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Macroinvertebrates as Indicators of The Water Quality of an Urbanized Stream, Kaduna Nigeria

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ABSTRACT: A survey was conducted from March to September, 2005 on a fourth order perennial Northern Nigerian stream to evaluate the water quality using the macrobenthic invertebrate community of the bankroot biotope. Physico-chemical variables were determined using standard methods. A total of 1304 macroinvertebrates were recovered. Twenty-seven taxa were recorded. The higher number of taxa (23) was recorded at station 2. The abundance of individuals was highest at station 3. The presence of low densities of pollution tolerant macroinvertebrate groups, the deteriorating water quality and the physco-chemical conditions of the water during the dry season months was a reflection of organic pollution stress caused by decomposing domestic refuse and inorganic fertilizer washed into the stream by irrigation. [Nature and Science. 2009;7(1):1-7]. (ISSN: 1545-0740).

KEYWORDS: Macroinvertebrates, water quality, urbanized stream, Kaduna

INTRODUCTION

Most major cities contain a number of waterways such as bays, harbors and rivers together with a small network of small streams. In Nigeria most of these streams have been subjected to an increasing pollution load from contaminated urban run-off water originating from industrial, agricultural, residential, commercial and recreational areas and institutions such as schools and hospitals (Adakole and Annune, 2003). Ogbogu and Hassan (1996) pointed out the effects of contaminants usually flushed into streams especially in areas of high human activities.

Macroinvertebrate organisms form an integral part of an aquatic environment and are of ecological and economic importance as they maintain various levels of interaction between the community and the environment (Anderson and Sedel, 1979). According to Marques *et. al.* (2003) knowledge of the structure of the benthic macroinvertebrate community provides precise and local information on recent events, which can be seen in their structuring. The use of invertebrates and fish as bioindicators of water quality has been advocated by several researchers (Victor and Ogbeibu, 1985; Ofojekwu *et. al.*, 1996; Edokpayi and Osimen, 2001; Adakole and Annune, 2003).

The Barnawa stream is the main drainage system of most parts of Barnawa, an urban settlement in Kaduna metropolis. Although it is not a major river for fisheries activities because of its size it is the major effluent receiving stream in Barnawa as many gutters are linked to it. It is also used in the irrigation of crops as well as source of drinking water for cattle during the dry season. As a river-let of river Kaduna (Beecroft, 1987) it has a significant contribution to its discharge and consequently its pollution load. The present study examines the effects of various activities associated with urban settlement on some water quality parameters and the macrobenthic invertebrate composition of the Barnawa stream.

MATERIALS AND METHODS

STUDY AREA

The Barnawa stream is located in the southern part of Kaduna Metropolis (Longitude $7^0$50'E and Latitude $10^0$50'N) about 645 meters above sea level. The stream takes its source within the Kalapanzy (Artillery) barracks in Kaduna south and joins other river-lets, which empty into River Kaduna (Mallo, 2001). It is a shallow, fast flowing stream. The area is characterized by flat land surface and easily worked sandy loam soil. The climate of Kaduna is tropical with a distinct rainy season (late April to October) and dry season (October to May) (Beecroft *et. al.*, 1987). The vegetation is guinea savanna, which has been cleared but only relic shrubs of *Isoherlinia doka* and few grasses are still striving.

The major human activity in the catchments area is dry season farming. Heaps of garbage, human excreta and cattle dung were found on slope of the bridge across the stream and these present ugly sites at various spots along the stream. Three sampling stations along a 2.5km stretch were chosen for the study. Station 1 is about 1km from the source. Here the stream is wider than deep. Heaps of refuse were seen by the sides of the bridge across the stream, with the decomposing refuse emitting foul smell around the catchments area. The vegetation is mainly grasses and creeping plants and there was no farming activity at

this station. Station 2 is about 500m from station 1. Human activities here include farming and washing of implements. Bankroot biotope included maize, vegetable crops like spinach, tomato, okra and herbaceous weed. The stream here is relatively wide. Station 3 is about 1km from station 2. The stream

Channel at this site is narrow and fast flowing. Mango trees, grasses and relic shrubs shade this area. The substratum is sandy loam soil. A mechanic workshop and block molding industry are located close to this station; Human activities include washing of block molding implements and motor spare parts.

SAMPLE COLLECTION AND ANALYSIS

Samples were taken at fortnightly intervals over a period of seven months (March – September, 2005) between 0900 hours and 1500hours from the sampling stations, Macrobenthic invertebrate fauna were collected by the kick method (Lenat *et. al.,* 1981; Victor and Ogbeibu, 1985). All invertebrates were killed in the field using small quantities of 40% formaldehyde and later preserved in 70% ethanol for further examination. Further analyses carried out in the laboratory include sieving (mesh size 1.4mm – 250mm), counting and sorting under suitable magnifications (7-40x). The macrobenthic invertebrates were identified using manuals of Pennak (1953); Needham and Needham (1962); Victor and Ogbeibu (1985); Egborge (1995).

Water samples for physico-chemical studies were also collected from stations 1, 2 and 3. Temperature, pH and conductivity were determined in the field using a portable pH/EC/TDS/temperature meter model H1-991301 while dissolved oxygen and Biochemical oxygen demand were determined by titration (APHA, 1985).

Water quality for each station was determined using the diversity (d) indices of Margalef. Magalef's water quality index >3.0 indicates clean condition; values <1.0 indicate severe pollution and intermediate indicate moderate pollution (Lenat *et. al.,* 1981).

All statistical procedures where appropriate were adopted from Zar (1984). SPSS 6.5 applications and Excel (Genstat release 4.03 packages) were used to calculate the two-way analysis of variance (ANOVA).

RESULTS

A summary of the physical and chemical parameters of the study area is given in Table 1. The mean, minimum and maximum values and the standard errors are shown. The water temperature followed closely that of the ambient temperature. Alkaline pH was recorded (pH between 8.5-8.7) during the dry season months. However the pH reduced from neutral to slightly acidic (5.3-7.0) during the rainy season months (July-September). The conductivity was lowest at station 1 and increased downstream. The highest conductivity value ($63.0\mu Scm^{-1}$), was recorded at station 2. Dissolved oxygen was low during the study period ($0.00\text{-}3.60 mgL^{-1}$) while the BOD ranged from $0.00\text{-}2.00 mgL^{-1}$.

The overall macrobenthic invertebrate composition, abundance and distribution in the study stations are summarized in table 2. Twenty-five taxa were identified from a total of 1304 individuals collected. Station 1 had 18 taxa, while stations 2 and 3 had 23 and 15 respectively. Also, station 1 contributed the highest (46.90%) of the total number of individuals and the least number of taxa (15) recorded (Table 2). A summary of the relative contribution of the major invertebrate groups to the overall macrobentthic population at the different stations is presented in Table 3, figure 1 . Station I was dominated by mollusc represented by *Bulinus* and *Biomphalaria* species while dipteran families dominated stations 2 and 3. The variations in taxa and number of individuals between stations were not significantly different ($P>0.05$). Aquatic insects represented 68.00% of all taxa and 65.97% of all individuals. Coleptera, Diptera, plecoptera and odonata mainly represent them. Three species of Annelida; *Tubifex*, *Nais*, and *Glassiponia* were recorded in all stations, but the fourth species *Stylaria* was absent at station I. Crustacea was poorly represented by a single taxon (Astacidae) in this study. Dipterans were dominated by Chironominae and Tanypodinae families, out of which *Chironomus* was the most abundant. *Simulium, Pentaneura and Anopheles* species were restricted to station 2. Two individuals of *Pseudocleon* species poorly represented Ephemeroptera.The indices of general diversity (H), evenness (E) and dominance calculated for the three stations are presented in table 4. Although diversity was higher at station 2 evenness and dominance were higher at station 1 and 3 respectively.

DISCUSSION

The physical and chemical properties of the stream showed some variations. However, there was no significant difference between the stations studied. Slight longitudinal variation in water level was observed. The water level of aquatic ecosystem is usually influenced by the rainfall pattern of the drainage

basin (Ikusima *et. al.*, 1982). Alkaline pH and low conductivity was recorded in all stations. High pH has been reported for most fluvial (Beecroft *et. al.*, 1987; Emere, 2000; Adakole and Annune, 2003) and Lacustrine ecosystems (Ufodike and Garba, 1992; Kemdirim, 2005) in Northern Nigeria. This may be due to the granite, which forms the basement rock of these water bodies. The low conductivity in this stream places it in class 1 of Talling and Talling's (1965).

Classification of African waters (the most dilute waters of conductivity < 600 mhoscm^{-1}): this class of water is said to be poor in nutrients.

The low dissolved oxygen concentration recorded agreed with values reported for some Nigerian waters (Ofojekwu *et. al.*, 1996; Bukar 2006 unpublished). The dissolved oxygen values revealed anoxic or septic condition during the dry season within the study period. Such low oxygen saturation has been reported in River Kaduna in dry season months when there was little or no flow (Beecroft, 1987; Emere 2000). Low dissolved oxygen has been reported to be deleterious to most aquatic fauna. Based on BOD classification of streams: unpolluted (BOD < 1.0mgL^{-1}), moderately polluted (BOD between 2-9mgL^{-1}) and heavily polluted (BOD > 10mgL^{-1}) (Vowels and Connel, 1980), the stream was moderately polluted during the study period.

The 27 taxa comprising of 1304 individuals recorded was low when compared with over 55 taxa reported for tropical streams (Victor and Ogbeibu, 1985; Edokpayi *et. al.*, 2000; Ogbeibu 2001; Adakole and Annune, 2003). The low species diversity could be due to some physico-chemical conditions like fast flow, high pH, low dissolved oxygen and low conductivity of the water. Odum (1971) had reported that diversity tends to be low in physically controlled systems. These factors probably caused disruption of life cycle, reproductive cycle, food chain and migrations or imposed physiological stress on even the tolerant macroinvertebrates (Adakole and Annune, 2003).

The taxonomic breakdown of the macrointerbrates indicated the dominance of arthropods in species richness followed by mollusc and annelids. *Biomphalaria* was the dominant mollusc. Among the arthropods aquatic beetles (Coleoptera), and dipterans, which include the rattail maggot (*Eristalis*), *Culex* Coatate pupa and *Chironomus* species occurred in all stations. Gaufin (1973) reported that most aquatic beetles can renew their oxygen supply directly from the atmosphere, they are thus unaffected by oxygen depleting wastes while others possess special adaptations for obtaining oxygen (Marques *et. al.*, 2003). All the macroinvertebrates reported in this study during the dry season months belong to the tolerant classes in water bodies, which indicate organic pollution. However, these groups did not show the expected pattern of opportunistic population, that is, few species and large number of individuals (Ogbeibu, 2001; Marques, 2003). This suggests that there maybe other factors, which caused oxygen depletion such as oxidation of iron, accumulation of sediment or inorganic fertilizer from irrigation run-off. Few species of Odonata and Ephemeroptera which are fauna associated with clean water quality were recovered only during the rainy season months. This could be due to dilution during the rains, which caused some improvement in the water quality. The occurrence of stonefly, though low in number was the only sensitive class present during the dry season. Since most species of stoneflies are clean water species (Gaufin, 1973), it is possible that this species occupied in a niche where the oxygen concentration was higher than values recorded for the stream.

