and the cooler the temperature in the core, the weaker the earth's magnetic field. The core is cooling, because it's not getting the fuel (crude oil) it once did, before man discovered crude oil, and new uses for it. Everything that generates energy, or expends energy needs fuel, and the earth is no different from any other machine. Animals derive their energy from food, automobiles from gasoline, computer from electricity, rockets from rocket fuel, thunderstorm, snowstorms, hurricanes, and tornados derive their energy from electricity, because these storms are electro- magnetic phenomenon. The earth generates a magnetic energy field, and it is derived from combustion of crude oil in its outer core. This is a man made situation, not the will of God. People take the earth's magnetic field for granted, because it's invisible, and silent. The magnetic field holds people, object, and the oceans to the surface of earth; it keeps the air we breathe from escaping into space. It protects life on this planet from the harshness, and radiation of space, it protects life on the surface of this planet from sun flares, it locks the earth in orbit around the sun, locks the moon in orbit around the earth, and keeps the earth at a safe distance from the sun.

Contrary to popular belief the electro- magnetic energy in thunderstorms, winter storms, hurricanes, and tornados are not generated by sunspots, neither sun flares, nor energy flares from deep space. The energy in these storms are generated by the earth magnetic field. The earth acts as a generator's armature. The earth turns at a thousand miles per hour, its magnetic field brushes against the magnetic field of the surrounding universe. The energy is trapped in the earth's atmosphere, and dispersed throughout the earth's atmosphere, and that's just some of the things earth's magnetic field does.

The earth is a self-contained biosphere. These fuel cells (oil wells) can be re-pressurized by igniting the methane gases in them. In fuel cells thought to be empty, such as spindle top in Beaumont, Texas. It will be necessary to pump in a mixture of air, and methane gas, and ignite the mixture. The gas will expand, when ignited creating the necessary pressure to force the remaining oil into the core. These fuel cells extend for thousands of miles, from the upper crust down to the outer core of the planet. The oil companies can only drill less, than ten miles down. There are millions of gallons of crude oil remaining in these fuel cells, and they are located all around this planet. This will increase the temperature in the outer core, and the outer core heats the inner core, which generates the earth's magnetic field. If left alone the temperature in the outer core will stabilize. This is the only way to save all life on this planet. The evidence that large quantities of crude oil is combusted, and sustain the high temperatures in the core. Every conceivable by-product of crude oil is ejected from volcanoes all around this planet, carbon dioxide, carbon monoxide, and sulfur dioxide, etc. There is a point of no return, because it will take decades to reheat the core back to normal temperatures. Volcanoes are the means by which the outer core rids itself of spent fuel, and volcanoes regulate the pressures in the outer core. Volcanoes extends from the surface of the planet down to the outer core. Volcanic eruptions in the past occurred more frequently, and they occurred in various parts around the planet, and were much more powerful, than volcanic eruptions in present days. This is more evidence the core is cooling. The more violent, and frequent the eruption, the higher the temperature in the core, and the cooler the temperature in the core, the less frequent, and the less violent the eruption. Many volcanoes are lying dormant, and haven't erupted in centuries. Crude oil is capable of generating temperatures found in the core, after all crude oil is a hydro- carbon, and hydrocarbons are used to melt, and manufacture steel. There are three types of hydro- carbons, crude oil- a liquid, methane- a gas, and coal- a solid. The tremendous pressures ejected from volcanoes are due to the combustion of crude oil in the outer core. The gases in all hydrocarbons expand, when ignited, and will create pressure in an

enclose vessel, such as the core of this planet. I pray someone read this letter, and is intelligent enough to see the truth. There are two different diagnoses, but only one right solution. My solution is the only correct one. The leading scientists are wrong about everything. Cleaning the air will not reverse global warming. Please keep an open mind. If we choose the wrong solution we will leave our grandchildren and great grandchildren a future that doesn't exist. The leading scientist will think of another excuse for global warming, when they realize they are wrong, and their plans are not working. Please don't let them do that, time is running out! Remember there is a point of no return.

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Assessment *In Vitro* Of The Biological Effect Of A Herbal Product Extract: Morphological And Radiolabeling Analysis

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Abstract: An increasing number of people in the world are using natural products as medicine which led many scientists to contribute to the research in this field. Also a few pharmacologists, after an initial phase of correct criticisms, today recognize the possibility of investigating the scientific value of medicinal products composed essentially of vegetable extracts. The constituents of herbal products can cause adverse effects. We evaluated the influence of a chayotte (*Sechium edule*) extract on the morphology of red blood cells and on the radiolabeling of blood elements with technetium-99m (99mTc). In our study, blood was withdrawn from *Wistar* rats. Samples of blood were treated with chayotte extract (decoct) in different concentrations (100; 50; 25; 12.5 and 6.25%v/v,) during 1 hour. After that blood was incubated with stannous chloride for more 1 hour. Elapsed this time 99mTc as Sodium pertechnetate was added and the incubation continued for more 10 minutes. Plasma (P) and blood cells (BC) were isolated, also precipitated with trichloroacetic acid and soluble (SF) and insoluble fractions (IF) separated. For the morphology analysis, samples of the blood were collected and smears were prepared. The blood smears were dried, fixed and stained. The analysis was done by video optical microscope using image pro-plus program. In our results it was observed that the referred extract was not capable of altering the radiolabeling of blood elements although it was capable to alter the morphology of red blood cells in the highest concentration. The effect of chayotte extract probably, could be explained by an effect which might alter the stabilizing activity of the red blood cell membrane. [The Journal of American Science. 2008;4(2):68-77]. (ISSN 1545-1003).

Key words: chayotte, red blood cells, morphology, technetium-99m, *in vitro*

Introduction

Natural medicines are increasingly used throughout the world, as they are considered to be effective and to have few side-effects (1). Traditional herbal medicines have been reported to cause serious hematological adverse effects. It is well-known, that lipid antioxidants can retard the oxidative rancidity of foods caused by atmospheric oxidation, and thus protect oils, fats, and fat-soluble components from their quality degradation. In the last few years, much emphasis has been put on the promotion and use of natural antioxidants, commonly occurring in many fruits and vegetables and thereby produced from various natural extracts (2). Many drugs and vegetable extracts have been reported to affect the radiolabeling of blood elements with 99mTc as well as the bioavailability of sodium pertechnetate (3,4). *Sechium edule* (chayotte) a subtropical vegetable with potent diuretic action, is a cucurbitaceous species which is used as food or as medication in popular medicine. It was reported a case of severe hypokalemia pregnancy and that a chayotte preparation was implicated, as the potassium level returned to normal, without recurrence of hypokalemia, once the ingestion of this vegetable was stopped (5, 6). Gordon (2000) described the antihypertensive effect of chayotte. Diré et al, 2001 have noticed that chayotte extract (macerated) was capable of altering the biodistribution of sodium pertechnetate. In a *in vivo* study, Diré et al, 2002 observed the extracts (decoct and macerated) of chayotte were capable to alter the radiolabeling of blood elements with 99mTc. The effects of natural products on the radiolabeling of blood elements have been studied by different researchers (9, 10, 11, 12, 13, 14, 15, 16). Mongelli et al, 1997 have showed that *Bolax gummifera* extract was used as a treatment of wounds probably due its properties related to the stabilizing activity of the red blood cell membrane. In a *in vitro* study developed by Oliveira et al, 2003 was remarked through a qualitative analysis that the *Fucus vesiculosus* extract altered the morphology of red blood cells. *F. vesiculosus* extract was capable to alter the radiolabeling of blood elements with 99mTc. In other study, Oliveira et al (2002), have shown that the *Paullinia cupana* extract have promoted alterations in the shape

of red blood cells and on the radiolabeling of blood elements with ^{99m}Tc . Similar results were obtained by Braga et al, 2000 in a comparative study with *Thuya occidentalis* and *Nicotiana tabacum*. In a qualitative *in vitro* study, was noticed a lightly morphological alterations on the red blood cells due to treatment of blood with *Maytenus ilicifolia*. In this same study was verified that the studied extract has altered the labeling of blood constituents with ^{99m}Tc (13). Tetechnetium-99m (^{99m}Tc) has been the most utilized radionuclide in nuclear medicine procedures (18) and it has also been used in basic research (19). The wide utilized in nuclear medicine is due to its optimal physical characteristics as half-life of 6h, gamma rays energy of 140 keV and minimal dose to the patients, convenient availability from $^{99}\text{Mo}/^{99m}\text{Tc}$ generator and negligible environmental impact. There are many applications of ^{99m}Tc -labeled red blood cells (^{99m}Tc -RBC), as in cardiovascular evaluations, in the detection of gastrointestinal bleeding and in the determination of the RBC mass in patients. RBC have been labeled with ^{99m}Tc through of *in vitro*, *in vivo* or *in vivo/in vitro* techniques (20, 21). The 3 foundations of nuclear medicine are radiation conscious personnel, specific radiopharmaceuticals and equipment. The trend in molecular radiopharmacy is to develop new radiopharmaceuticals targeting peptides and receptors. ^{99m}Tc -radiopharmaceuticals give important clinical and molecular information especially in endocrinology, oncology and cardiology. Nevertheless, there is not a well established model to study the influence of drugs (synthetic or natural) on the labeling of blood elements as well as molecules as peptides and receptors. Here, we have evaluated the influence of a chayotte extract on the labeling of blood elements with ^{99m}Tc using an *in vitro* technique and on morphology of the red blood cells (22).

Material and Methods

Characterization of the chayotte sample:

Chayotte was purchased from a local market in Rio de Janeiro city, RJ, Brazil. To prepare the extract, 50 g of skin of chayotte were mixtured with 500 mL of water in an electric extractor. This preparation was filtered and this extract was considered 100%.

The presence of toxic compounds was evaluated and we did not find them in the extracts of chayotte used in our experiments. The method to verify the presence of these toxic products is based on inhibition of acetylcholinesterase in the presence of the pesticides. In this method, brain acethylcholinestarse is utilized as an *in vitro* detector of organophosphorus and carbamate insectides. Briefly, a preparation of acetylcholinesterase was obtained after extraction of a rat brain microsomal fraction with Triton X-100 and was incubated with the extract of chayotte. Enzyme assay was performed by a potentiometric method based on the formation of acetic acid in the incubation mixture (preparation of acetylcholinesterase and extract of chayotte)

Preparing of the extract:

Heparinized whole blood was withdrawn from *Wistar* rats. Samples of 0.5 ml of blood were incubated with 100 μl of different concentrations (100; 50; 25; 12.5 and 6.25% v/v) of a preparation (decoct) of chayotte. To prepare the decoct of cahyotte, this vegetable (50 g) was put in an Erlenmeyer with 500 mL of saline solution (0.9% NaCl) and it was boiled on slow heat for ten minutes. After that, the solution was filtered and the watery extract was obtained.

Radiolabeling process:

Sechium edule preparation was incubated with samples of blood (0.5 mL) for 1 hour at room temperature. Samples of heparinized blood (0.5mL) which were incubated with saline solution (NaCl 0.9%) were utilized as control. Then, 0.5 mL of stannous chloride (1.2 $\mu\text{g}/\text{mL}$), as $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ (Reagen, Quimibrás Indústrias Químicas SA, Brazil) was added and the incubation continued for another 1 hour. After this period of time, ^{99m}Tc (0.1 mL), as sodium pertechnetate, recently milked from a $^{99}\text{Mo}/^{99m}\text{Tc}$ generator (Instituto de Pesquisas Energéticas e Nucleares, Comissão Nacional de Energia Nuclear, Brazil), was added and the incubation continued for another 10 min. These samples were centrifuged (clinical centrifuge) and plasma (P) and blood These samples were centrifuged and plasma (P) and blood cells (BC) were separated. Samples (20 μL) of P and BC were also precipitated with 1 mL of trichloroacetic acid (TCA) 5% and soluble (SF) and insoluble fractions (IF) were separated. The radioactivity in P, BC, IF-P,

SF-P, IF-BC and SF-BC were determined in a well counter. After that, the % of radioactivity (%ATI) was calculated. Statistical analysis (Kruskal Wallis test) was utilized to compare the experimental data.

Preparing of the blood smears

The blood homogenized was distended in the surface of a smear (26x76mm), after adequate cleaning, forming an angle of 45°. The distention was dried, agitating the smear in the air. The rapid desiccating is indispensable for a good conservation of the morphology of the cells and other elements.

Staining: May-Grünwald-Giensa method

The May-Grünwald-Giensa is a technique of staining which was utilized in this study. It has permitted us to visualize the cells through their distinct characteristics of staining, namely, according to the affinity of various cellular compounds towards the used dyes (23).

Technique of staining

The smears were putted upon a appropriated support and the staining technique followed: (i) distention with the dye of May-Grünwald-Giensa (Merck, Germany), for a period of 5 minutes, homogenized with a pipe; (ii) after that, the smear was covered with the dye of GIENSA (Merck, Germany), and again the surface of the smear was homogenized and left the dye acting for 10 minutes; (iii) the dye was despised and the smear was washed in the fluent water, dried in the room temperature in the vertical position.

The smears were evaluated under optical microscope of clear field (Eclipse E 400™), in the immersion objective (100x), with photographer ocular.

Morphometric analysis

The aim of this study was to evaluate the effect of the different concentrations of a chayotte extract on the morphology of red blood cells. It was analyzed the following parameters: the area (μm^2); maximum diameter (μm); minimum diameter (μm); perimeter (μm); sphericity (no dimensional-[/]) e perceptual of area (no dimensional - [/]), which correspond to the percentage of the number of cells counted per area. The quantification of the data was realized by the following equipment: Software image pro plus (media Cybernetics), according to the procedures: (1) capture of images in the gray schedule of 256 tons; (2) transformation of the image from gray schedule to binary schedule according to the calibrating threshold to detach red blood cells; (3) counting of cells with the use of the function *count/size* of the program which has permitted us: (i) title the objectives by the size; (ii) seep the objectives by the relation major and minor axis (iii) measure automatically the area, the perimeter and the sphericity; (4) the gauges were impressed in the archives ASCII, being exported to electronics tables and formatted to the programs STATISTICA® e SPSS® where the statistical tests were performed.

In the morphometric analysis were employed: (i) optical microscope of clear field (Nikon); (ii) photographic ocular; (iii) video camera CCD Sony DXC-151 A; (iv) microcomputer Pentium MMX 166 MHz, with 64 Mb of RAM memory, 256 de memory *Cache* and equipped with *frame grabber Matrix Vision* to the capture and to process the image; (vi) auxiliary monitor SONY KX-14CP1, (vii) printer HP 692C.

Results

Table 1 has shown the effect of a chayotte extract on the distribution of the radioactivity on the red blood cells and in the plasma. The analysis of the results indicates that there is not an alteration ($p > 0.05$) in the uptake of $^{99\text{m}}\text{Tc}$ by the RBC in the plasma.

Table 1- Effect of a chayotte extract on the labeling of blood cells and plasma with $^{99\text{m}}\text{Tc}$.

<i>Sechium edule</i>	Blood Cells	Plasma
	Percentage radioactivity	
control	94.81 ± 2.57	5.19 ± 2.57
6.25%	90.94 ± 6.18	9.06 ± 6.18

12.5%	93.93 ± 2.93	6.07 ± 2.93
25%	92.65 ± 3.45	7.35 ± 3.45
50%	90.68 ± 5.66	9.32 ± 5.66
100%	93.04 ± 4.97	6.96 ± 4.97

Sample of blood were incubated with different concentrations of chayotte extract. In the control blood was treated with saline solution (NaCl 0.9%). After that, stannous chloride and 99mTc were added. The ATI% was calculated. A statistical analysis (Kruskal Wallis test, n=5) was used to compare the results.

Table 2 has shown the effect of a chayotte extract on the distribution of the radioactivity in the plasma proteins. The analysis of the results indicates that there is not an alteration (p>0.05) in the liaison of 99mTc in the plasma proteins.

Table 2- Effect of a chayotte extract on the labeling of plasma proteins with 99mTc.

<i>Sechium edule</i>	Insoluble fraction	Soluble fraction
	Percentage radioactivity	
control	77.67 ± 7.44	22.33 ± 7.44
6.25%	81.53 ± 4.45	18.47 ± 4.45
12.5%	76.96 ± 8.63	20.01 ± 8.63
25%	72.22 ± 10.40	27.78 ± 10.40
50%	74.03 ± 9.76	25.97 ± 9.76
100%	72.69 ± 9.55	27.31 ± 9.95

Sample of blood were incubated with different concentrations of chayotte extract. In the control blood was treated with saline solution (NaCl 0.9%). After that, stannous chloride and 99mTc were added. The ATI% was calculated. A statistical analysis (Kruskal Wallis test, n=5) was used to compare the results.

Table 3 has shown the effect of a chayotte extract on the distribution of the radioactivity in the blood cells proteins. The analysis of the results indicates that there is not an alteration (p>0.05) in the fixation of 99mTc in the blood proteins.

Table 3- Effect of a chayotte extract on the labeling of cell proteins with 99mTc.

<i>Sechium edule</i>	Insoluble fraction	Soluble fraction
	Percentage radioactivity	
control	91.26 ± 3.57	8.74 ± 3.57
6.25%	90.03 ± 2.63	9.97 ± 2.63
12.5%	89.15 ± 3.34	10.85 ± 3.34
25%	91.37 ± 3.44	8.63 ± 3.44
50%	91.94 ± 1.77	8.06 ± 1.77
100%	91.36 ± 2.29	8.64 ± 2.29

Sample of blood were incubated with different concentrations of chayotte extract. In the control blood was treated with saline solution (NaCl 0.9%). After that, stannous chloride and 99mTc were added. The ATI% was calculated. A statistical analysis (Kruskal Wallis test, n=5) was used to compare the results.

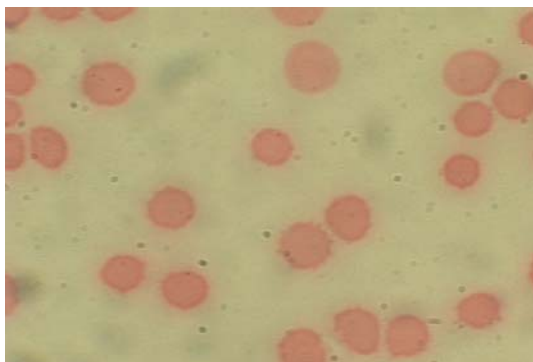


Figure 1. Photomicrography of blood smears prepared with samples of whole blood used to label blood elements with 99Tc. Samples of whole blood were incubated with NaCl 0.9% solution (control) for 60min. After that, stannous chloride was added and the incubation continued for 60min. Then, 99mTc, as sodium pertechnetate was added. Blood smears were carried out, dried, fixed and staining. Afterwards, the morphology of red blood cells was evaluated under an optical microscope (x1000).

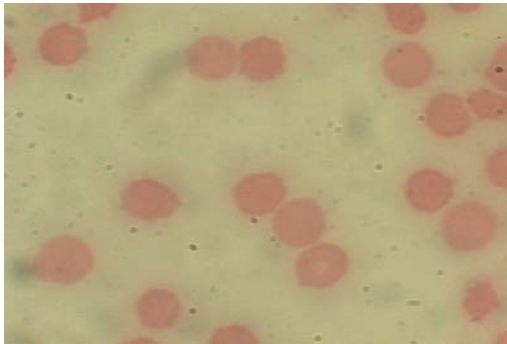


Figure 2. Photomicrography of blood smears prepared with samples of whole blood used to label blood elements with ^{99}Tc . Samples of whole blood were incubated with chayotte extract (6.25%) for 60min. After that, stannous chloride was added and the incubation continued for 60min. Then, $^{99\text{m}}\text{Tc}$, as sodium pertechnetate was added. Blood smears were carried out, dried, fixed and staining. Afterwards, the morphology of red blood cells was evaluated under an optical microscope (x1000).

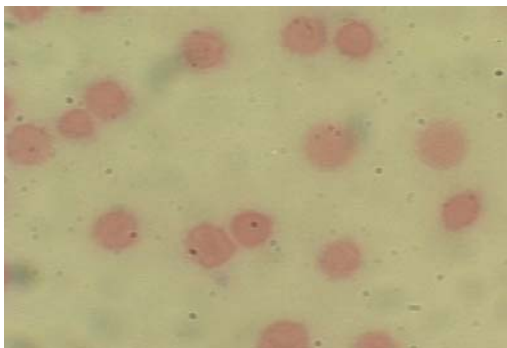


Figure 3. Photomicrography of blood smears prepared with samples of whole blood used to label blood elements with ^{99}Tc . Samples of whole blood were incubated with chayotte extract (12.5%) for 60min. After that, stannous chloride was added and the incubation continued for 60min. Then, $^{99\text{m}}\text{Tc}$, as sodium pertechnetate was added. Blood smears were carried out, dried, fixed and staining. Afterwards, the morphology of red blood cells was evaluated under an optical microscope (x1000).