Table 1. Summary Of Some Physical And Chemical Conditions Of The Barnawa Stream Study Stations (March-September, 2002)

	Stations								
PARAMETERS	1 Mean ±SE	Min. Max		2 Mean ± SE	Min.	Max.	3 Mean ± SE	Min.	Max.
Air temperature (^{0}C)	28.5± 1.00	26.0	32.0	27.5 ± 0.8	24.0	32.0	28.0 ± 0.7	25.0	31.0
Water Temp. (^{0}C)	27.5 ±0.72	25.0	32.0	27.4 ±1.00	24.0	31.8	26.5 ± 1.11	22.0	31.0
Conductivity (µScm^{-1})	34.5±2.54	32.0	39.0	52.7±1.11	42.0	63.0	54.3 ± 3.6	48.0	56.0
pH	7.0	5.3	8.7	7.6	6.6	8.7	7.7	6.7	8.7
Dissolved oxygen mgL^{-1}	1.58 ± 0.98	0.00	3.0	1.25 ± 0.62	0.00	2.1	2.50 ± 1.5	0.50	3.60
B O D (mgL^{-1})	1.48 ± 0.28	0.00	1.68	0.67 ± 0.60	0.00	1.5	1.15 ± 0.30	1.15	2.00

Table 2. Overall Abundance And Distribution Of Macrobenthic Invertebrates At The Study Stations In Barnawa Stream (Feb. To Sept., 2005)

	Stations			
	1	**2**	**3**	**Total**
MOLLUSCA				
Bulinus spp	12	8	2	22
Biomphalaria	58	12	8	78
Physa spp	-	8	-	8
COLEOPTERA				
Sphaerodema	10	12	2	24
Hydrous sp	2	8	2	12
Phihydrus sp	10	2	4	16
DITERA				
Enstalis larvae	6	16	14	36
Simulium	-	8	-	8
Chironomus	15	143	405	563
Pentaneura	-	40	-	40
Coatate pupa	4	16	18	38
Chronomus pupa	6	21	21	48
Pupa type 1	10	-	-	10
Anopheles larva	-	4	-	4
HEMIPTERA				
Notonecta	-	-	1	1
PLECOPTERA			-	4
Neoperla	2	6	-	8
Brachythermis	12	2	-	14
ODONATA (ZYGOPTERA)				
Coenagrion	-	18	-	18
Aeschna		-	-	-
Pseudagrion	3	3	6	12
Enallagma	-	6	-	6
EPHEMEROPTERA				
(*Pseudocleon)*	2	-	-	2
CRUSTACEA				
Astacidae	20	11	6	37
ANNELIDA POLYCHAETA				
Tubifex	40	10	12	62
Nais	9	24	63	96
Stylaria	-	66	45	111
HIRUDINEA				
Glossiphonia	10	16	4	30
TOTAL	232	460	612	1304
PERCENTAGE	17.8%	35.3%	46.9%	100%

Table 3. Percentage Contribution Of Major Invertebrate Groups In The Barnawa Stream, March To September 2005. The Values Are Percentages; ≥15% Dorminant; ≥ 5% To < 15% Subdominant

Taxa	Staion1	Station2	Station3	Overrall
MOLLUSCA	5.37	2.15	0.77	8.29
COLEOPTERA	1.69	1.69	0.61	3.99
DIPTERA	3.14	19.00	35.10	57.24
PLECOPTERA	1.07	0.61	-	1.68
ODONATA	0.38	2.07	0.46	2.91
EPHEMEROPTERA	0.15	-	-	0.15
CRUSTACEA	1.53	0.84	0.46	2.83
ANNELIDA	4.52	8.89	9.20	22.61

Table 4. Diversity Of Invertebrates In The Study Stations Of Barnawa Stream, March-September 2005

	Stations		
	1	**2**	**3**
	n = 14	n = 14	N = 14
No. of Taxa	18	23	15
No. of Individuals	232	460	612
Margalef's diversity (d)	3.12	3.56	3.24
Evenness (E)	0.173	0.156	0.146
Dominance (D)	0.03	0.12	0.22

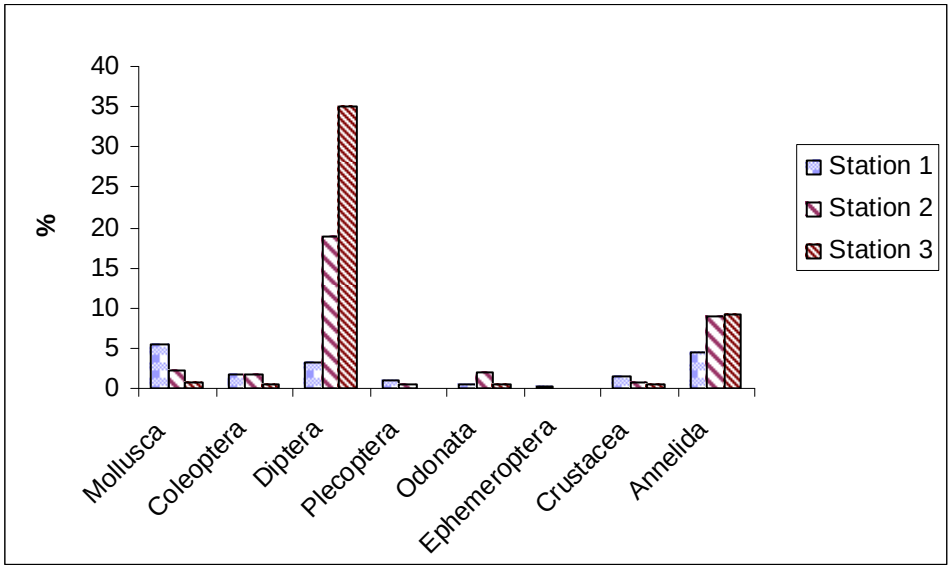

Figure 1. Relative contribution of the major macro invertebrate groups in the Barnawa stream, March to September, 2005.

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Determination of capacity building by life stage for the farmers in Bangladesh

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Abstract: Several researchers have identified capacity building is essential for sustainable development of the farming community in developing countries. It is assumed that improvement of the capacity depends on physical, technical and managerial skills of the farmers at different stages of their life, which has not yet studied for developing countries. The study found that children of the marginal and small farmers engage earlier in assisting physical activities in farming than medium farmers in Bangladesh because they have less opportunity to educate their children. Majority of the marginal and small farmers are well ahead in improving physical and technical skill at a high level than the medium farmers and therefore, they enter into the Gehilfen stage of capacity building. Moreover, they have started to build these skills independently at the early age of their farming. Medium farmers are, on the contrary, reluctant to engage independently in farming activities than the marginal and small farmers. None of the farmers enter into Meister stage of capacity building. The study also found that physical skill is the dominant factor followed by technical skill for increasing capacity of the farmers in Bangladesh. The marginal and small farmers could make agricultural productivity better than the medium farmers owe to skill development. [Nature and Science. 2009;7(1):8-16]. (ISSN: 1545-0740).

Keywords: Capacity building, life stage, physical skill, Bangladesh

Introduction

Capacity is often defined in terms of ability and performance. For example, the United Nations Development Program (UNDP) defines capacity as `the ability to perform functions effectively, efficiently and sustainably` (UNDP 1997). This definition of capacity can also be used in agricultural production activity. Agricultural production activity comprises of physical involvement, application of technical knowledge, procurement efficiency of raw materials, efficient management of land and labor, short term and long term farm development plan, financial management using accounting knowledge, and efficient management of assets and property. Therefore, how efficiently a farmer can perform these activities determines his capacity to do the job. Capacity building is associated with increment in these activities or performance through transformation process of different activity.

In the present study capacity building is associated with efficient performance of different agricultural activities to increase agricultural production in a further extent. Efficient performance of a different agricultural activity is a dynamic process instead of static one. For example, younger farmers may concern about the technology of different crop production such as appropriate planting time, requirement of irrigation, usages of organic and inorganic matter, harvesting methods along with involvement of physical skill. Middle aged farmers may think about the marketing strategy, efficient management of financial resources along with receiving and payment of credit, development of short term and long term farm planning etc. Therefore farmers build their capacity in different stages of life. According to the German literature there are three stages of capacity building for the farming activity. The stages are Lehrling, Gehilfen and Meister. The farmers, who have high skill in the technical aspect along with efficient performance of physical activities, are in Lehrling stage. The farmers who have high capability of cash, capital and production management are in Gehilfen stage. The farmers are in Meister stage; they can analyze their farming activities well, can do long term financial management in efficient way, and have capacity to manage assets and property efficiently, and ability to prepare short and long term farm development plans. Some farmers achieve successfully high level of capacity building arriving at Meister stage and some farmers achieve low level with Lehrling stage. Different literatures published in German and Japanese language show that many farmers in developed country are in Meister stage. However, there are no literatures found for the farmers of developing countries regarding in which stage of capacity

building they are. Therefore, present study is undertaken to determine capacity building by life stage for the farmers in Bangladesh as a representative of developing country.

In developed countries like Germany and Japan agriculture is mostly capital intensive because of using heavy machines like tractor, combined harvester or greenhouses. For using these machines or plants farmers need huge capital and they have to borrow capital from different financial institutions like banks, agricultural cooperatives etc. For receiving and payment of huge amount of loan farmers need to have financial management skill. The economy of Bangladesh is still dominated by agriculture sector. Around 19.6% of gross domestic product comes from agriculture (BBS 2006) in which crop and horticulture contributed 11.5% (BBS 2006). Among total labor force 48.1% employed in agriculture sector (BBS 2006). Approximately 79.4% farmers are landless (≤0.20ha), marginal (0.20ha to ≤0.40ha) and small farmers (0.40ha to ≤1.00ha) along with a dependency ratio of 3.60 and family size 5.19 (BBS 2007). Of them 25.2% are landless, and 31.4% are marginal farmers. With this salient feature the present study will also identify the current situation of Bangladesh agriculture whether it is capital or labor intensive and at which stage of capacity building he/she is.

There are some researches available in which managerial ability is found an important factor for improving efficiency of farming. Johanson (2007) empirically estimated the impact of personal aspects and decision making characteristics on farm level efficiency, in a sample of Swedish dairy farms. Individual beliefs of a person which can influence his decision are taken as a personal aspect. Öhlmer (1998) and Öhlmer *et al.* (1997) found a connection between the ability of a farmer and his or her locus of control i.e. individual beliefs. Rougoor *et al.* (1998) considered managerial capacity as consisting of both personal aspects of the manager (in terms of drives and motivations, abilities and capabilities, and biography) which affect decision making and which in turn affects the performance of a farmer. Solano *et al.* (2006) studies the impact of a series of biographical variables and decision making profiles, as a representative of the managerial capacity of the farmers, on the management and performance of their farm. They found that managerial capacity positively influences the performance of the farm. Trip *et al.* (2002) measured managerial efficiency for the commercial greenhouse growers. They considered decision making process as reflected by producers` goal, planning, data recording and evaluation. Kularatne and Takeya (2005) examined the management factor in relation to perennial crops or measured the implementation process to evaluate the management. There are no analytical studies found so far which considered physical, technical and managerial skill as factors for estimating capacity building of the farmers in developing countries. Therefore, the present study is focused on two aspects. First, determination of capacity building by life stage for the farmers in Bangladesh and second, identifying some factors which affect capacity building of the same farmers. It is expected that the findings of the study have some potentials to add some important knowledge on existing literature of capacity building study.

Methodology

Sample selection and data collection

Comilla, Bogra and Jessore districts of Bangladesh are selected as study areas for the present research. From these three districts 46 marginal farmers, 36 small farmers and 18 medium farmers were chosen by random sampling as samples. The data were collected using a pretested interview schedule through face to face interview.

Regression analysis

A multiple linear regression analysis was used to identify factors affecting capacity building of vegetable farmers in Bangladesh. The regression model is as follows (Gujarati, 2001).

$$\mathbf{Y_i = \beta_0 + \beta_1 X_{1i} + \beta_2 X_{2i} + \beta_3 X_{3i} + \beta_4 D_{1i} + \beta_5 D_{2i}}$$

Where, Y_i = Total return from vegetable production of i^{th} farm, X_{1i} = Physical skill score of i^{th} farm, X_{2i} = Technical skill score of i^{th} farm, X_{3i} = Managerial skill score of i^{th} farm, D_{1i} = Communication skill dummy for i^{th} farm (1 for mobile phone using, 0 for otherwise), D_{2i} = Communication skill dummy for i^{th} farm (1 for using broadcast media, 0 for otherwise). Interpersonal communication is taken as base for using dummy variables.