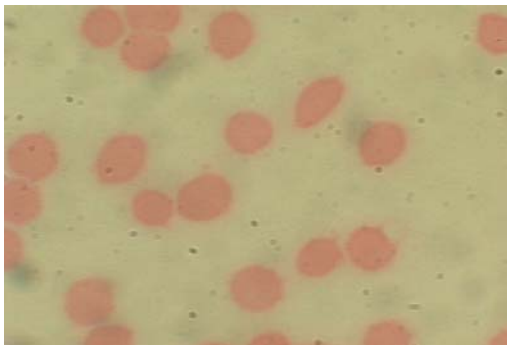


Figure 4. Photomicrography of blood smears prepared with samples of whole blood used to label blood elements with ^{99}Tc . Samples of whole blood were incubated with chayotte extract (25%) for 60min. After that, stannous chloride was added and the incubation continued for 60min. Then, $^{99\text{m}}\text{Tc}$, as sodium pertechnetate was added. Blood smears were carried out, dried, fixed and staining. Afterwards, the morphology of red blood cells was evaluated under an optical microscope (x1000).

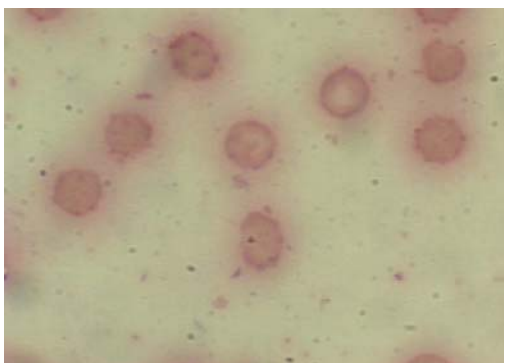


Figure 5. Photomicrography of blood smears prepared with samples of whole blood used to label blood elements with ^{99}Tc . Samples of whole blood were incubated with chayotte extract (75%) for 60min. After that, stannous chloride was added and the incubation continued for 60min. Then, $^{99\text{m}}\text{Tc}$, as sodium pertechnetate was added. Blood smears were carried out, dried, fixed and staining. Afterwards, the morphology of red blood cells was evaluated under an optical microscope (x1000).

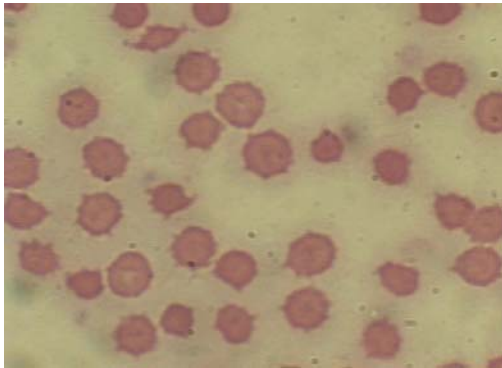


Figure 6. Photomicrography of blood smears prepared with samples of whole blood used to label blood elements with ^{99}Tc . Samples of whole blood were incubated with chayotte extract (100%) for 60min. After that, stannous chloride was added and the incubation continued for 60min. Then, ^{99}mTc , as sodium pertechnetate was added. Blood smears were carried out, dried, fixed and staining. Afterwards, the morphology of red blood cells was evaluated under an optical microscope (x1000).

Table 4 has shown the effect of a chayotte extract on the morphology of red blood cells. The analysis of the results indicates that there is an alteration ($p < 0.05$) on the shape of the cells.

Table 4- Effect of a chayotte extract on the morphometry of red blood cells.

Concentration %	Perimeter/ Area ($\mu\text{m}/ \mu\text{m}^2$)
Control	0.72 ± 0.07
6.25	0.71 ± 0.03
12.5	0.74 ± 0.01
25	0.76 ± 0.04
50	0.81 ± 0.09
100	0.91 ± 0.08

The blood smears were observed under optical microscope. In the treated group blood was incubated with chayotte extract (different concentrations 6.25; 12.5; 25; 50 and 100%) during 1 hour. In the control group blood was incubated with saline solution (NaCl 0.9%). The morphometric results were compared employing the ANOVA and Dunnet tests.

Discussion

The red blood cells may have alterations in their morphology which can indicate states of abnormality of the organism. It is of the fundamental importance the acquaintance of these alterations so as to the clinical full measure can have more efficacy due to the diagnostic of the patient. (24). Extracts obtained from various medical plants can alter the labeling of blood elements with ^{99}mTc as well as the morphology of red blood cells (3, 4, 9, 10, 11, 12, 13, 14, 15, 16). The developing of models that permit

evaluation of biologic properties of natural products is worthwhile. The evidence that drugs can affect either the radiolabeling as the biodistribution of red blood cells or the morphology of them in the context of nuclear medicine clinic has come to light only comparatively recently and it is an important factor in the interpretation of scintigraphic images. A great number of workers have turned their attention to *in vitro* and *in vivo* evaluation of drugs in the process to label blood cells and in the biodistribution of radiopharmaceutical (25, 26, 27, 28).

We have studied the effect of *Sechium edule* extract (decoct): (i) on the labeling of blood elements and (ii) on the morphology of red blood cells. In the labeling process of blood elements with ^{99m}Tc needs a reducing agent, and probably the stannous ion would be oxidized. In *in vitro* studies was verified that extracts of *Thuya occidentalis* (9), *Nicotiana tabacum* (10) and *Maytenus ilicifolia* (13), possibly, would have oxidants compounds, and the labeling of blood elements decrease in the presence of these extracts. By of a qualitative analysis it was remarked that *N. tabacum* and *T. occidentalis* have altered the shape of red blood cells and the radiolabeling of blood elements (12). In qualitative study, Oliveira et al, 2000 have shown that the extract of *M. ilicifolia* was only induced a lightly morphological alterations of red blood cells. In a research was verified that *Paullinia cupana* extract was capable to alter the radiolabeling of blood elements as well as to alter quantitatively the shape of red blood cells (15). In other *in vitro* study with *Fucus vesiculosus* extract was noticed that the referred extract has induced a qualitative alterations on the morphology of red blood cells together with alterations on the labeling of blood elements with ^{99m}Tc (16). In a *in vivo* studies Diré et al, 2002, have demonstrated that the chayotte extracts were capable to alter the radiolabeling of blood elements. Sastre et al, 1998, described that *Ginkgo biloba*, used for treating cognitive disturbances, has been reported to produce an anticoagulant effect by inhibition of platelet activating factor. In other study, Moreno et al, 2002, eyed that in a *in vitro* study the extract of *Ginkgo biloba* altered the morphology of red blood cells, the opposite, was observed in a *in vivo* study which this fact may be explained by the generate of metabolites *in vivo* without direct action on the morphology of red blood cells. It was reported by Santos-Filho & Ribeiro et al, 2002, that the extracts of *Mentha crispa* L. (mint) and *Piper methysticum* (Kava Kava) were capable to alter the morphology of red blood cells notwithstanding mint extract has also altered the radiolabeling process. Braga et al, 2000, in a *in vitro* study demonstrated that *Peumus boldus* did not alter the labeling of blood elements with ^{99m}Tc . Lima et al, 2002 in a *in vivo* study have shown that an extract of cauliflower (leaf) was not capable of altering the labeling of blood elements with technetium-99m. Diré et al, 2002, in a *in vitro* study eyed that the chayotte extracts were not capable to alter the radiolabeling of blood constituents. In the procedure of labeling RBC with ^{99m}Tc , the stannous and pertechnetate ions pass through the plasma membrane (19, 32). Then, as reported to the tobacco extract (10) and *Maytenus ilicifolia* extract (13), histological alterations of red blood cells could be responsible for the modifications on the labeling of RBC with ^{99m}Tc . Furthermore, we can speculate that if the chemical compounds present in the extracts could complex with these ions as a chelating agent, this fact could explain the decrease in the fixation of radioactivity on the blood elements. Diré et al, 2001, in a qualitative analysis *in vivo*, have eyed that a chayotte extract (macerated) has induced alteration on the shape of red blood cells. In this study the chayotte extract did not alter the radiolabeling although it was capable to induce qualitative and quantitative alterations on the shape of red blood cells, in question to this fact, we can suggest like observed by Mongelli et al, 1997, in a study with *Bolax gummifera* extract, that the chayotte extract is able to stabilizer the active of red blood cell membrane even though the morphology of the cell has been altered.

Conclusion

In general, we can conclude that *Sechium edule* extract is not capable of altering the labeling of blood elements with ^{99m}Tc although it has altered the morphology of red blood cells. This fact could be due to the presence of compounds which were not strong enough to complex with pertechnetate and stannous ions but the sufficient to modify the architecture of plasma membrane without interfere in the transport of ions by the cell.

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Deterioration of Soil Organic Components and Adoptability of Green fallows for Soil Fertility Replenishment

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Abstract: We studied the adoptability of green fallow technology in soil fertility regeneration in Owerri agricultural zone, southeastern Nigeria in 2005. A well-structured interview schedule was used in collecting socio-economic data. Using target soil survey sampling technique, soil samples were collected to evaluate fertility status in continuously cultivated soils. Soil samples were air-dried and passed through 2-mm sieve before they were subjected to routine laboratory analyses. Socio-economic and soil data were analyzed statistically using some descriptive and inferential statistical tools. Results indicated the influence of age, education and farm size on willingness to adopt green fallow periods while soil fertility indices of organic matter and total nitrogen showed that soils were highly deteriorated. While soil organic matter showed significant ($p=0.05$) relationship with available phosphorus and cation exchange capacity, total nitrogen exhibited strong relationship with available phosphorus, pH and cation exchange capacity at 5% level of significance. Studies on the restorative capacities of some tropical plant species should be conducted to ascertain their efficacy. [The Journal of American Science. 2008;4(2):78-84]. (ISSN 1545-1003).

Keywords: Adoption, degradation, organic matter, socio-economy, soil fertility regeneration

Introduction

The rapidity of soil organic matter decline in tropical soils is worrisome as it is a principal factor in soil quality of the biome. Low content of soil organic components has been attributed to shortened fallow cycles (Aikorie et al., 2003). Poor management practices (Pagliai et al., 1998), changes in microbial population (Whalley et al., 1995), decline in microbial chemistry (Piovanelli et al., 1998), bush burning (Reich et al., 2001), deforestation (Cattaneo, 2002), long-term tillage (Hooker et al., 2004., Wright and 2005, Hons, 2005), harvest of forest products (Hassled and Zak,), mining (Onweremadu, 2007) and poverty (Place et al., 2005; Smith et al., 2006). Of all these causes of low organic composition of soils, deforestation takes a great toll in sub-Saharan Africa, and indeed the tropical world. A comprehensive assessment of the state of the world's forest released by the Food and Agriculture Organization of the United Nations (FAO) indicates that total forested area continued to decline significantly in the 1990s (FAO, 1999). Based on this analysis, deforestation is concentrated in the developing countries, which lost approximately 62 million hectares between 1990 and 1995, with an average annual loss of 12.5 million hectares.

In central southeastern Nigeria, there is increased deforestation and resultant erosion damages of soil resource (Oti, 2007). Erosive activities in the agro-ecology have led to a decline in organic matter (Mbagwu and Obi, 2003). Consequently, there is reduced biological activity, adverse changes in physical properties of soils, adverse changes in soil nutrient status and build-up of toxicities.