Definition and measurement of variables

Considering the German concept of capacity building farmers need to develop their physical,

technical and managerial skill. In this study physical skill is comprised of physical involvement of labor doing several agricultural activities and knowledge on doing these activities. Physical involvement of labor is divided into three parts; (1) land preparation, (2) Intercultural operation and (3) harvest and post harvest activity. Land preparation includes: ploughing, seed bed preparation, sowing/transplanting. Intercultural operation includes: establishment of bamboo stack/plastic net, fertilizing, irrigating, hilling, weeding, spraying pesticides. Harvest and post harvest activity includes: harvesting, handling, and grading. Technical skill includes use of recommended dose of seed/plant following required spacing, different kinds of chemical fertilizer with organic matter, application of required irrigation water, use of integrated pest management and spraying of plant protection chemicals at tolerance level. Managerial skill comprises of marketing skill, short term farm planning, labor management and financial management.

Marketing skill includes selling of products at wholesale market with appropriate grading, collection of spot price information using mobile phone. Marginal and small farmers usually make short term farm planning for one year only because they lease lands from medium and large farmers. Medium and large farmers are almost keeping them absent from farming and they do not make any farm plan for a long term. Labor management includes employment of family and hired labor in different activities, determination of wage rate. Farmers have no book keeping experience to prepare day book, balance sheet and profit & loss statement. Therefore, financial management includes rough estimation of income and expenditure from farming, receiving and payment of loan. Capacity building by life stage is determined differently in two stages; first, how and when family members of a farm assist the farmer in different farming activities and second, how and when a farmer operate different farming activities independently by his/herself.

The dependent variable of the multiple regression analysis is measured in monetary terms to avoid an aggregation problem. Some vegetables are sold in number of pieces and some of them are sold in weight basis. Therefore, total return is used in monetary terms instead of physical quantity as a dependent variable. The farmers consume a portion of their vegetable products by themselves, distribute some portions to the relatives and sell the balance to the markets. Therefore, total return is calculated by aggregating the market values of consumed, distributed, and sold quantities. There is a positive relationship between total return from vegetable cultivation and capacity building of a farmer (Kamruzzaman and Takeya 2007). Therefore, total return is used as a proxy of capacity building for estimating the multiple regression model.

Physical skill score = Capability of physical involvement in j^{th} activity + technical knowledge on j^{th} activity. Thirteen agricultural activities are considered in this study where physical involvement is necessary along with technical knowledge. At first how many hours of physical involvement required for each activity are calculated and then average hours needed for each activity along with standard deviation are calculated for the samples of the study. High physical involvement is determined as; less than (average – 1/2 of standard deviation), medium as; within (average ± 1/2 standard deviation) and low as; greater than (average + 1/2 standard deviation). High, medium and low involved scored as 3, 2 and 1 respectively for each activity. Technical knowledge for each activity divided into two categories; low and high. If the farmer has adequate technical knowledge then a score of 2 is given and if inadequate then 1 is given. Therefore, a maximum of 65 and a minimum of 26 score can be obtained from 13 activities by each farmer for evaluating his physical skill.

Technical skill is calculated as; if a farmer follow recommended practice for transplanting, fertilizer application, irrigation water applied, integrated pest management, weeding and spraying of plant protection chemicals then a score of 2 is given for his high technical skill and if a farmer does not follow recommended practice for these activities then a score of 1 is given for his low technical skill. With this idea a farmer can obtain a maximum score of 12 and a minimum of 6.

Managerial score calculated as; if a farmer makes short term farm planning, grades their product according to size and shape, distributes labor according to their skill, receives loan, pays the loan in due time, maintains income and expenditure statement then a score of 2 is given, if they do not do it then a score of 1 is given. In addition, a score of 3, 2, and 1 is given if a farmer sells his product to the wholesale market, intermediary and local market respectively. Therefore, a farmer can obtain a maximum score of 15 and a minimum of 7. Communication skill is separated from managerial skill, because it is assumed that communication skill has a vital role in earning total return from farming. Therefore, high communication skill is treated for a farmer if he uses mobile phone for buying raw materials and selling his products, and obtains a score of 3 for his high communication skill, if a farmer uses broadcast media and interpersonal communication for this purpose then he obtains a score of 2 and 1 respectively.

Categorization of level of skill

Technical and managerial skills as well as the physical skill are categorized into three groups according to their scores obtained. Those whose score is ≥0.5 standard deviation below the mean score are categorized as "low" in each skill. Farmers whose score is ≤0.5 standard deviation on either side of the mean are categorized as "medium" in each skill and farmers with ≥0.5 standard deviation above the mean are categorized as "high" in each skill.

Results and discussion

Intensity of farming

Majority of rice farmers used human labor for conducting several agricultural activities for rice production. Of the farmers, marginal and small farmers used more than 50% of their total cost of rice production for human labor (Table 1). Among these farmers, around 40% of total cost was covered by the family members of marginal and small farmers whereas only 14% was shouldered by family labor for medium farmers. Power tillers are used for ploughing of land by the medium farmers and a small portion of marginal and small farmers used a power tiller hiring from other farmers for ploughing of their land. Therefore, most of the agricultural activities are still depended on human labor which indicates labor intensive farming is still dominated in Bangladesh. However, in Japan, only 36.4% of total cost of rice production is covered by labor cost (MAFF 2005) and in Germany only 10% of total cost of agricultural production is covered by labor cost (FMFACP, 2006).

Table 1. Cost of rice production (per hectare) across different category of farmers

Unit: Taka

Input use	Marginal		Small		Medium	
	Cost	%	Cost	%	Cost	%
Family labor (A)	10780	40.4	11550	40.2	4950	14.0
Hired labor (B)	3190	11.9	3630	12.6	6980	27.4
Human labor (A+B)	13790	52.3	15180	52.8	14630	41.5
Animal/Mechanical power	3750	14.0	3920	13.6	7450	21.1
Seed	1470	5.5	1520	5.3	1745	4.9
Fertilizer	4850	18.2	5050	17.6	6370	18.1
Irrigation	2210	8.3	2450	8.5	4250	12.0
Plant protection	450	1.7	625	2.2	840	2.4
Total cost	26700	100.0	28745	100.0	35285	100.0
Total return	44658		47521		58750	
BCR	1.67		1.65		1.66	

Source: Collected data by authors from interviews. Data in all tables and figures are the same as Table 1.

Vegetable production in Bangladesh is more labor intensive than rice because around 68% of the total cost of vegetables was covered by human labor for the marginal and small categories of farmers (Table 2). Majority of labor comes from family source for them. Medium farmers also have to spend around 58% of total cost for human labor, but a significant portion of human labor is used on hired basis for vegetable production. Because, family members of medium sized farms engaged themselves in non-farm business and in service out of agriculture. Vegetable production is also highly labor intensive compared to developed countries like Japan where, 40.6% of total cost is covered by labor for upland vegetable production (MAFF 2005).

Table 2. Cost of vegetable production (per hectare) for different categories of farmer

Unit: Taka

Input use	Marginal		Small		Medium	
	Cost	%	Cost	%	Cost	%
Family labor (A)	27515	53.4	27726	52.7	7012	11.0
Hired labor (B)	7264	14.1	7738	14.7	29975	47.2
Human labor (A+B)	34779	67.5	35464	67.4	36986	58.2
Animal/Mechanical power	3197	6.2	3305	6.3	9832	15.5
Seed	955	1.9	946	1.7	1103	1.8
Fertilizer	8329	16.2	8448	16.0	9972	15.7
Irrigation	2357	4.6	2464	4.7	3242	5.1
Plant protection	1886	3.7	2006	3.8	2439	3.8
Total cost	51504	100.0	52632	100.0	63575	100.0
Total return	197934		178835		130927	
BCR	3.85		3.42		2.06	

Involvement of labor for vegetable cultivation

Vegetable production is labor intensive. It shows high profitability (Sahabuddin and Dorosh 2002) and high correlation with capacity building ability (Kamruzzaman and Takeya 2007). Therefore, vegetable farmers are considered for this study to determine their capacity building by life stage. The results show that 2650, 2702 and 2818 hours of labor are engaged in per hectare of vegetable production for marginal, small and medium farmers respectively (Table 3). There are thirteen farming activities identified which requires human labor to be employed for vegetable production. Among these activities weeding and harvesting are the most labor intensive job. Because farmers have to uproot each individual weed around the plants and harvesting is done periodically depending on the maturity stage and high price getting opportunity. Therefore farmers have to develop their physical skill to perform well in the thirteen farming activities. It is assumed that farmers can build their capacity if they develop their physical skill at an early stage.

Table 3. Labor involvement (hours/ha) in different farming activity across farm category

	Marginal	Small	Medium
Land preparation	66	69	75
Seed bed preparation	59	59	67
Sowing/transplanting	170	173	180
Bamboo stack/net	38	40	44
Irrigation	158	162	173
Fertilizer	66	69	75
Weeding	960	966	980
Spraying pesticides	43	46	51
Harvesting	706	715	729
Handling	122	127	141
Grading	124	132	144
Carrying	90	94	103
Total	2650	2702	2818

Capacity building by life stage

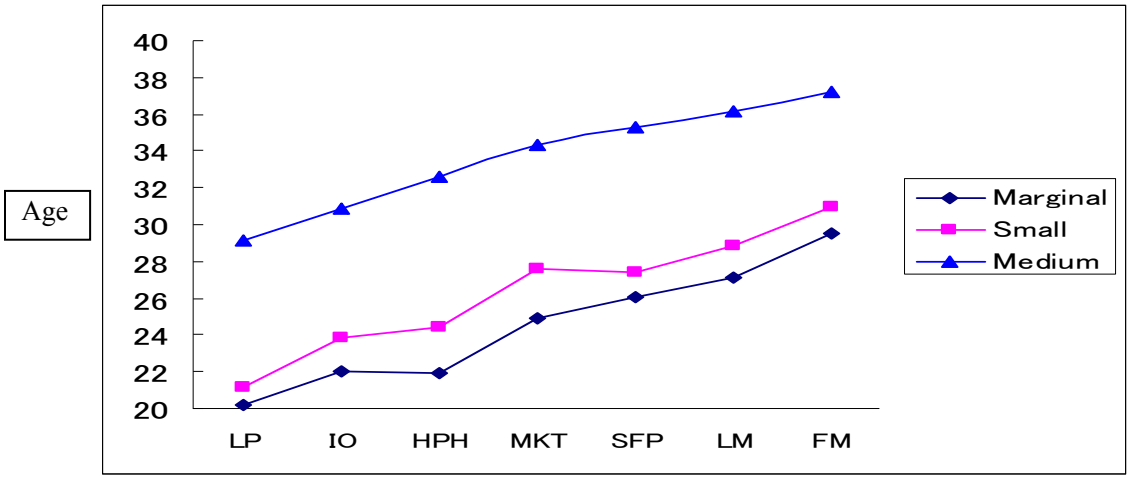
Family members of the marginal and small farmers begin to assist the farmer in different farming activity at the age of 12.0, whereas medium farmers begin at the age of 15.5 (Table 4). Marginal and small farmers face disadvantages in terms of their limited resource base and small scale operations for producing of diversified crops, therefore family members of these groups of farmers engaged earlier in different farming activities to build up their capacity to earn additional income. Whereas, family members of the medium farm category are less interested to do farming business and their tendency is to do non-farm

business and engage in service sector for earning more income. They also treated farming activity as an activity for poor people and they tried to maintain their social status not by doing farming but doing some non-farm business. Another reason is that younger family members of the medium farmers are usually go to high school and they also tend to think that agricultural activities is for the poor people who are irrational and they should engage in agricultural activities for its better performance. The medium farmers mostly used hired labor and machines for their agricultural activities and therefore, some of their family members started to assist the farmer a little later than marginal and small farmers.