In the light of the above, several soil fertility enhancing practices have been suggested with little success due to increasing population and poverty which consequently resulted in pronounced degradation of soil resources. Common soil conservation practices in the area include mulching, mixed cropping, terracing and ridging (Ogbonna et al., 2006) but whose efficacy has declined (Matthews-Njoku and Onweremadu, 2007). Adverse climatic conditions coupled with fragile soils of the study area require a conservation technique that minimizes high erosivity of rainfall in the agroecology. It is based on this premise that we suggest the use of green fallows. Green fallow periods restore soil fertility quickly and

reduces the competitiveness of weeds on farmlands (Van Scholl, 1998). However, adoption of this technology is inter alia a function of socio-economic factors (Ogbonna et al., 2006; WOCAT,2007). The major objective of this study was to investigate the status of organic components of soils of the study site while estimating the adoptability of green fallow periods as soil fertility-enhancing strategy.

Materials and Methods

Study Area: The study was conducted in Owerri agricultural zone in 2005. Owerri agricultural zone (Latitudes 5° 15' -5°45'N; Longitudes 6°45' -7°30'E) is located in Imo State, Southeastern Nigeria. It has a land area of about 3000 km² and consists of eleven local government areas. Soils of the area are derived from Benin Formation (Coastal Plain Sands). The area has a humid tropical climate with an average annual rainfall of about 2500 mm and mean annual temperatures ranging from 26-30 °C. It has 3 distinct months of dry spell. Owerri agricultural zone is characterized by a highly depleted rainforest vegetation due to high demographic pressure. The Imo River and others such as Otamiri, Mbaa, Uramiriukwa, Ogochic, Okitankwo and Nworie contribute to hydrology of the agricultural zone. A variety of socioeconomic activities abound ranging from farming, cottage industrialization, fishing, hunting, sand mining and automobile servicing . Owerri agricultural zone houses the seat of government, and this influences socio-economic activities of the area. However, a majority of traditional farming practices including slash- and- burn clearing are retained in its agriculture. But, increase in population has altered the traditional long fallows to shortened ones and in severe cases, continuous cultivation is practiced irrespective of declining yield.

Field Studies: Field sampling was conducted in 2006, involving three local government areas namely Ikeduru, Ezinihitte Mbaise and Owerri North. These local government areas were purposively selected based on the intensity of deforestation, and consequent land degradation. Three towns; Amakohia, Akabo and Eziam (Ikeduru), Onicha, Amumara and Udo (Ezinihitte Mbaise), and Emii, Nekede and Ulakwo (Owerri North) were randomly selected. A total of 180 project farmers constituted the sample size for the study. The target population was about 21, 000 project- farmers in Owerri agricultural zone.

A well- structured interview schedule was developed and used in the study,. The structured interview schedule was validated using the content validity technique (Chuta, 1992). All items contained in the draft interview schedule for the study were subjected to thorough examination and criticism by three lecturers of Department of Agricultural Extension, Federal University of Technology, Owerri, Nigeria. The final structured interview was certified by the expert opinions of these lecturers. Socioeconomic variables studied include gender, age, education, membership of social organization and farm sizes

In addition to the above 10 surface soil samples were collected from continuously cultivated owner-managed farm in each town, giving a total of 60 soil samples for the study. These soil samples were air-dried, gently crushed and passed through 2-mm sieve in readiness for laboratory analysis.

Laboratory Analysis: Exchangeable basic cations (Ca, Mg, K and Na) were estimated by inductively coupled plasma atomic emission spectrometer (ICP- AES- Integra XMO, GBC, Arlington Heights, Il). Cation exchange capacity was determined by repeated saturation using 1 M NH₄OAc followed by washing, distillation and titration (Soil Survey Staff, 1996). Available phosphorus was measured by Olsen method (Emteryd, 1989). Total nitrogen was determined by Kjeldahl digestion with a Kjeltac Auto 1030 System (Tecator Hogan as, Sweden. Total carbon was determined by combustion on a Leco Model 521- 275 (Leco Corporation, Svenka AB Upplands, Vasby, Sweden) and soil organic matter was estimated by multiplying carbon content by a factor of 1.724. Soil pH was measured (1:1 soil/ water) in water (Thomas, 1996). Particle size distribution was determined by hydrometer method (Gee and Or, 2002).

Statistics: Descriptive statistical tools were used in analyzing socioeconomic and soil data. Willingness to adopt green fallow period technique was regressed to some socio-economic characteristics. Multiple regression model was used as shown below.

$$Y = a + b_1x_1 + b_2x_2 + b_3x_3 + b_4x_4 + e \dots 1$$

Where Y= willingness to adopt green fallows

a = intercept
 b1-b4 = regression coefficients
 x₁ = age
 x₂ = education
 x₃ = membership of social organization
 x₄ = farm size
 e = error term

Results

Respondent-farmers were mainly females (70 %) of youthful age (21-40) (70 %) with a majority of them attaining secondary education (Table 1). In addition to the above, these farmers belonged to 1-4 social organizations (85.9 %) but possessing about 2 hectares of farm size (80.1%). Farm size, education and age significantly (P = 0.05) influenced willingness of respondent-farmers to adopt green fallow technique. Soil fertility indicators, namely organic matter, cation exchange capacity, pH, total nitrogen, available phosphorus and base saturation are shown in Table 3, indicating poor fertility status of soils using existing standards (FMANR, 1990; SPDC, 2003). Soils exhibited high degree of sandiness when compared with other particle sizes. Significant relationships (P= 0.05) were established among soil properties (Table 4). Soil organic matter was related with total nitrogen, cation exchange capacity, available phosphorus, clay and silt content. Soil pH had good relationships with available phosphorus, cation exchange capacity, total nitrogen, sand, silt and base saturation

Table 1. Distribution of respondents according to socio-economic characteristics (180).

Socioeconomic characteristics	Percentage
Gender	
Male	30
Female	70
Age (years)	
21-30	20
31-40	50
41-50	25
51-and above	5
Education	
No formal education	2.2
Primary education	36.3
Secondary education	52.6
Post- Secondary education	8.9
Membership of social organizations	
1-2	46.1
3-4	39.8
5-6	14.1
Farm size (Ha)	
1.0	42.0
1.1-2.0	38.1
2.1-3.0	19.9
Less than 3.1	14.6

Source: Field Survey Data, 2006.

Table 2. Multiple regression analysis on the relationship between willingness to adopt green fallow and socioeconomic variables (n=180).

Independent Variable	Coefficient	SE	T-Value	F-ratio	R ²
Constant	112	0.77	14.26*	3.26	0.38
Age	-7.48	0.09	-6.24*		
Education	11.88	0.03	7.22*		
Membership of Social Organization	7.16	0.08	0.96*		
Farm Size	-15.43	0.04	-8.36		

Table 3 Soil properties of studied sites (mean value of sites)

Location	Sand (%)	Silt (%)	Clay (%)	pH (water)	OM (%)	BS (%)	TN (%)	Av.P (p.p.m)	CEC (meq/100g)
Ikeduru	86	2	12	4.7	2.6	38	0.12	6.2	5.6
Amakohia									
Akabo	84	6	10	4.6	2.4	35	0.109	5.7	5.2
Eziama	89	2	9	4.2	1.9	28	0.016	4.0	4.8
Ezinihitte									
Mbaise									
Amumara	83	4	13	4.8	2.8	36	0.128	6.8	6.0
Onicha	90	1	9	4.4	2.0	29	0.019	4.4	6.6
Udo	89	4	7	4.4	2.1	29	0.100	4.6	5.0
Owerri									
North									
Emii	82	3	15	4.9	2.9	36	0.17	7.2	6.1
Nekede	88	8	4	4.0	1.7	21	0.011	3.7	4.4
Ulakwo	85	3	12	4.6	2.3	33	0.102	5.3	5.0

OM= organic matter, BS= base saturation; TN= total nitrogen, Av.P= available phosphorus, CEC = cation exchange capacity.

Table 4. Correlation matrix for linear relationships between soil parameters (n = 90)

	OM	TN	Av.P	CEC	pH	Clay	Silt	Sand	BS
OM									
TN	0.72*								
Av.P	0.68*	0.46*							
CEC	0.73*	0.38*	0.61*						
pH	0.28 ^{NS}	0.41*	0.79*	0.43*					
Clay	0.51*	0.35 ^{NS}	0.48*	0.74*	0.46*				
Silt	0.43*	0.22 ^{NS}	0.23 ^{NS}	0.51*	0.19*	0.19 ^{NS}			
Sand	0.15 ^{NS}	0.19 ^{NS}	0.09 ^{NS}	0.16*	0.56*	0.44*	0.09 ^{NS}		
BS	0.20 ^{NS}	0.17 ^{NS}	0.33 ^{NS}	0.42*	0.39*	0.46*	0.25 ^{NS}	0.24 ^{NS}	

OM= organic matter, TN=total nitrogen Av.P =available phosphorus, CEC = cation exchange capacity, BS=base saturation, significant at P=0.5, NS= not significant

Discussion

Dominance of the female population is indicative of the need for their consideration in agricultural policies. The implication of this result is the extension services needed to spread the adoption campaign for green fallows must focus on women associations, especially those that are farmer-oriented. Women had been identified as having capabilities and resources for generating food security for their families in the sub-Saharan African countries (Brown et al., 2001). But, these potentials of women are hindered by socio-cultural factors in the southeastern Nigeria (Mgbada, 2007). Interestingly, majority of the farmer-respondents dominated by the feminine gender attained secondary education, suggesting greater possibility of adoption of green fallows since educated person understands innovations faster than the illiterate one. Further agricultural extension services can be directed to the social organizations since many of them belong to minimum of one social grouping. In addition, the ownership of 2 hectares of farmland by 80.1% of the surveyed population portends greater propensity to adopt since such farm sizes under intensive management could be fairly profitable in small to medium scale arable agriculture, notwithstanding the crop type. These statements are confirmed by the significant ($P = 0.05$) influence of farm size, education and age (Table 2) on the adoptability of green fallow periods. Unavailability of land or reduced farm size was identified as a principal reason for the discontinuance of a technology in southeastern Nigeria (Nnadi and Akwiwu, 2007).