Table 4. Physical involvement of vegetable farmers in different farming activity across farm category

Activity	Marginal		Small		Medium	
	Mean	Range	Mean	Range	Mean	Range
Land preparation	14.4	12.5-17.5	15.3	12.8-17.0	16.4	15.5-17.3
Intercultural operation	15.1	12.5-18.1	16.7	15.1-18.5	18.0	17.0-18.5
Harvest and post harvest	15.3	13.0-19.8	16.2	14.3-17.3	17.3	16.8-18.5
Marketing	15.7	12.0-18.0	18.1	13.5-20.0	18.8	17.0-21.0
Farm planning	18.5	16.0-21.3	19.9	17.0-21.7	21.5	20.3-22.7
Accounting	19.9	17.0-23.0	21.3	18.5-24.0	24.4	22.5-25.5

Similar trend is found for these groups of farmers when they started different farming activity independently (Fig. 1). The result shows that majority of the marginal and small farmers are well ahead in Gehilfen[1] stage of capacity building than the medium category. Because, resource poor marginal and small farmers have to do their farming for meeting up their subsistence need and to improve their standard of living by increasing capacity of farm production.



Note: LP = Land preparation, IO = Intercultural operation, HPH = Harvest and post harvest activity, MKT = Marketing, SFP = Short term farm planning, LM = Labor management, FM = Financial management

The result also shows that farmers cannot enter into the Meister stage because marginal and small farmers cannot make long term farm development and financial management plan, maintenance of assets and properties etc. The marginal and small farmers have a small piece of owned land and they have to lease some lands from medium and large farmers. When they lease lands they cannot make a long term plan because land owners can take lands any time for their own purpose. Moreover, farm development plan requires specialized education in agriculture but the farmers in Bangladesh have no formal education at agricultural high school or college. The long term financial management plan also requires specialization in farm business management which is absent for the farmers in Bangladesh. Almost 80% of the farmers cannot use heavy machines like tractor, combined harvester, greenhouses because of their very limited resources. Therefore, farmers in Bangladesh can hardly enter into the Meister stage of capacity building.

Factors affecting capacity building

Physical and technical skill score is higher for marginal and small farmers than medium farmers (Table 5). Family members of the marginal and small farmers utilized their labor very efficiently because of their limited resource base. They participated in some training programs on how to produce vegetable in a scientific way, how to manage soil fertility, how to do integrated pest management etc given by department of agricultural extension or non-government organizations. Therefore, they have some technical knowledge on agricultural production which increases their technical knowledge base along with technical skill.

Table 5. Average score of physical and technical skills for the farmers across farm category.

Skills	Marginal	Small	Medium
Physical	56.5 (12.6)	52.0 (13.6)	33.9 (7.6)
Technical	10.5 (2.2)	9.5 (2.1)	6.8 (1.0)
Managerial score	13.4 (2.5)	12.1 (2.8)	9.0 (2.0)

Note: Maximum value for physical, technical and managerial score is 65, 12, and 15 respectively. Figures in the parentheses indicate respective standard deviation.

Regarding physical skill 67.4% of marginal and 58.3% of small farmers have high skill, whereas there are no medium farmers who have high skill (Table 6). Technical skill level was also high for marginal farmers (67.4%) followed by small farmers (44.4%). The tendency is also similar for managerial skill. The marginal and small farmers devoted themselves in farming activities to sustain their life using very limited resources. They have utilizes their family members owe to skill development to have high physical and technical skill of farming activities. Though physical and technical skill score is higher for marginal farmers than small and medium farmers but there is an increasing tendency of having high skill for small farmers also.

Table 6. Distribution of different categories of farmers by physical, technical and managerial skill level.

Skill level	Marginal		Small		Medium	
	Count	%	Count	%	Count	%
Physical skill						
Low	12	26.1	11	30.6	15	83.3
Medium	3	6.5	4	11.1	3	16.7
High	31	67.4	21	58.3	0	0
Technical skill						
Low	12	26.1	12	33.3	17	94.4
Medium	3	6.5	8	22.2	1	5.6
High	31	67.4	16	44.4	0	0
Managerial skill						
Low	10	21.7	11	30.6	14	77.8
Medium	5	10.9	11	30.6	4	22.2
High	31	67.4	14	38.9	0	0

The result of multiple regression analysis shows that if physical score increase by 1 point then total return from vegetable production increases by Tk. 1762 (USD 25). The result also shows that mobile phone users for buying raw materials and selling products have a possibility of earning Tk. 18082 (USD 262) than the farmers who use interpersonal communication for this purpose (Table 7). The result also shows that physical skill is the most important factor with a standardized value of 0.58 for increasing total

return than technical skill (0.21) and high communication skill dummy (0.20). The managerial skill factor does not show any significant factor for increasing total return. In earlier discussion we saw that vegetable production is highly labor intensive and it needs physical involvement of labor with some technical knowledge. The farmers also need to have some ideas on recommended practice of some agricultural activities which is defined as technical score also vital for increasing vegetable production. Communication skill also shows a significant role in increasing total return because farmers can sell their product at a high price prevailing in the market through mobile phone and broadcast media. Managerial skill in the study area can not play an important role because farmers do not use machines, greenhouses for vegetable production. Moreover, they cannot make any long term farm development plan because of lease lands from the large and medium farmers, and medium farmers are almost out of farming business, engaging in non-farm business. Therefore, it can be concluded that physical skill is the dominant factor for increasing capacity building of the farmers in terms of earning total return.

Table 7. Factors affecting capacity building of the farmers in the study area

Variable	Coefficient	Standard value	Sig. level
Constant	38042.4		
Physical skill	1762.3	0.58	8.16
Technical skill	3808.8	0.21	2.48
Managerial score	552.5	0.04	0.74
High communication skill dummy	18082.0	0.20	4.54
Medium communication skill dummy	5595.5	0.05	2.22

R^2 is 0.98 and F-value is 1734 with 5 and 94 degrees of freedom.

Conclusion

The findings of the present study revealed that some of the farmers in Bangladesh are in the second stage (Gehilfen) of capacity building and there is a minimum possibility to enter into final stage (Meister) of capacity building. Majority of Marginal and small farmers are well ahead in entering into the second stage of capacity building than the medium farmers. The study also identified that physical skill is the dominant factor for increasing capacity in vegetable production for the farmers. Therefore, marginal and small farmers could make agricultural productivity better than the medium farmers owe to skill development. This sort of studies has not yet done by any researchers for the developing countries. Moreover, the findings of the present study is based on the characteristics of Bangladeshi farmers, therefore, there is a possibility of including more developing countries to verify the present findings.

Significance of the study:

Different literatures published in German and Japanese language show that many farmers in developed country are in Meister stage. However, there are no literatures found for the farmers of developing countries regarding in which stage of capacity building they are. Therefore, present study is undertaken to determine capacity building by life stage for the farmers in Bangladesh as a representative of developing country. Furthermore, There are no analytical studies found so far which considered physical, technical and managerial skill as factors for estimating capacity building of the farmers in developing countries. Therefore, the present study is focused on two aspects. First, determination of capacity building by life stage for the farmers in Bangladesh and second, identifying some factors which affect capacity building of the same farmers. It is expected that the findings of the study have some potentials to add some important knowledge on existing literature of capacity building study.

Note: [1]Lehrling, Gehilfen and Meister are the established stages of capacity building in developed countries like Germany and Japan. Farmers of those countries have to pass an examination with some designated experience to enter into next stage from the previous one. In Bangladesh, there are no formal licensing systems of capacity building by life stage like Germany and Japan. Therefore, it is hypothesized that the farmers who have high physical and technical skills are entering into the Gehilfen stage of capacity building from Lehrling stage.

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Physico-chemical analysis of ground water of selected area of Ghazipur city-A case study

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Abstract: A laboratory study was conducted to monitor the ground water quality of selected sites of Ghazipur city by examining the various physico-chemical parameters like pH, T.D.S., D.O.& CO_2 etc.. A comparision with ICMR standard shows that the water is nearly suitable for drinking purpose, the DBPs (Disinfection by products) analysis is required to corroborate the present study. [Nature and Science. 2009;7(1):17-20]. (ISSN: 1545-0740).

Key words: Physico-chemical parameters, Water characteristics, Ground water analysis, Potable water.

1. Introduction

Ground water is ultimate, most suitable fresh water resource with nearly balanced concentration of the salts for human consumption. Over burden of the population pressure, unplanned urbanization, unrestricted exploration policies and dumping of the polluted water at inappropriate place enhance the infiltration of harmful compounds to the ground water. Studies regarding the ground water quality analysis has been made by many authors like B. K. Gupta and R. R. Gupta (1999), M. Rajasekara et al. (2005), M. R. Rajan and I. Paneerselvam. (2005), S. B. Thakare et al. (2005), Shikha Bisht et al.(2007).They concluded that it is the high rate of exploration then its recharging, inappropriate dumping of solid as well as liquid wastes, lack of strict enforcement of law and loose governance are the cause of deterioration of ground water quality. Municipal Corporation of Ghazipur facilitates the drinking water in limited area, in alternate to this people keeps option as hand pumps and jet pumps etc. from last few years it has been seen that the water quality of the alternative sources like hand pumps, wells has been deteriorating and its responses are in the form of yellowish and uncommon odor of the water people in this area using chlorine tablets for disinfect the drinking water. The objective of this work is to assess the quality of drinking water in Ghazipur city.

2. Materials and Methods:

2.1 Study area:

The experiment was conducted at Deptt. of Environmental Science, P.G.College,Ghazipur. This is suburban area and district head quarter, located in the eastern gangetic plain of the Indian sub continent at $25^0$19`and $25^0$54`N latitude, $83^0$4`and $83^0$58`E longitude and 67.50 m above the sea level. The coldest months here are December-January and the hottest months are May-June. The Temperature varies from 5° to 17° centigrade in winters and 30° to 42° in summers. But some times winter temperature ebbs to 3° C and summer temperature shoots up to 45° C. In the summers, which begin from March and last till Mid June the temperature starts rising and sometimes it reaches 45° C. The annual rainfall in the district was between 800 mm. and 1200 mm and in 1997 the rainfall was 1034 mm. On the average there are 49-55 rainy days (days with rain fall of 2.5 mm or more) in a year in the district July and September the relative humidity are high being over 70 %. During the Post-Mansoon and winter season the humidity is high in the morning. By summer, the relative humidity become very low i.e. less than 25 %.Anonymous (2007).It having 25 wards with some extension areas of the city five sites are selected for the study as mentioned in Fig.:-1 Map View. The average boring depth of the city is 45-60 meter

2.2 Sampling and sampling sites:

A fluorinated plastic bottle of capacity 2 litre has been used to collect the sample, before sampling evacuation of the stored water in the pipelines has been made to take the fresh ground water sample the selected sampling sites are populated and urban areas of the city depicted in the Fig.:1 A map view of ghazipur city as site 1 to 5.The sampling has been carried out in the month of April year 2007.

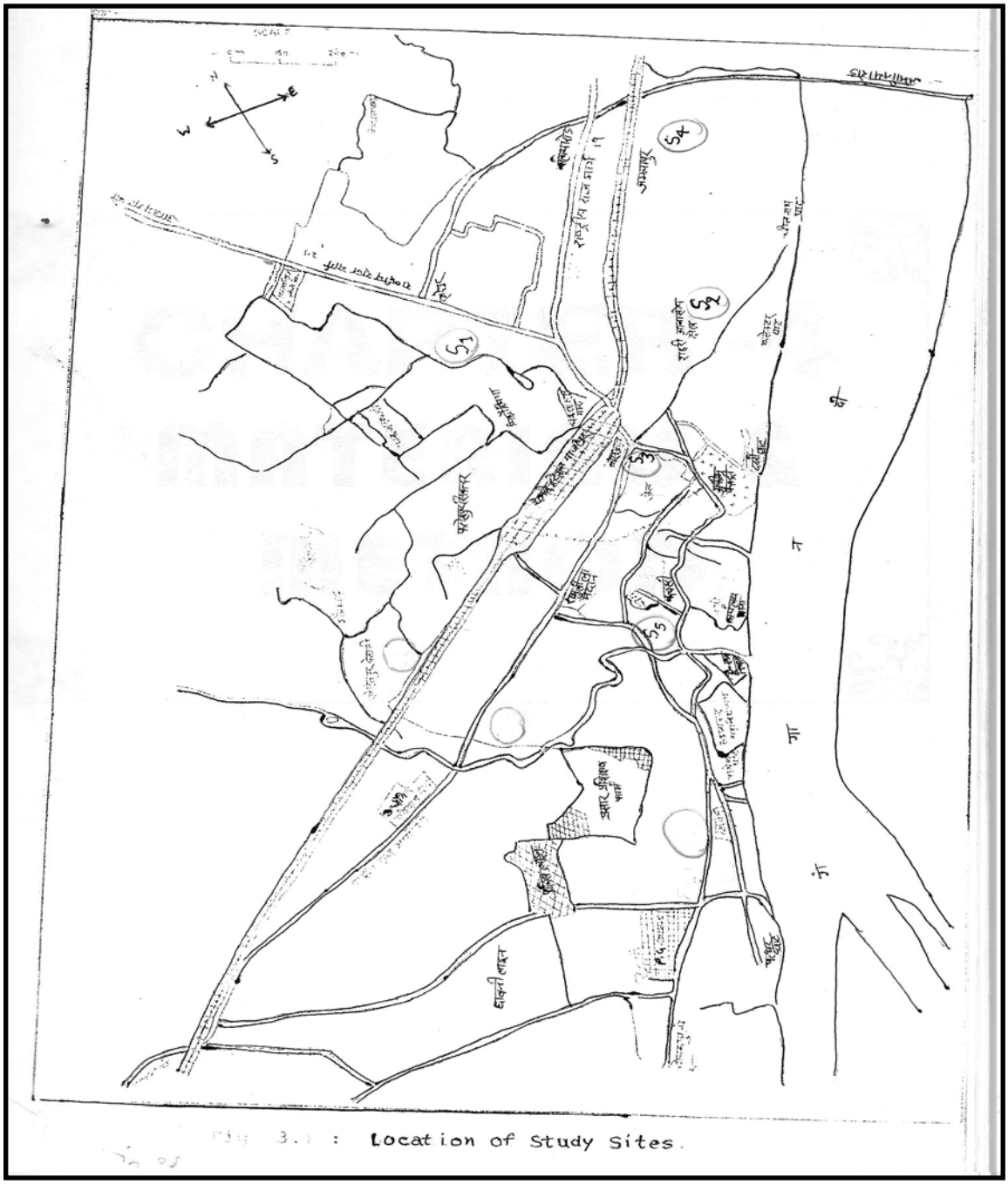

Figure 1: A map view of study site Ghazipur city.

2.3 Methodology:

pH was measured with the help of pH meter (Model no. 101 E) of Electronic India , standardized with pH buffer 4,7 and 9.2. TDS was estimated by evaporation method at 180^0C, Alkalinity, Hardness,

D.O., Chloride, CO_2 and all parameters were analyzed by standard procedure mentioned in APHA (1995). The elemental analysis carried out by digital flame photometer.

2.4 Statistical analysis:

The data were subjected to one way ANOVA analysis of variance using SPSS ver. 10 software Ducan's multiple range test performed to test the significance difference among the treatments.

3. Result:

Table 1: Reading of water quality parameters at different sites in Ghazipur city.

Parameters	S1	S2	S3	S4	S5	ICMR
pH	7.4±.00^{c}	7.2±.00^{c}	6.8±.12^{d}	8±.11^{b}	8.3±.00^{a}	7.0-8.5
T.D.S.	200±6.5^{c}	175±2.5^{d}	145±2.8^{e}	225±2.8^{b}	245±7.6^{a}	500
T. H.	256±.1^{c}	235±.11^{d}	240±4.04^{d}	266±1.15^{b}	304±3.05^{a}	300
Cal. Hard.	108±.11^{c}	99±.7^{d}	106±2.3^{c}	140±.35^{b}	158±3.05^{a}	-
D.O.	3.4±.005^{e}	4.1±.006^{b}	3.6±.00^{d}	4±.00^{c}	5±.00^{a}	4-6
Cl	78±.30^{e}	100±1.5^{b}	83±1.1^{d}	91±.57^{c}	106±.17^{a}	200
Alk.	120±.10^{b}	140±7.5^{a}	110±5.77^{b}	140±.17^{a}	149±1.7^{a}	200
Co_2	7.42±.009^{c}	7.84±.003^{a}	7.92±.002^{a}	7.02±.002^{d}	7.67±.00^{b}	-
Na	23±.17^{b}	28±.005^{b}	25±.00^{b}	42±6.7^{a}	46±2.3^{a}	-
K	4±.00^{e}	4±.00^{d}	6±.00^{c}	8±.00^{b}	10±.00^{a}	75

Different letters in each group shows significant difference at P<0.05 levels.(Mean ± stand. error)
S1- Rauza, **S2**-AamGhat, **S3**-Vishweshwar Ganj, **S4**-Shastri Nagar, **S5**-GoraBazar.

4. Discussion:

The value of pH range among 6.8 to 8.3. It is in the prescribed limit of ICMR. A little bit increase in pH level may depress the effectiveness of the disinfectants like chlorinations thereby requiring the additional chlorines. The value of total dissolved solid ranges from 145-245 mg/l all the values of total dissolved solid is in the prescribed limit of ICMR it is due to high dissolved salts of Ca, Mg and Fe it requires specific cation and anion analysis. Total hardness ranges from 235-304 mg/l, total hardness is with in the prescribed limit of ICMR except the site-5 which is 304 it fall in hard water category it means it contains appreciable amount of Calcium and Magnesium ions. Calcium hardness ranges from 99-158 mg/l. Dissolved Oxygen ranges from 3.4-5 mg/l, D.O. indicating the nearly pure symptoms. Chloride content is 78-106. Chloride content is also in the limit of ICMR. Alkalinity ranges from 110-149 mg/l. Alkalinity is the cause of carbonate and bicarbonate ion and its salts. It is in the prescribed limit of ICMR. Cabon dioxide content is from 7.02-7.92 ppm. According to Henry's law the gaseous dissolution has been determined by partial pressure of gases, soluble salt content and ambient temperature. Increase in CO_2 content may be by high dissolved salt contents. One more possibility is there that is the degradation of

DOC (dissolved organic carbon).Higher DOC on post disinfectant application causes some DBPs (Disinfection byproducts) like THM (Trihalomethanes),HAA (Haloaceticacids) etc. Some of them are potential carcinogens, and a short-term exposure can lead to dizziness, headaches, as well as to problems associated with the central nervous system. so it is more relevant for those areas where OM contaminations are high with high use of disinfectants. Quality of ground water under study is nearly fit for drinking purpose, but it is recommended that ground water analysis should be carried out from time to time to monitor the rate and kind of contamination along with analysis of DBPs to corroborate the present study.

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Biometrical Studies On Genetic Diversity Of Some Upland Rice (*Oryza Sativa* L.) Accessions

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ABSTRACT: Field experiments were conducted in 2005 in the Experimental Farm Station of the University of Agriculture. Makurdi, Nigeria to evaluate the performance and genetic diversity of some upland rice accessions. Preliminary results indicates highly significant ($P<0.01$) differences on all traits studied except for grain length, grain width, grain length/width ratio and 1000 grain weight, indicating genetic diversity among these accessions. Grain yield ranged between 0.54 t/ha (TOX 1870-30-102) and 3.7 t/ha (TOX 1010-21-5-124). Genotypic coefficient of variability (GCV) was generally lower than phenotypic coefficient of variability (PCV). Days to 50% heading, days to maturity, flag leaf area, panicle weight, panicle length, number of branches/panicle, number of seeds/panicle, grain weight/panicle and seed yield showed very low differences between their PVC and GCV values. Also these traits had high estimates for heritability and genetic advance. Genotypic correlation analysis of yield with other traits revealed that yield had a significantly positive correlation with flag leaf area, number of tillers, number of panicles, panicle weight, panicle length, number of branches/panicle, number of seeds/panicle and seed weigh/panicle, grain length and 1000 seed weight. The direct and indirect effect of the rice traits on yield was assessed. The implications of these results for varietal recommendations and crop improvement are highlighted. [Nature and Science. 2009;7(1):21-27]. (ISSN: 1545-0740).

Keywords: Diversity; Coefficient of variability; Heritability; Genetic advance; Correlation; Path coefficient

INTRODUCTION

Rice in Nigeria is the sixth major crop in area cultivated after sorghum, millet, cowpea, cassava and yam (FAO, 1994). It is grown in four major rice growing environment: Upland, rainfed lowland, irrigated lowlands, and deep water. Singh *et al*., 1997. Rainfed upland is the major rice growing ecology in West Africa, accounting for nearly 60% of the total regional rice area. In Nigeria, upland rice comprises around 32% of the total rice area (Singh *et al*. 1997).

Chaudhary and Nanda (1986) estimated 4.6 million ha as potential areas for rice cultivation in Nigeria. The rice area has increased tremendously since 1989. The average annual growth rate from 1983 to 1992 was 14.2% (WARDA 1996) due to ban on importation in 1986. There is still vast potential for increasing the rice area; especially for upland ecologies.

Rice production increased from 0.94 million tonnes to 2.54 million tonnes in 1994 (Singh *et al*., 1997). This increase is however due to mark expansion and not increased productivity per unit area, which remain around 1.5 t/ha.

Rice consumption is on increase, the annual growth rate in rice consumption average 7.7% from 1983 to 1992 (Singh *et al*., 1997). To meet up with this about 0.35 million tonnes valued at 91 million US dollars was imported in 1993 (Singh *et al*, 1997). Local production has not met the demand due to lack of adequate suitable flood plains and unavailability or affordable irrigation facilities to the local farmers, hence limiting lowland rice area expansion in addition to other bio physical constraints. Also human health risk poses important constraints to rapid development of the lowland areas.

Increase in rice production can only be achieved through area expansion and increase yield per unit area by employing high yielding varieties. To sustain local production and rice area expansion the potential of upland rice varieties to fit into the length of growing period, and it's cultivation ease (requiring less land clearance) has to be utilized.

Rice varieties had been evaluated in the country across rainforest and the Sudan Savanna agro-ecological zone (Kehinde *et al*., 1989). Vange *et al*., 1999; 2000, studied lowland rice genotypes in the Southern Guinea Savanna zone. Such evaluation Offers Scientist opportunity to select varieties/lines that are promising for breeding purpose or for on-farm farmers participatory varietal selection (a dual mechanism for obtaining feed back on farmers preferences in new rice varieties) and for technological transfer. These form the objective of this present study.

MATERIALS AND METHODS

Field experiments were conducted during 2005 cropping seasons at the Experimental Farm of the University of Agriculture, Makurdi (7.40^{o}N, 8.37^{o}E, altitude 106.m) Nigeria. Prior to the experimentation, soil analysis of the site was done (83.5% sand, 8.6% silt, 7.7% Clay and Bulk density 1.40 with pH (H_20) 6.19, 13.12% organic matter and 0.09% total N). Total rainfall data during the crop season June, to November, 2005 was 872.70 mm.