Values of soil fertility indices (Table 3) call for soil fertility regeneration measures including green fallow period technology. Earlier studies in the same agroecology identified Ca: Mg imbalances (Oti, 2002), low values of basic cations (Onweremadu, 2007), preponderance of acidic cations (Esu, 2005), soil structural degradation (Onweremadu et al., 2007) and low organic matter content (Mbah et al, 2007). Sandiness, strong acidity, low organic matter composition, low values of available, low base saturation as well as extremely low values of cation exchange capacity in soils of the study site are a result of interaction between harsh tropical climate, increasing demographic pressure and fragile nature of soils. However, these changes in soil properties varied in space (Onweremadu and Akamigbo, 2007) but are aggravated by soil erosion by the agency of water (Igwe, 2003). In the studied soils, organic matter had significant ($P = 0.05$) correlation with some soil fertility indices (total nitrogen, available phosphorus and cation exchange capacity), implying that adoption of green fallow period technology will certainly promote organic matter accumulation in these soils thereby improving soil fertility.

Conclusion

The study showed that socio-economic factors of age, education and farm size influenced willingness to adopt green fallow technology. Again, generated soil data indicated soil infertility in the study area while identifying organic matter as principal factor, having significant ($P = 0.05$) effect on the status of total nitrogen, available phosphorus and cation exchange capacity. Further studies should consider efficacious and adaptable plant species useful in green fallow technology in the study area.

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Feb 27, 2008 9:28 PM

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Know Thyself

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Abstract: An interpretation of quantum mechanics will be introduced in harmony with the theory of relativity. Similarities between the paradoxes of the theory of relativity and quantum mechanics will be discussed in order to demonstrate that the theories although different in essence have an identical form. The system of arithmetic also appears to have a similar form although it is much more transparent and will therefore be used as guidance. All three systems appear to be casts of the same mould or matrix and the outside of this matrix will be explored. [The Journal of American Science. 2008; 4(2):85-87].

Key words: contradiction; paradox; self-evident; spookiness; incompleteness; Matrix

Introduction:

Though many different interpretations of quantum mechanical behaviour have been created, all of them have one thing in common: they create some kind of undesired by-product. Instead of looking for a new interpretation a combination of two existing interpretations will be used to synthesize a hybrid interpretation. The combining will be achieved by mixing Everett's Many-worlds interpretation with the Copenhagen interpretation. This hybrid interpretation will contain the positive parts of both interpretations while leaving out the negative ones. The positive part of the Many-worlds interpretation lies in the fact that its worlds are real. The negative part is the absurdity of the multitude of universes and will therefore be left out. In the Copenhagen interpretation the negative part is the often debated collapsing of the wave function and will therefore be disposed of, while its single universe will be used. The created hybrid or Many-worlds-one-Universe interpretation can be understood as follows: all parallel real worlds are only different perceptions of the same universe. It is needless for a wave function to collapse because all possible observations happen in different parallel worlds. It is needless for a universe to split into a multiverse because all parallel worlds are only different perceptions of the same universe. All parallel worlds, although all different are equally real and therefore bound to be unreal.

This Many-worlds-one-Universe interpretation is actually already in use for the relativity theory. In that every coordinate system can be experienced as a real world. There exists a multitude of real worlds all describing the same universe, though each world resulting in a different reality (lorentz contraction, time dilation). All these different worlds are only different perceptions of the same universe. All these different worlds are equally real and therefore bound to be unreal.

Discussion:

Although the essence of the theory of relativity and quantum mechanics is different their form appears to have similarities. There is another logical system which has a shape similar to the former two systems, but which is more transparent. This logical system is arithmetic and it too has a special and a general case. The special case occurs if one only looks at the differences between numbers (only addition and subtraction). When you're inside the system of arithmetic, you look around, what do you see? Numbers, signs, symbols, equations all appear to be under control. But if one is willing to freeze the most basic equation and take a step backwards one can become aware of the following paradox.

$$0 - 0 = 0$$

The difference between equals, equals sameness.

This paradox is the transparent form of the clock paradox in special relativity; the difference between a moving clock and an unmoving clock equals sameness. Similarly, the paradox of Schrödinger's cat; the difference between a living cat and a dead cat equals sameness [1]. Both paradoxes take place in the special case of each theory (without acceleration between the clocks and without entanglement between observer and cat) and can be seen as different casts of the same mould. The source of these paradoxes lies in this: the observer tries to accept both parallel worlds as real without being entangled with either one of them. Thus the observer tries to detach from reality while still being part of it. The observer therefore supposes that the realness of reality is equivalent to the complete reality. But reality is also responsible for its own realness and therefore proves its own truthfulness. Kurt Gödel already demonstrated with his incompleteness theorem that such a self-evident self-fulfilling systematic realisation of reality will either be inconsistent or incomplete [2].

It can now be seen clearly why, in the end, the agents representing the scientific community could only come up with calculations as an answer to Herbert Dingle's inquiry to this clock paradox [3]. The calculations are self-evident and avoid answering the paradox, which lies beyond the self-evident. Dingle's proposition though, of deleting relativity can be compared with arithmetic going back to the roman numbering system. His failure of realising the true nature of this paradox resulted in a mind being imprisoned by ignorance.

One can expand the special system of arithmetic into a general system, by allowing relationships between numbers (multiplication and division). In this case the following question can be formulated revealing the incompleteness of arithmetic accompanied with its paradoxical statement:

$$0 \div 0 = ?$$

The relationship between identicals, identifies unrelatedness.

This shows that within arithmetic one can formulate a question whose answer is un-embodied, thus revealing the incompleteness of arithmetic. This un-embodiment can be referred to as the spookiness of a system. The paradox is the transparent form of Einstein, Podolsky and Rosen's (EPR) paradox and also the twin paradox in the general case [4] [5]. The EPR paper does indeed detect the incompleteness of quantum mechanics, because Einstein's spooky action is un-embodied by the system of quantum mechanics, though a direct result of this system. It shows that the reasoning done by the EPR trio is indeed consistent and the spooky action is its inevitable result. This spookiness was already prophesised four years earlier by Gödel's incompleteness theorem as the shape of inevitability. The EPR trio's twisting conclusion at the end of the EPR paper of dodging spookiness and praising local action reflects a kind of cubicle bliss instead of a thorough comprehension of Gödel's work. The failure of realising the true nature of this paradox results in the mind being imprisoned by ignorance.

This spookiness can also be found in Einstein's solution of the twin paradox, although Einstein himself left the spookiness unmentioned. Einstein's explanation incorporates a homogeneous gravitational field that appears and disappears instantaneously and such an appearance, although an inevitable consequence, is un-embodied by the theory of relativity [6]. This spookiness was pointed out by Geoffrey Builder in 1957, though he too was led astray and failed to realise the true nature of the paradox [7]. The rejection of Einstein's solution of the twin paradox continues to cause a glitch within the scientific program. <System Failure>

The action of an individual measurement which makes a collapsing wave function appear can be compared with the action of an individual acceleration which makes a homogeneous gravitational field appear (continuous measurement or acceleration consists of many individual measurements or accelerations). The self-evident consciousness of the observer is in both cases uploaded with a "new" parallel world. It is the popping up of this "new" parallel world that creates these non-local appearances. It will be impossible for the observer to return to the "former" parallel world and hereby undo the consequences of measurement or acceleration. The appearance of particle entanglement and the appearance of time dilation are as equally real as everything else in the world and are therefore impossible to undo by uploading "backwards". This continuous popping up of a "new" reality is experienced by the self-evident conscious observer as the flux of time.

Conclusion:

Both relativity and quantum mechanics appear to have similarities in their form and can be seen as casts of the same mould or matrix. The system of arithmetic also seems to be moulded by this matrix, hereafter called Matrix [8]. The system of arithmetic is transparent enough to be used as a platform or training program in order to develop an understanding about human understanding. Kurt Gödel already demonstrated the value of this training program with his incompleteness theorem. This theorem prophesises the inevitable incompleteness of non-trivial sufficiently strong consistent logical systems (e.g. arithmetic) and demonstrates a useful pathway for facing paradoxes.

The human intellect appears to be born inside some kind of Matrix, for understanding appears to be moulded inside it. Everything that has an inside has an outside and paradoxes are the gateway towards this outside. For as long as a human being is attached to the bliss of ignorance (and of course ignores this very fact) paradoxes will be experienced as contradictions and the intellect sees the gateway as a blind alley. These "contradictions" are like a splinter in the mind, separating the obvious (self-evident) inside from the spooky (unself-evident) outside. Hence for the uncontrolled mind the Matrix appears as control, and in this way the Matrix separates and protects. When the mind loses ignorance it becomes more clear, contradictions start transforming into paradoxes and a gateway appears. The Matrix opens and unprotects. Once one is able to completely morph the inside and the outside into one without bending the Matrix, then finally can the faceless be faced.

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8. Online Etymology Dictionary, Douglas Harper, 2001. Matrix: 1373, from Old French matrice, from Latin matrix (gen. matricis) "pregnant animal," in Late Latin "womb," also "source, origin," from mater (gen. matris) "mother." Sense of "place or medium where something is developed" is first recorded 1555; sense of "embedding or enclosing mass" first recorded 1641. Logical sense of "array of possible combinations of truth-values" is attested from 1914.

Sterol Regulatory Element Binding Proteins (SREBPs)

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Abstract: Sterol regulatory element binding proteins (SREBPs) are transcription factors that bind to the sterol regulatory element DNA sequence TCACNCCAC. SREBPs belong to the basic-helix-loop-helix leucine zipper class of transcription factors. Unactivated SREBPs are attached to the nuclear envelope and endoplasmic reticulum membranes. In cells with low levels of sterols, SREBPs are cleaved to a water soluble N-terminal domain which is translocated to the nucleus. These activated SREBPs then bind to specific sterol regulatory element DNA sequences which upregulate the synthesis of enzymes involved in sterol biosynthesis. Sterols in turn inhibit the cleavage of SREBPs and therefore synthesis of additional sterols is reduced through a negative feed back loop. [The Journal of American Science. 2008;4(2):88-94]. (ISSN 1545-1003).

1. Introduction

Sterol regulatory element binding proteins (SREBPs) are transcription factors that bind to the sterol regulatory element DNA sequence TCACNCCAC (Chen, Chen et al. 2006; Rasmussen, Blobaum et al. 2008). SREBPs belong to the basic-helix-loop-helix leucine zipper class of transcription factors (Brown and Goldstein 1997). Unactivated SREBPs are attached to the nuclear envelope and endoplasmic reticulum membranes (Sakai, Nohturfft et al. 1997). In cells with low levels of sterols, SREBPs are cleaved to a water soluble N-terminal domain which is translocated to the nucleus (Zhang, Shin et al. 2005). These activated SREBPs then bind to specific sterol regulatory element DNA sequences which upregulate the synthesis of enzymes involved in sterol biosynthesis (Yokoyama, Wang et al. 1993; Wang, Sato et al. 1994). Sterols in turn inhibit the cleavage of SREBPs and therefore synthesis of additional sterols is reduced through a negative feed back loop (Wikipedia, 2008).