Upland rice varieties obtained from International Network for Germplasm Evaluation (INGER) Africa, West Africa Rice Development Association (WARDA) and National Cereal Research Institute (NCRI) Badeggi-Nigeria were laid in a Randomised Complete Block Design in 4 replications with plot size of 12m^2/plot.

The experimental site was ploughed and harrowed twice before seeding. The seeds were broadcasted (farmers most adopted seeding method) at 50kg/ha (60g/plot). Fertilizer was applied at 75Kg N, 60 Kg P and 60 kg K in 3 split doses at 2nd harrowing, 5th week after planting and at panicle initiation with NPK 15:15:15 for the first 2 doses and Urea (46N:0:0) at top dressing. Weeds were controlled manually by weeding at 4 weeks after planting and subsequently as the weeds appear.

The observations were recorded from 5 random plants from each plot. The characters studied were grain yield, panicles/m^2, 1000-grain weight, grain weight/panicle, Total number of panicles branches (both primary and secondary branches), production tillers (%), plant height, days to 50% heading and days to maturity.

All data were subjected to analysis using relevant analysis of variance according to Steel and Torrie (1980), means were separated with List significant Difference (LSD) at P≤0.05.

Genotypic and phenotypic variance was estimated by the formulae suggested by Singh and Chandhary (1977), while broad since heritability was estimated on a replicated plot mean basis according Burton and De Vane (1953). Also genetic and phenotypic coefficient of variability was estimated according to Burton and De Vane (1953). The estimated genetic Advance was estimated using the formula given by Allard (1960) at 50% selection intensity. Path coefficient analysis and genotypic correlation between yield and yield components was computed.

RESULTS AND DISCUSSION

The genotypes showed significant genotypic variation for all traits studied except for grain length, grain width, grain length/width ratio and 1000-grain weight (Table 1). This indicates wide variability among genotypes especially for the traits that are significant thus genetic improvement through selection could be promising. Singh *et al.,* 1986, reported genetic variability in 98 upland rice cultivars they studied with respect to seedling height, days to 50% flowering, culm angle, leaf angle, leaf length, plant height (cm), panicle length (cm), shealth length (cm), tillers/plant, grains/panicle and grain yield (g). Mehetre *et al.*, 1994, reported similar findings on upland rice they studied.

The mean values are presented in Table 2. TOX 1010-21-5-12-4 (TOX 1010) had the highest yield of 3.70t/ha. TOX 1010 also had the highest panicle length, number of panicles/m^2 seed weight/panicle, grain length and grain width with high 1,000-grain weight, and number of seeds/panicle, and panicle weight. Similar trend were obtained for ITA 315, WAB 36-34-FX, ITA 150, and WAB 96-1-1 that had grain yield of 2.67, 2.63, 2.09, 2.08 t/ha respectively.

TOX 1870-30-102 gave the lowest yield of 0.54t/ha, the lowest 1000 grain weight, and generally low seed weight per panicle, panicle weight and panicle length. WARDA (1990) reported avenge yield of 2211kg/ha and 1738kg/ha for WAB-56-104 and WAB 6-125 in their replicated on Station yield trials.

The results of the phenotypic coefficient of variability (PVC) and genotypic coefficient of variability (GCV) revealed that PCV was generally higher than the GCV in the genotypes studied (Table 3). The difference was low for Days to 50% heading, panicle length, no of Branches/Panicle, No of seeds/panicle, and weight of seeds per panicle. Seed yield had a moderate amount of difference between PCV and GCV, while plant height had a considerable high difference between PCV and GCV. These results implies that traits with low difference in PCV and GCV shows that variability is due more to genetic cause. Heritability and Genetic advance estimates was observed to be high for Days to 50% heading, Days to maturity, flag leaf area. Panicle weight, panicle length, No of branches per panicle, no of seeds/panicle, seed weight/panicle at seed yield (Table 3). High heritability coupled with high Genetic

advance observed for these traits indicates a predominance of additive gene effects. Vange and Ojo 1997 reported similar results in lowland rice genotypes.

The results of the path coefficient analysis and genotypic correlation of yield with other traits revealed generally that, the genotypic correlation estimates was positive and significantly correlated with flag leaf area, number of tillers, number of panicles, panicle weight, panicles length number of branches/panicle, number of seeds/panicle, seeds weight/panicle, grain length and 1000 seed weight. Number of tillers (0.30174), number of panicles (0.28615), panicle weight (0.27933), and seed weight per panicle (0.2294) had positive high direct effect on yield while Flag leaf area (0.17938), grain length (0.16251) and 1000 seed weight had direct effect on yield. Number of tillers had high positive indirect effect via number of panicles while panicle weight had indirect effect on yield via panicle length, number of panicle branches, number of seeds per panicle and seed weight per panicle. These traits can serve as indicators in selecting for high yield in the material studied. Ramalingam *et al.* (1993) assert that traits that have high positive correlations, very high direct effects and positive indirect effects on yield through many traits should be emphasised for selecting yield. Panicle length, number of panicle branches, number of seeds per panicle hand negative direct effect on yield. While days to 50% heading, Days to maturity, and productive tillers seem to have limited practical usefulness as indicators for selecting high yield in these genotypes. Chauhan *et al.* (1986), Suarez *et al*. (1989), Vange *et al.* (1999; 2000) reported similar results.

In conclusion: TOX 1010, WAB 36-34-Fx, and WAB 96-1-1 appear promising while Number of tillers, number of panicles, panicle weight, seeds weight/panicle, number of seeds/panicle could be use for indirect selection criteria for grain yield improvement.

Table 1: MEANSQUARES FROM ANALYSIS OF VARIANCE FOR AGRONOMIC TRAITS, YEILD AND YIELD COMPONENTS OF 19 UPLAND RICE GENOTYPES

Sources of Variation	Replications	Genotypes	Error	F pr.
Df	3	18	54	
Days to 50% Heading	45.14	298.79**	8.94	< 0.001
Days to Maturity	47.63	295.87**	9.05	< 0.001
Plant Height (cm)	475.8	219.20**	101.90	< 0.016
Flag Leaf Area (cm^3)	66.64	741.83**	97.10	< 0.001
No. of Tillers/M^2	427.2	1429.6**	357.8	< 0.001
No. of Panicles/M^2	508.3	883.1**	308.5	< 0.001
Productive Tillers (%)	149.09	280.12**	46.28	< 0.001
Panicle Weight (g)	0.715	2.685**	0.424	< 0.001
Panicles Length (cm)	2.74	41.528**	4.235	< 0.001
No. of Branches/Panicle	1.93	38.00**	6.672	< 0.001
No. of Seeds/Panicle	296.7	5623.5**	541.5	< 0.001
Seeds Weight /Panicle (g)	0.497	1.8819**	0.345	< 0.001
Grain Length (mm)	0.27	0.484	0.287	0.072
Grain Width (mm)	0.0695	0.0771	0.107	0.777
Grain Length/Width ratio	0.154	0.1299	0.1932	0.822
1000 Grain Weight (g)	44.11	45.86	31.81	0.150
Seed Yield (t/ha)	0.285	2.903**	0.788	< 0.001

** = significant at P = 0.01

TRAITS, YIELD AND YIELD COMPONENTS

Genotypes	Source	Days to 50% Heading	Days to Maturity	Plant Height (cm)	Flag Leaf Area (cm^2)	No. of Tillers/M^2	No. of Panicles/M^2	Productive Tillers (%)	Panicle Weight (g)	Panicles Length (cm)	No. of Branches/ Panicle	No. of Seeds/Panicle	Seeds Weight /Panicle (g)	Grain Length (mm)	Grain Width (mm)	Grain Length / Width Ratio	1000 Grain Weight (g)	Seed Yield (t/ha)
WAB 96-1-1	NCRI	83	118	89.8	107.6	97.2	88	98	3.4	23.85	13	159	2.45	9.55	3.00	3.18	23.3	2.08
WAB 99-1-1	NCRI	65	100	79.7	60.2	113.6	91	81	2.1	20.35	10	65	1.38	9.05	3.00	3.03	28.0	1.24
WAB 181-11	NCRI	64	99	83.2	51.2	97.2	64	72	2.5	23.28	11	70	1.35	9.40	2.95	3.20	26.4	0.67
WAB 36-34-Fx	NCRI	69	104	102.2	62.3	116.0	104	90	2.9	23.85	14	94	1.70	10.25	3.15	3.53	34.5	2.63
WAB 56-128-Fx	NCRI	66	102	92.4	80.3	109.6	93	86	3.4	27.90	17	148	2.40	9.85	3.00	3.25	25.4	2.22
WAB 56-144-Fx	NCRI	66	100	89.2	59.1	103.6	73	78	2.1	20.00	11	81	1.33	9.45	3.00	3.08	26.3	0.97
WAB 56-21-Fx	NCRI	71	106	87.4	55.7	111.2	91	83	2.6	20.85	12	113	2.03	9.45	3.00	3.15	29.6	1.85
TOX 1010-21-5-12-4	IITA/WARDA	85	120	85.6	81.7	140.0	124	99	3.9	25.95	12	135	2.90	10.10	3.05	3.30	32.3	3.70
ITA 150	IITA/WARDA	65	100	97.9	60.6	124.0	102	83	3.0	24.35	11	120	2.03	9.90	3.00	3.28	29.8	2.09
ITA 315	IITA/WARDA	80	115	76.2	74.1	111.2	96	87	4.2	24.80	18	162	3.00	9.00	3.00	3.05	27.0	2.67
WAB 18-844	NCRI	83	118	76.0	74.5	67.2	65	97	3.4	24.60	14	124	2.53	9.35	3.15	3.08	25.0	1.02
WAB 35-1-Fx2	NCRI	72	107	83.9	48.0	92.2	86	93	1.3	15.55	7	35	0.68	9.55	3.05	3.05	25.3	1.23
WAB 56-1-Fx2	NCRI	70	105	96.8	55.5	90.5	89	98	1.6	15.50	8	53	1.15	9.30	2.90	3.13	28.9	0.63
ITA 337	IITA/WARDA	87	122	86.3	69.0	85.5	80	93	4.0	24.45	18	159	2.86	9.50	3.05	3.10	24.7	1.45
TOX 1870-30-102	IITA/WARDA	76	111	88.9	63.4	96.7	91	96	1.6	20.85	9	74	0.95	9.55	2.85	3.45	19.3	0.54
ITA 343	IITA/WARDA	83	118	89.4	58.3	89.5	90	100	3.1	23.05	12	122	2.43	9.00	3.05	2.95	29.9	1.37
WABIS 675	INGER	81	116	85.2	60.8	82.2	79	96	3.1	24.10	12	127	2.33	9.15	3.50	2.70	26.4	1.12
ITA 143	INGER	85	120	75.8	66.5	66.5	66	98	2.3	19.60	11	95	1.65	9.40	2.90	3.25	27.1	0.65
FARO 43	NCRI	88	123	80.5	59.9	77.7	76	98	2.4	22.40	13	115	1.95	9.25	2.90	3.15	24.7	0.89
$LSD_{(0.05)}$		4.3	4.3	14.4	14.09	27.0	25.1	9.7	0.9	2.94	3.69	33.2	0.84	0.77	0.47	0.63	8.06	1.27
C.V (%)		3.95	2.72	11.66	15.00	19.20	20.3	7.50	23.6	9.20	21.1	21.6	30.18	5.66	10.8	13.9	20.87	58.0