Beginning with the discovery of the SREBPs in 1993, a productive combination of biochemistry, molecular biology and genetics, has brought to light the complex mechanisms by which animal cells maintain the proper levels of intracellular lipid (fats and oils) in the face of widely varying circumstances (lipid homeostasis) (Brown and Goldstein 1999; Brown, Ye et al. 2000). These studies exposed a signaling mechanism of beguiling complexity that is responsible for the end-product feedback regulation of gene transcription. For example, when cellular cholesterol levels fall below the level needed, the cell makes more of the enzymes necessary to make cholesterol. A principal step in this response is to make more of the mRNA transcripts that direct the synthesis of these enzymes. Conversely, when there is enough cholesterol around, the cell stops making those mRNAs and the level of the enzymes falls. As a result, the cell quits making cholesterol once it has enough.

The defining feature of the SREBP pathway is the proteolytic release of a membrane-bound transcription factor, SREBP. Proteolytic cleavage frees it to move through the cytoplasm to the nucleus. Once in the nucleus, SREBP can bind to specific DNA sequences that are found in the control regions of the genes that encode enzymes needed to make lipids. This binding to DNA leads to the increased transcription of the target genes.

The ~120 kDa SREBP precursor protein is anchored in the membranes of the endoplasmic reticulum and nuclear envelope by virtue of two membrane-spanning helices in the middle of the protein. The precursor has a hairpin orientation in the membrane, so that both the amino-terminal transcription factor domain and the COOH-terminal regulatory domain face the cytoplasm. The two membrane-spanning helices are separated by a loop of about 30 amino acids that lies in the lumen of the endoplasmic reticulum. Two separate, site-specific proteolytic cleavages are necessary for release of the transcriptionally active amino-terminal domain. Regulation of SREBP cleavage employs a notable feature of eukaryotic cells, subcellular compartmentalization defined by intracellular membranes, to ensure that cleavage occurs only when needed.

2. SREBP-1 and SREBP-2

The mammalian gene for SREBP-1 contains two promoters that control the production of two proteins, SREBP-1a and -1c, and each contains a unique N-terminal transcriptional activation domain, but they are otherwise identical. The relative level of each mRNA varies from tissue to tissue and they respond differently to regulatory stimuli. SREBP-1c is more abundantly expressed in liver, where its level is also regulated by insulin and liver X receptor activators, and it is also autoregulated by SREBPs. In contrast, SREBP-1a mRNA levels are relatively low and constant in different tissues and few studies have specifically analysed its pattern of expression and regulation. According to the studies by Zhang and Shin, the promoter for SREBP-1a is contained in a very small promoter-proximal region containing two Sp1 sites. The small and relatively simple structure for its promoter provides an explanation for the low level of SREBP-1a expression. Additionally, since Sp1 has been implicated in the modest regulation of several genes by insulin, its involvement in the expression of the SREBP-1a promoter provides an explanation for the modest insulin regulation observed in animal experiments (Zhang, Shin et al. 2005). SREBP-2 regulates the genes of cholesterol metabolism.

SREBP-1a is a unique membrane-bound transcription factor highly expressed in actively growing cells and involved in the biosynthesis of cholesterol, fatty acids, and phospholipids. Because mammalian cells need to synthesize membrane lipids for cell replication, the functional relevance of SREBP-1a in cell proliferation has been considered a biological adaptation (Nakakuki, Shimano et al. 2007).

The 5' end of the mRNA-encoding SREBP-1 exists in two forms, designated 1a and 1c. The divergence results from the use of two transcription start sites that produce two separate 5' exons, each of which is spliced to a common exon 2. Mutations in the sterol regulatory element binding protein gene (SREBF)-1 may contribute to insulin resistance states. However, the variants described to date do not affect the SREBP function (Vernia, Eberle et al. 2006).

3. SREBP and diabetes

Diabetic renal disease is associated with lipid deposits in the kidney. In 2002, Sun et al made the study to determine whether there is altered regulation of the sterol regulatory element-binding proteins (SREBPs) in the diabetic kidney and whether SREBPs mediate the abnormal renal lipid metabolism and diabetic renal disease. In streptozotocin-induced diabetes in the rat, there were marked increases in SREBP-1 and fatty acid synthase (FAS) expression, resulting in increased triglyceride (TG) accumulation. Treatment of diabetic rats with insulin prevented the increased renal expression of SREBP-1 and the accumulation of TG. The role of hyperglycemia in the up-regulation of SREBP-1 was confirmed in renal cells cultured in a high glucose media. High glucose induced increased expression of SREBP-1a and -1c mRNA, SREBP-1 protein, and FAS, resulting in increased TG content. To determine a direct role for SREBP in mediating the increase in renal lipids and glomerulosclerosis, they studied SREBP-1a transgenic mice with increased renal expression of SREBP-1. The increase in SREBP-1 was associated with increased expression of FAS and acetyl CoA carboxylase, resulting in increased TG content, increased expression of transforming growth factor beta1 and vascular endothelial growth factor, mesangial expansion, glomerulosclerosis, and proteinuria. Their study therefore indicates that renal SREBP-1 expression is increased in diabetes and that SREBP-1 plays an important role in the increased lipid synthesis, TG accumulation, mesangial expansion, glomerulosclerosis, and proteinuria by increasing the expression of transforming growth factor beta and vascular endothelial growth factor (Sun, Halaihel et al. 2002).

SREBP-1c is intimately involved in the regulation of lipid and glucose metabolism and SREBP-1c gene might influence diabetes risk and plasma cholesterol level (Laudes, Barroso et al. 2004).

ABC transporter A1 (ABCA1) mediates and rate-limits biogenesis of high density lipoprotein (HDL), and hepatic ABCA1 plays a major role in regulating plasma HDL levels. HDL generation is also responsible for release of cellular cholesterol. In peripheral cells ABCA1 is up-regulated by the liver X receptor (LXR) system when cell cholesterol increases. However, cholesterol feeding has failed to show a significant increase in hepatic ABCA1 gene expression, and its expression is up-regulated by statins (3-hydroxy-3-methylglutaryl-CoA reductase inhibitors), suggesting distinct regulation. Compactin activated the novel liver-type promoter in rat hepatoma McARH7777 cells by binding SREBP-2. In contrast, compactin repressed the previously identified peripheral-type promoter in an LXR-responsive element-dependent but not E-box-dependent manner. Thus, compactin increased the liver-type transcript and decreased the peripheral-type transcript. The same two transcripts were also dominant in human and mouse livers, whereas the intestine contains only the peripheral-type transcript. Treatment of rats with pravastatin and a

bile acid binding resin (colestimide), which is known to activate SREBP-2 in the liver, caused a reduction in the hepatic cholesterol level and the same differential responses in vivo, leading to increases in hepatic ABCA1 mRNA and protein and plasma HDL levels. The dual promoter system driven by SREBP-2 and LXR regulates hepatic ABCA1 expression and may mediate the unique response of hepatic ABCA1 gene expression to cellular cholesterol status (Tamehiro, Shigemoto-Mogami et al. 2007).

4. SREBP protein and gene structure

(1) Human SREBP1 protein sequence (Olsen, Blagoev et al. 2006):

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1 mdeppfseaa leqalgepcd ldaalltdie dmlqlinnqd sdfpglfdpp yagsgaggtg
61 paspdtsspg slspppatls sseafslgp qaapsplspp qpaptplkmy psmpafspgp
121 gikeesvpls ilqtptqppl pgallpqsfp apappqsst pvlgypppg gfstgspgpn
181 tqqlpglpl asppgvppvs lhtqvqvvp qllvtvaap taapvtvtv sqiqqvpvll
241 qphfikadsl lltamktgda tvkaaglspl vsgettvtgp lptlvsggti latvplvda
301 eklpinrlaa gskapasqs rgekrthna iekryrssin dkielkdlv vgteaklnks
361 avlrkaidyi rflqhsnqkl kqenlsrta vhsksklkl vsacgsgnt dvlmegvkte
421 vedltppps dagspfqssp lsrgsrgsgs gsgsdsepd spvfedskak peqrpslhr
481 gmldrsrlal ctvlflclsc nplaslgar glpspsdts vyhspgnrvl gtesrdpggw
541 aqwlppvvw llngllvls lvllfygep vtrphspav yfwrhrkqad ldlargdfaq
601 aaqqlwlar algrplptsh ldlacslwn lirlhqlrw vgrwlagrag glqqdcalrv
661 dasasardaa lvyhklhqlh tmgkhtgghl tatnlalsal nlaecagdav svatlaeiyv
721 aaalrvktsl pralhfltrf flssarqacl aqsgsvppam qwlchpvghr ffdgdwsvl
781 stpweslysl agnvpdplaq vtqlfrehll eralnvtqp nspgsadgd kefsdalgy
841 qlnscsdaa gapaysfsis ssmattgvd pvakwwasl avvihwlrrd eaaerlcp
901 vehlprvlqe serlpraal hsfkaarall gcakaesgpa slticekasg yldslattp
961 assidkavq lficdlvvv rtslwrqqqp papapaaqgt sspqasale lrgfqrldss
1021 lrrlaqsfrp amrrvflhea tarlmagasp trthqlldrs lrragpggk ggavaepr
1081 ptrrehaeal llascylppg flsapqrv mlaeaartle klgdrrllhd cqqlmlrlgg
1141 gttvtss
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(2) Human SREBP2 protein sequence (Sjoblom, Jones et al. 2006):

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1 mddsgelggl etmettelg delilgdide mlqfvsnqvg efpdlfseql cssfpgsggs
61 gsssgssgs ssssnrgss sgavdpsvr sftqvtlpsf spsaaspqap tlqkvvspts
121 vpttpratpi lqprpqpqp pqtqlqqqv mitptfsttp qtriiqqpli yqnaatsfv
181 lqpqvsvlt ssqvqvptiq qqvqvtaqr vltqtangtl qtlapatvqt vaapqvqvvp
241 vlvqqiikt dslvtlkt dgsppmaavq npaltaltq iqtaalqvpt lvgssgtilt
301 tmpvmmgqek vpiqvpggv kqleppkege rrthniiek ryrssindki ielkdvlmgt
361 dakmhksvgl rkaidyikyl qqvnhklrqe nmvlkiank nkllkgidlg slvdnevdlk
421 iedfnqnvll mspasdsqs qagfypsids sepgsplldd akvkdepdsp pvalgmvdrr
481 rillcvlftl clsnfplsl lqwgahdsd qhphsgsgrs vlsfsgsgg wfdwmmptll
541 lwlvngvivil svfvkllvhg epvirphsrs svtfwrhrkq adldlargdf aaaagnlqtc
601 lavlgralpt srldacsls wnviryslqk lrlvrllkk vfqcratpa teagfedea
661 tsardaalay hrlhqlhitg klpagsacd vhmalcavn l aecaekipp stlveihlta
721 amglkrcgg klglasyfl sraqslcpe hsavpdslrw lchplgqkff merswsvksa
781 akeslycaqr npadpiaqv qafcknller aieslvkpqa kkkagdqeef scfssaley
841 kllhsfvds vgvmsplsr ssvlksalgp diicrwwtsa itvaiswlqg ddaavrshft
901 kveripkale vtesplvkai fhacramhas lpgkadgqqs sfchcerasg hlwsslsvsg
961 atsdpalnhv vqllctdlil slrtalwqkq asasqavget yhasgaelag fqrldgslr
1021 lahsfrpayr kvilheatvr lmagasprt hqllhslrr rttqstkhge vdawpgqrer
1081 atailacrhl lplslsspg qravllaea rtlekvgdrr scndcqqmiv klgggtaiaa
1141 s
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(3) Human SREBP1 gene sequence (Furuta, Pai et al. 2008):

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1 agcagagctg cggccggggg aaccagttt ccgaggaact ttcgccggc gccggggcgc
61 ctctgaggcc agggcaggac acgaacgcgc ggagcggcgg cggcgactga gagccggggc
121 cgccggcggc ctcctagga agggccgtac gagcggcgg gcccggcgg cctccgggag
181 gagcggctg cgccatggac gagccacct tcagcgaggc ggctttggag caggcgctg
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241 gcgagccgtg cgatctggac gggcgctgc tgaccgacat cgaagacatg cttcagctta
301 tcaacaacca agacagtac ttccctggcc tatttgacc acctatgct gggagtgggg
361 cagggggcac agaccctgcc agccccgata ccagctcccc aggcagcttg tctccactc
421 ctgccacatt gagctctct ctggaagcct tctgagcgg gccgcaggca ggcctcac
481 ccctgtcccc tcccagcct gcaccactc cattgaagat gtaccctcc atgcccctt
541 tctcccctgg gcctgtatc aaggaagagt cagtccact gagcatctg cagacccca
601 cccacagcc cctgccagg gcctctctc cacagagctt cccagccca gcccaccgc
661 agttcagtc caccctgtg ttaggctacc ccagccctc gggaggctt tctacaggaa
721 gccctcccgg gaacaccag cagccgctgc ctggcctgc actggctcc cggcagggg
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841 tcacagtgc cccacggca gccctgtaa cgaccactgt gacctgcag atccagcag
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1441 gcgtgaagac tgaggtggag gacactga cccaccccc ctggatgct ggctacctt
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3421 agctggagcc gcggccacg cggcgggag acgcggaggc ctgctgctg gcctctgt
3481 acctgcccc cgttctctg tggcggccc ggcagcgtt gggcatgct gctgaggcgg
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3961 atttcctgt gctgacggaa gccaaacttg ctttcccga ctgcaagcag ggctctgccc
4021 cagaggcctc tctctccgtc gtgggagaga gacgtgtaca tagttaggt cagcgtgctt
4081 agcctcctga cctgaggctc ctgtgctact ttccctttg caaatcttat ttcatagat
4141 tgagaagttt tgtacagaga attaaaaatg aaattattha taatctggaa aaaa

(4) Human SREBP2 gene sequence (Lee and Kong 2007):

1 gccctttctg tgcggcgccc gggcgcaacg caaacatggc ggcgggtggc acccgtcgg
61 gaggggtg cggggggggg ttgtcgggtg tcatggcggg tggcgacggc accgccccg
121 cgtctccctg agcgggacgg cagggggggc ttctgcgtg agccgggca tggacgacag
181 cggcgagctg ggtggtctgg agaccatgga gacctcagc gagctgggag acgagctgac
241 cctgggagac atcgacgaga tctgcaatt tctcagtaat caagtgggag agttccctga
301 ctgtttca gaacagctgt gtagctcctt tctggcagt ggtgtagtg gtagcagcag
361 cggcagcagt ggcagcagca gcagcagcag caatggcagg ggcagcagca gcggagctgt
421 ggaccttca gtgcaacggt cattcacca ggtcacatta cctccttct ctcctcggc
481 ggctcccca caggctccaa ctctgcaagt caagtttct cccacctcag tccccacc
541 acccagggca actcctatc ttagccccg cccccagccc cagcctcaac ctcaactca
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661 catccagcag cctttgatat accagaatgc agtactagc ttcaagtcc ttagcctca
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1681 agggggccc gactctgacc agcaccaca ctacggctct gggcagctg tctgtcatt
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2281 agaggagaag atcccaccg gcacactgt tgagatccat ctactgctg ccatggggct
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4321 aaaaa

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Study on the Influence of wetland Media on the Purifying the micro-polluted Raw Water

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ABSTRACT: In order to study the role of wetland media on the purifying the micro-polluted water, media-bacteria system is built to study experimentally in Yuqing Lake in Jinan. The removal process of a simulation wetland was studied in the absence of aquatic plants. The results show the average removal rates of COD, TN, NH₄⁺-N and TP were 46.67%, 34.86%, 39.83% and 45.12%, respectively. The 16m long system was divided into four equal units along the direction of inflow. The removal of COD, TN, NH₄⁺-N and TP mainly occurred in the first unit, with a removal of 21.5%, 13.5%, 19.85 and 16.64% respectively. This efficiency was much greater than those in the subsequent three units. In addition, the accumulation of P was found in media and TP of media would reach a peak. [The Journal of American Science. 2008;4(2):95-100]. (ISSN 1545-1003).

Key words: medium-bacteria system; micro-polluted raw Water; treatment effect

1. Introduction

Most of the water supply comes from Yellow River as the foundation in Jinan city of Shandong province. Due to the fast development of industry and agriculture around drainage area of Yellow River and large sewage, the water quality of Yellow River comes to deterioration. The status of Yellow River as the drinking water quality is not optimistic. The present techniques of water supply factory in Jinan can't remove COD in light polluted raw water and polluted substances such as nitrogen and phosphorous, so we need pretreatment to the raw water.

Because constructed wetland is a kind of water treatment method as a lower cost, lower energy, lower technical-demanding (Winthrop, 2002; Ji, et al., 2002; Scholes L, et al. 1998), so we adopted this method to treat. Nowadays, the research on constructed wetland system always attaches importance on the effects on the biological role during the purifying process, and the non-biological role such as the media that the plants and microorganism live by are neglected (Shen Dongsheng, 1996; Andrew, 2007). In order to explore the influence of wetland medium on purifying the light-polluted water, a media-bacteria system is built in Yuqing Lake in Jinan. At the same time, the research is in process.

2. Materials and Methods

2.1 Description of the media-bacteria system

The measurement of the system is 16m×6m×0.8m, the average depth of water is 0.2 m, The 16 m long system was divided into four equal sampling exit along the direction of inflow with 4 m, 8 m, 12 m, 16 m. The wetland bottoms are plastered by concrete to reduce the seepage of the water losses, with brick built up in layers and mortar plastered. Both Water distribution and discharge adopt triangular weir, in order to distribute the water evenly. And each one set a water gathering pool. The media of the system was local soil in which Reed grow, which is naturalized in, April 2005.

2.2 Running of the system

The system began to run in the middle of June, 2005, with a load of 75 L/h, and the hydraulic retention time of 1.5 days. The raw water comes from effluent Grit Chamber of forepart of Yuqing Lake Reservoir. The system was completed in December.

2.3 Analytical method

Water samples were collected both from the influent and effluent at regular, short intervals (6-8 per month). COD, TN, TP, $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$ and $\text{NO}_2^-\text{-N}$ were measured according to the standard methods. Temperature and pH were measured with electrodes.

3. RESULTS AND DISCUSSION

3.1 Total purification efficiency

Table 1 shows after six month research, the experimental results show that the removal rates of COD and TP in the system were more 45%, that of $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$ and $\text{NO}_2^-\text{-N}$ were nearly 40%, that of TN were nearly 35%, respectively. According to the China standard for surface water resources (GB3838-2002), by purification function of the system, COD in water turns from Grade IV to Grade II, TP from Grade III to II, TN from much higher to Grade V into close to V, and $\text{NH}_4^+\text{-N}$ from Grade III into II.

Table 1. The characteristic of inflow and outflow and the removal rate of nutrition

Parameter	Inflow /mg/L			Outflow /mg/L			Removal Rate /%
	Min.	Max.	Mean (Std. Deviation)	Min.	Max.	Mean Std. Deviation	
COD	10.9000	44.3000	23.8042 (9.5664)	5.5590	18.6060	11.8988 (3.2433)	46.67
TP	0.0047	0.4202	0.1006 (0.1287)	0.0026	0.2185	0.0530 (0.0656)	45.12
TN	1.4664	4.6100	3.2575 (0.8800)	0.8477	3.2047	2.1399 (0.6874)	34.86
$\text{NH}_4^+\text{-N}$	0.0370	1.1700	0.5136 (0.2683)	0.0178	0.7956	0.3260 (0.2031)	39.83
$\text{NO}_3^-\text{-N}$	0.0309	3.9620	2.9934 (0.8118)	0.0151	2.3376	1.8291 (0.5345)	39.36
$\text{NO}_2^-\text{-N}$	0.0140	0.9800	0.0722 (0.1941)	0.0092	0.7056	0.0476 (0.1405)	39.17

3.2 Purification efficiency in different unit

In order to illuminate Purification effect in different unit, we calculated average Purification efficiency in different unit. In Figure 1、Figure 2、Figure 3 and Figure 4, 1, 2, 3 and 4 of abscissa show four equal units along the direction of inflow. The removal rates of TP in different units were 16.64%, 15.5%, 7.41% and 5.57%, respectively; those of COD in different units were 21.5%, 11.2%, 9.5% and 4.47%, respectively. The removal rates of TN in different units were 18.32%, 7.5%, 5.3% and 3.74%, respectively; those of $\text{NH}_4^+\text{-N}$ in different units were 19.85%, 8.7%, 6.8% and 4.48%, respectively. These show removal of contamination mainly occurred in the first half unit.