Table 3: GENETIC PARAMETERS FOR 13 TRAITS IN UPLAND RICE GENOTYPES

Traits	Means	Standard Error	Broad Sense Heritability (h^2)	Genotypic Coefficient of Variability	Phenotypic Coefficient of Variability	Genetic Advance as % of mean
Days to 50% Heading	75.61	2.11	0.89	11.26	11.93	21.88
Days to Maturity	110.64	2.13	0.89	7.654	8.122	14.86
Plant Height (cm)	86.63	7.14	0.22	6.251	13.22	6.087
Flag Leaf Area (cm^3)	65.72	6.97	0.62	19.32	24.45	31.44
No. of Tillers/M^2	98.52	13.38	0.43	16.62	25.39	22.4
No. of Panicles/M^2	86.58	12.42	0.32	13.84	24.56	16.07
Productive Tillers (%)	90.74	4.81	0.56	8.426	11.28	12.97
Panicle Weight (g)	2.76	0.46	0.57	27.24	36.04	42.42
Panicles Length (cm)	22.38	1.46	0.69	13.64	16.45	23.31
No. of Branches/Panicle	12.22	1.83	0.54	22.9	31.16	34.67
No. of Seeds/Panicle	107.9	16.45	0.70	33.03	39.45	56.98
Weight of Seeds/Panicle (g)	1.95	0.42	0.53	31.79	43.79	47.54
1000 Grain Weight (g)	27.03	3.99	0.10	6.934	21.99	4.504
Seed Yield (t/ha)	1.53	0.63	0.40	47.53	74.99	62.06

TABLE 4: A PATH COEFFICIENT ANALYSIS† AND GENOTYPIC CORRELATION OF YIELD WITH 10 YIELD RELATED TRAITS

	Flag Leaf Area (cm^3)	No. of Tillers/M^2	No. of Panicles/M^2	Panicle Weight (g)	Panicles Length (cm)	No. of Branches/Panicle	No. of Seeds/Panicle	Seeds Weight /Panicle (g)	Grain Length (mm)	1000 Grain Weight (g)
Flag Leaf Area (cm^3)	**0.17938**	0.022602	0.042513	0.110139	0.099556	0.087896	0.126642	0.109242	0.04054	-0.03319
No. of Tillers/M^2	0.038019	**0.30174**	0.25316	0.067288	0.085091	0.013277	0.028364	0.038924	0.15962	0.153284
No. of Panicles/M^2	0.067818	0.24008	**0.28615**	0.078691	0.070393	0.015452	0.057802	0.072682	0.150229	0.146509
Panicle Weight (g)	0.171509	0.062291	0.076816	**0.27933**	0.238827	0.239386	0.257822	0.270112	0.034637	0.057821
Panicles Length (cm)	-0.08881	-0.04513	-0.03936	-0.13682	**-0.16002**	-0.1245	-0.13106	-0.12706	-0.04609	-0.01472
No. of Branches/Panicle	0.071075	0.006382	0.007833	0.124308	0.112849	**0.14505**	0.123147	0.117926	0.007833	0.002611
No. of Seeds/Panicle	-0.09479	-0.01262	-0.02712	-0.12392	-0.10996	-0.11399	**-0.13426**	-0.12701	-0.00765	0.001477
Seeds Weight /Panicle (g)	0.139759	0.029604	0.05829	0.221917	0.182215	0.186575	0.217098	**0.22949**	0.001836	0.040849
Grain Length (mm)	0.036727	0.085968	0.085318	0.020151	0.046803	0.008776	0.009263	0.0013	**0.16251**	0.057529
1000 Grain Weight (g)	-0.025	0.068661	0.069202	0.027978	0.012435	0.002433	-0.00149	0.024058	0.047847	**0.13516**
Genotypic Correlation with Yield (t/ha)	0.49568*	0.7596**	0.8128**	0.6690*	0.5782**	0.460*	0.5533*	0.6097**	0.55131*	0.54733*

† = Direct (underlined) and indirect effect of rice traits on rice yield.

*, ** = Significant at P = 0.05 and P = 0.01 respectively.

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Biodesulfurization of Kerosene by *Desulfobacterium indolicum*

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ABSTRACT

Recalcitrant organosulfur compounds such as Dibenzothiophene (DBT) and its derivatives in real petroleum fractions such as kerosene cannot be removed by convectional hydrodesulfurization (HDS) treatment using metallic catalysts as well as extremes of conditions of high pressure and temperature. The desulfurizing bacterium *Desulfobacterium indolicum* was isolated and subsequently identified by the Department of Botany & Microbiology; University of Lagos, Nigeria exhibited very high desulfurizing ability towards kerosene at 30°C and normal atmospheric pressure. The biodesulfurization of kerosene by *Desulfobacterium indolicum* resulted in reduction of sulfur from 48.68 ppm to 13.76 ppm over a period of 72 hours. Gas chromatography analysis with a pulse flow photoatomic detector revealed that the peaks of Thiophene and 2, 5 - dimethyl Thiophene significantly decreased after biodesulfurization. Therefore, *Desulfobacterium indolicum* could effectively desulfurized kerosene and thus may be a promising biocatalyst for practical biodesulfurization of kerosene. [Nature and Science. 2009;7(1):28-35]. (ISSN: 1545-0740).

INTRODUCTION

The problem with fossil fuels is that the combustion products are harmful to the planet. Carbon dioxide emissions have been implicated in global warming. Nitrogen oxides and sulfur oxides emissions have been shown to be responsible for acid rain, which destroys buildings, kills forests and poison lakes. Governments throughout the world have recognized the problems associated with these emissions and moved to reduce them through legislation. Regulations for the sulfur level in diesel oil have become increasingly strict and it is planned to reduce the level to 50 ppm by 2005 in the European Union and Japan. The sulfur content in diesel will probably be less than 10 or 15 ppm (w/w) in the United States and Europe by 2010 (Constants et al, 1994).

The concentration of sulfur in crude oil is typically between 0.05 and 5.0% (by weight), although values as high as 13.95% have been reported (Speight 1981). In general, the distributions of sulfur increase along with the boiling point of the distillate fraction. As a result, the higher the boiling range of the fuel, the higher the sulfur content will tend to be. Upon combustion, the sulfur in fuels can contribute to air pollution in the form of particulate material and acidic gases, such as sulfur dioxide. To reduce sulfur-related air pollution, the level of sulfur in fuels is regulated, and to meet these regulations sulfur must be removed from fuels during the refining process. The availability of low-sulfur crude has decreased over the last decade as a consequence of the increasing reserves of heavy crude (Grossman et al, 2001).

Refineries remove organic sulfur from crude oil-derived fuels by hydrodesulfurization (HDS). HDS is a catalytic process that converts organic sulfur to hydrogen sulfide gas by reacting crude oil fractions with hydrogen at pressures between 1 and 20 MPa and temperatures between 290 and 455 °C, depending upon the feed and level of desulfurization required. Organic sulfur compounds in the lower-boiling fractions of petroleum, e.g., the gasoline range, are mainly thiols, sulfides and thiophene, which are readily removed by HDS. However, middle-distillate fractions, like diesel, kerosene and some fuel oil range, contain significant amounts of benzothiophenes and dibenzothiophenes (DBTs), which are considerably more difficult to remove by HDS (Chang et al, 1998). Among the most refractory of these compounds are DBTs with substitutions adjacent to the sulfur moiety. Compounds of this type are referred to as sterically hindered compounds because the substitutions are believed to sterically hinder access of the sulfur atom to the catalyst surface due to their resistance to HDS; sterically hindered compounds represent a significant barrier to reaching very low sulfur levels in middle and heavy-distillate-range fuels (Kirimura et al, 2003). The high cost and inherent chemical limitations associated with HDS make alternatives to this technology of interest to the petroleum industry. Moreover, current trends toward

stricter regulations on the content of sulfur in fuels provide incentive for the continued search for improved desulfurization processes. The hydrogen sulfide produced as a result of HDS is a corrosive gaseous substance, which is stripped from the fossil fuel by known techniques. Elevated or persistent levels of hydrogen sulfide are known to poison (inactivate) the HDS catalyst, thereby complicating the desulfurization of petroleum crude and products that are high in sulfur. Organic sulfur in petroleum fossil fuels is present in a myriad of compounds, some of which are unstable in that they cannot readily be desulfurized or refractory because they do not easily yield to conventional desulfurization treatment by HDS. Increasing the severity of HDS also elicits undesirable effects on fuel quality as other chemical components are reduced at the higher temperatures and pressures needed to achieve low sulfur levels.

MATERIALS AND METHODS

The microorganism *Desulfobacterium indolicum* with the ability to desulfurize oil was isolated from oil contaminated soil by enrichment culture. It was suspended in 9 ml of 0.1M phosphate buffer solution (pH 7.0) and 1 ml of diesel for the biodesulfurization experiment in a 100 ml Erlenmeyer flask (Rhee et al, 1998). The optical density at 510 nm (OD_{510}) was 1.5 the experiment was performed at 30°C with a moderate shaking of 180 rpm. Also, the growth of the sulfur bacterium *Desulfobacterium indolicum* in the experimental tube was monitored as described previously (Chukwu and Nwachukwu, 2005).

Thiophene, 2,5- dimethyl thiophene, benzothiophene and Dibenzothiophene were analyzed using gas chromatography 5890 Hewlett Packard, equipped with a pulse flow photoatomic detector (PFPD).

RESULT AND DISCUSSION

Desulfobacterium indolicum is a motile, oval to rod like, gram negative, non spore forming anaerobic microorganism. Biochemical test has shown that it is capable of utilizing various kinds of sugar as a source of carbon. In the biodesulfurization experiment, the organism was suspended in a sulfur free phosphate medium and the kerosene. It is easier for the organism to utilize carbon in glucose which is in aqueous state in which the organism is also suspended if available than kerosene which is oil. Thus one may conclude that the biodesulfurization of thiophene and 2, 5 - dimethyl thiophene took place via a sulfur-specific degradation pathway.

The GC analysis revealed that the kerosene contained 6.955 mg/l of thiophene and 41.724 mg/l of 2, 5 - dimethyl thiophene. No benzothiophene and dibenzothiophene were found in kerosene. Figures 1 and 2 below show

Figure 3 below shows the concentration-time profile for the biodesulfurization of benzothiophene. It showed that *Desulfobacterium indolicum* steadily desulfurized the benzothiophene decreasing its concentration to 1.72 mg/l at the end of 72 hours.