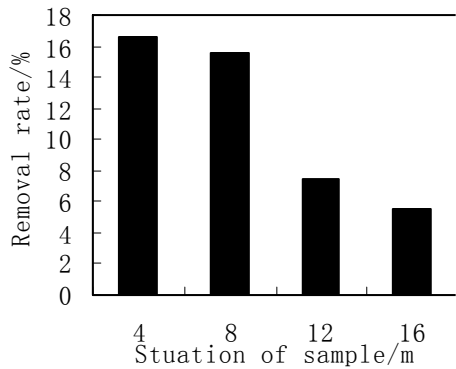


Figure 1. The average Removal rate of TP in different units

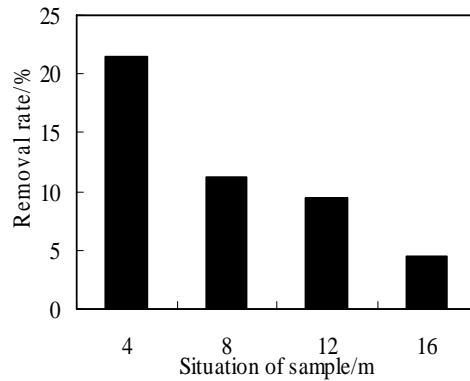


Figure 2. The average Removal rate of COD in different units

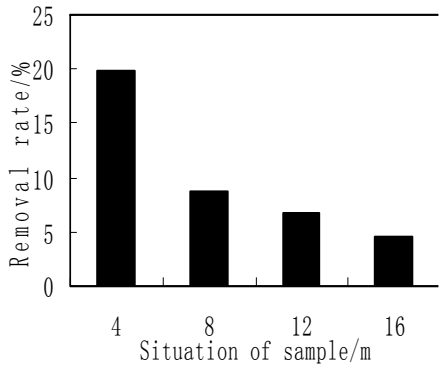


Figure 3. The average Removal rate of NH₄⁺-N in different units

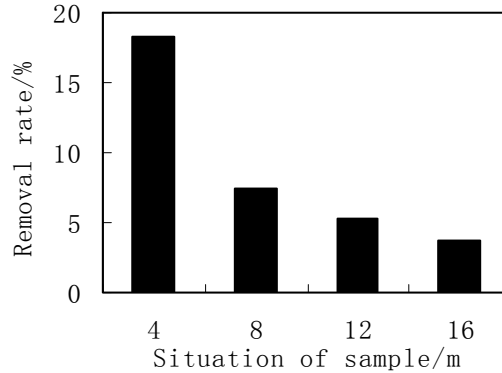


Figure 4. The average Removal rate of TN in different units

3.3 Concentration variety of TN and TP of media of different experimental phase

In experimental phase of early, metaphase and final, TN and TP of media were measured (Corstanje, 2007), respectively. The results are shown in Table 2 and Table 3. Increase of media's TN is isochronous in different units. There were a lot of nitrobacteria, de-nitrobacteria by identifying, which facilitate removal of N.

From experimental early phase to metaphase, TP of media distinctly increase in the first unit of system. However, TP of media has no obvious increase in the final stage. In the later stage of experiment, concentration of TP distinctly increase in the following second, third, and fourth units of system. This shows there is a saturation of sorption sites in media. TP Concentration of media increase in proper order from early phase to the fourth unit, and the early phase comes to saturation at first.

Table 2. TN concentration of media (%)

Situation of situation	4m	8m	12m	16m
Early phase	0.071	0.072	0.069	0.071
Metaphase	0.121	0.113	0.108	0.095
final phase	0.152	0.135	0.128	0.118

Table 3. TP concentration of media (%)

Situation of situation	4m	8m	12m	16m
Early phase	0.046	0.045	0.048	0.047
Metaphase	0.121	0.87	0.064	0.56
final phase	0.135	0.119	0.093	0.074

3.4 Temperature influence

Any microbe has a proper temperature of growth. In the range of temperature, with growth of temperature, the microbe grow rapidly. Correspondingly, contamination is eliminated rapidly. Not only does temperature influence organic matter Biodegradation by altering microbial metabolism velocity, but also influence solubility of organic matter. Therefore, in actual treatment, Controlling microbial proper temperature of growth can better improve restoring function.

Table 5 shows in the whole motion of the system, the temperature of water varies from 0 to 32, and the removal of contamination in water is influenced obviously by the system under the temperature. As the temperature goes down, the removal rate of contamination is eliminated rapidly.

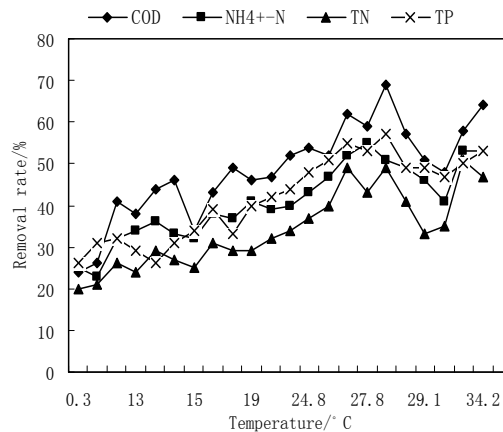


Figure 5. The impact of temperature on pollutant removal efficiency

3.5 Removal relations of contamination Concentration

Figure.6 to Figure.9 show there is a liner relation between the variations of influent and effluent COD, TN, TP and $\text{NH}_4^+\text{-N}$ concentrations. Moreover, TP have a good pertinence. Removal concentration, with the increase of contamination and Concentration of influent have steady increase. On the whole, there is depressive inflexion. This shows current load has no problem on over loading. This also shows potential of taking on more loads in the system.

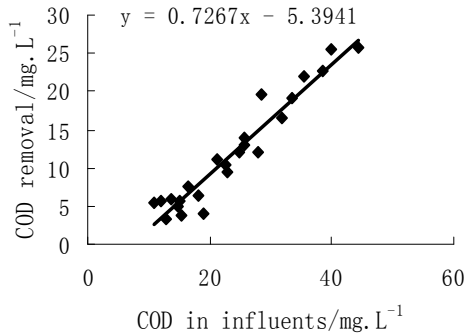


Figure 6. Relationship between COD in influents and COD removal

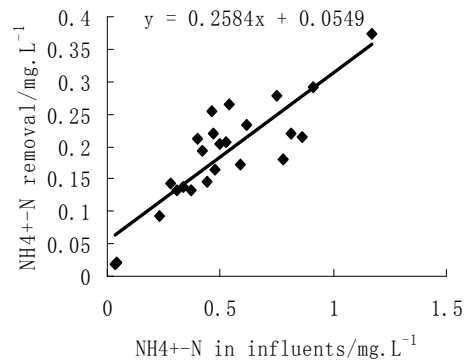


Figure 7. Relationship between $\text{NH}_4^+\text{-N}$ in Influent and $\text{NH}_4^+\text{-N}$ removal

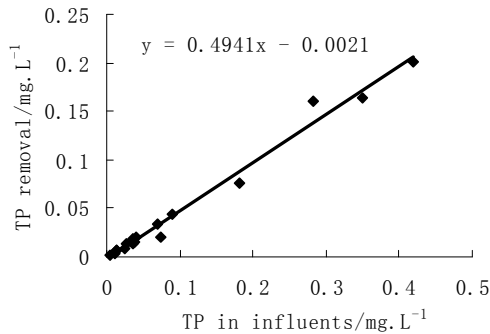


Figure 8. Relationship between TP in influents and TP removal

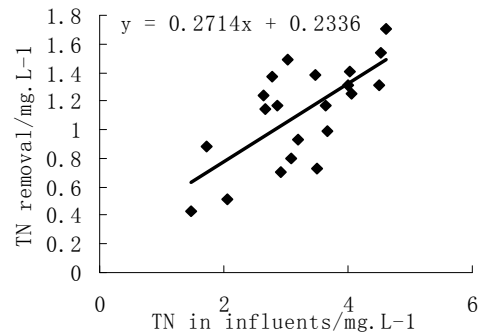


Figure 9. Relationship between TN in influents and TN removal

4. CONCLUSIONS

4.1 Without regard to role of plant, micro-polluted water has better treatment effect by the constructed wetland. This shows media play important role in micro-polluted water treatment.

4.2 Micro-polluted water flew the whole system, with different disposal in different phase. The disposal efficiency decreased under the increase of system. The early and final phases have the highest disposal efficiency.

4.3 In course of system running, there is an accumulation of P there is a saturation of sorption sites, the phenomenon of accumulation shifted gradually from the early phase to the final phase of the system.

4.4 Temperature has a positive effect on the removal efficiency. A higher removal rate can be obtained when the water temperature is higher.

4.5 Because load is low and the removal function of system can't be fully used, so the future experiment can improve load in suitable time and fully used the removal effect of system.

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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 Maastricht 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 Maastricht is carried out in Maastricht Physics, Maastricht Trials, Maastricht School, and Maastricht 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 than 1500 patients included.

The lab has 4 permanent scientists, 5 technicians, more than 5 PhD students and is fully equipped for cell culture, molecular biology, flow cytometry, hypoxia, gene expression, proteomics and microscopy. Maastricht 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.

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[Cover Page](#), [Introduction](#), [Contents](#), [Call for Papers](#), [All papers in one file](#)

Contents

- [1. Practical Technique of Western Blotting](#)
Jenny Young, Ma Hongbao 1-3
- [2. Prevalence of Enterohaemorrhagic Eschericia Coli 0157:H7 Causing Severe Urinary Tract Infection in Abeokuta, Nigeria](#)
Akinduti P.A, Akinbo J.A, Ejilude O.A, Mannie-Udoh M.I, Umahoin K.O, Ogunbileje J.O, Folarin B.O 4-9
- [3. The Role Played By Blocking over the Northern Hemisphere in Hurricane Katrina](#)
Y. Y. Hafez 10-25
- [4. Anthropogenic Impacts on Protected Area of Burundi. Case Study of Ruvubu National Park](#)
Ntovenimana Remegie, Gu Yansheng 26-33
- [5. Feeding behaviour of wild Asian Elephants \(*Elephas maximus*\) in the Rajaji National Park](#)
Ritesh Joshi, Rambir Singh 34-48
- [6. Comparison Of Dac-Elisa And Dot-Blot-Elisa For The Detection Of Cucumber Mosaic And Banana Streak Viruses Infecting Banana](#)
P. Rajasulochana, R. Dhamotharan & P. Srinivasulu 49-57
- [7. Research on the New Accounting Control Based on the Environment of IT](#)
TAO Ping, LI Wen-hua 58-64
- [8. The Truth about Global Warming](#)
Willie J. McDonald 65-67
- [9. Assessment *In Vitro* Of The Biological Effect Of A Herbal Product Extract: Morphological And Radiolabeling Analysis](#)
G. Diré, E. Lima, M. Gomes, D. Mattos and M. Bernardo-Filho 68-77
- [10. Deterioration of Soil Organic Components and Adoptability of Green fallows for Soil Fertility Replenishment](#)
E.U. Onweremadu: E.C. Matthews-Njoku, F.C. Nnadi², F.C. Anaeto: F.O. Ugwuoke, D.O. Onu M.A.C.A. Odii 78-84
- [11. Know Thyself](#)
Kees Beukering 85-87
- [12. Sterol Regulatory Element Binding Proteins \(SREBPs\)](#)
Ma Hongbao, Cherng Shen 88-94
- [13. Study on the Influence of wetland Media on the Purifying the micro-polluted Raw Water](#)
Xu Yang, Shuili Yu, Yongsheng Ma, Yan Zhao, Xiaoju Yan, Cunhai Xiu 95-100

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