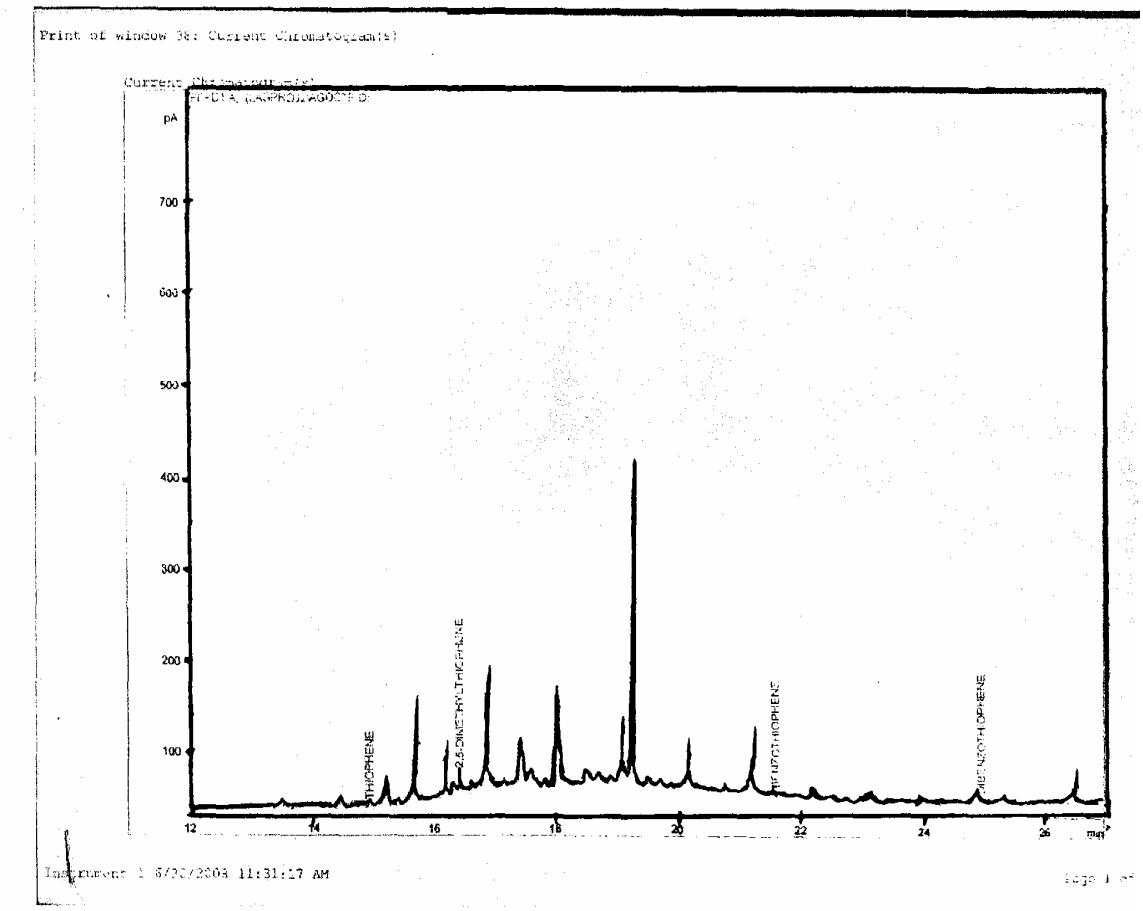

Figure 1: GC-PFPD Chromatograms for Kerosene before Biodesulfurization. the GC-PFPD peaks for all of the sulfur compounds in the kerosene (approximately 48.679 mg/l sulfur initially) before the biodesulfurization by *Desulfobactenum indolicum.* after treatment of the kerosene for 72 hours, all of the peaks significantly decreased. It is important to note that the sulfur compounds with retention times longer than 5 minutes nearly disappeared. Such characteristics of desulfurization by cells of *Desulfobactenum indolicum* are opposite or complimentary to those of hydrodesulfurization, in which sulfur compounds with a shorter residence time are more easily desulfurized (Dzidic with a shorter residence time are more easily desulfurized (Dzidic et al, 1988). Based on these results, cells of *Desulfobacterium indolicum* are considered to have a sufficiently broad substrate specificity to desulfurize major organic sulfur compounds contained in diesel.

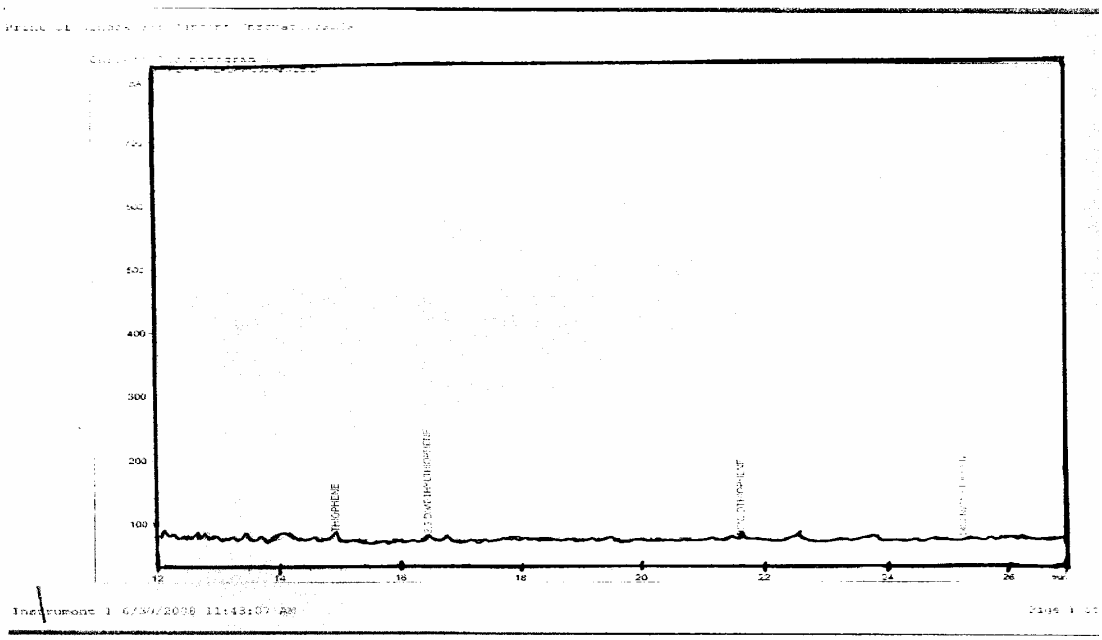

Figure 2: GC-PFPD Chromatograms for Kerosene 72 hours after Biodesulfurization

This is a remarkable feat at a reaction temperature of only 30°C, extremes of reaction conditions would have been employed in hydrodesulfurization to attain the same level of desulfurization.

Similarly, Figure *4* below shows that *Desulfobacterium indolicum* also desulfurized dibenzothiophene steadily reducing its concentration to 31.692 mg/l at the end of 72 hours.

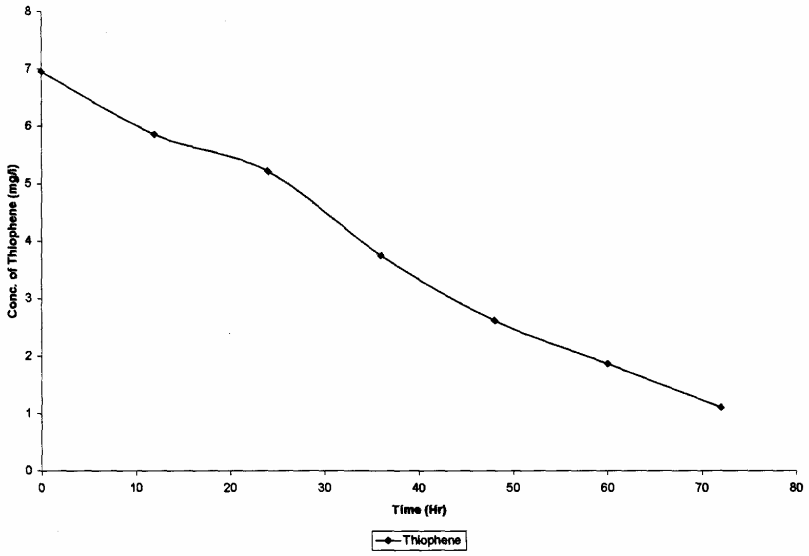

Figure 3: The Concentration-Time Profile of Thiophene Biodesulfurization by *Desulfobacterium indolicum*

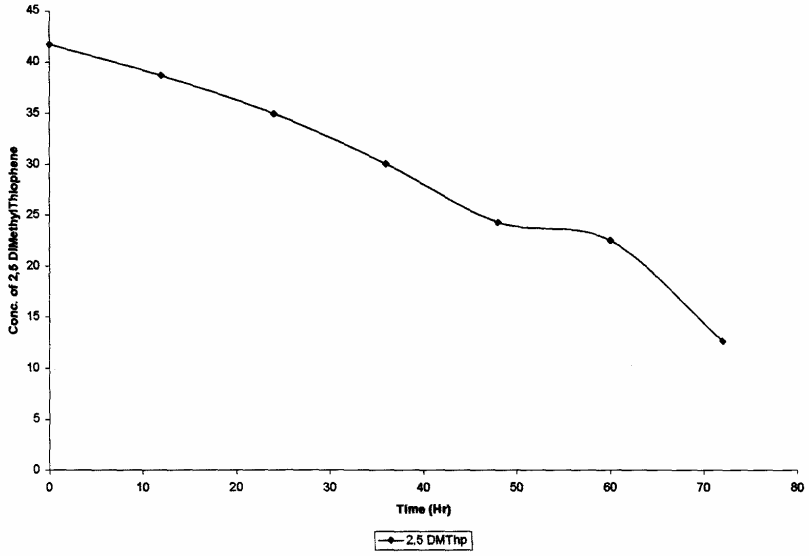

Figure 4: The Concentration-Time Profile of 2, 5 – Dimethyl Thiophene Biodesulfurization by *Desulfobacterium indolicum*

From the viewpoint of a practical process, biodesulfurization at ambient temperature and pressure of kerosene containing various types of thiophene derivative is advantageous, since cooling treatment of the oil to ambient temperature would be unnecessary.

Figures 3 and 4 above show the concentration-time of biodesulfurization of thiophene and 2, 5 - dimethyl thiophene in kerosene. It was observed that at all times, the percentage of thiophene desulfurized is higher than 2, 5 - dimethyl thiophene. This is expected because the methyl substituents at

positions 2 and 5 would constitute a steric hindrance to the organism from reaching the sulfur atom in the thiophene ring. At the end of 72 hours, 84% of thiophene has been desulfurized while 70% of 2, 5 - dimethyl thiophene was desulfurized. This is shown in figure 5 below.

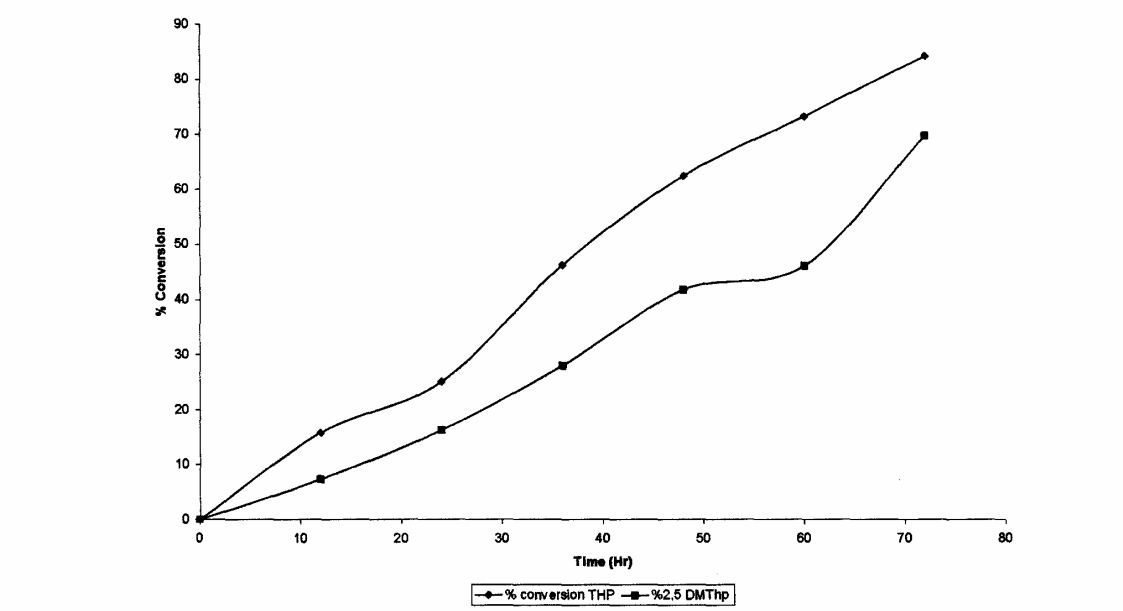

Figure 5: The Percentage Desulfurization -Time Profile of Thiophene & 2, 5 - Dimethyl Thiophene Biodesulfurization by *Desulfobacterium indolicum* The population density of *Desulfobacterium indolicum* is increasing as biodesulfurization of kerosene progresses. The Log™ of the population of the cells of A versus time is shown in figure 6 below.

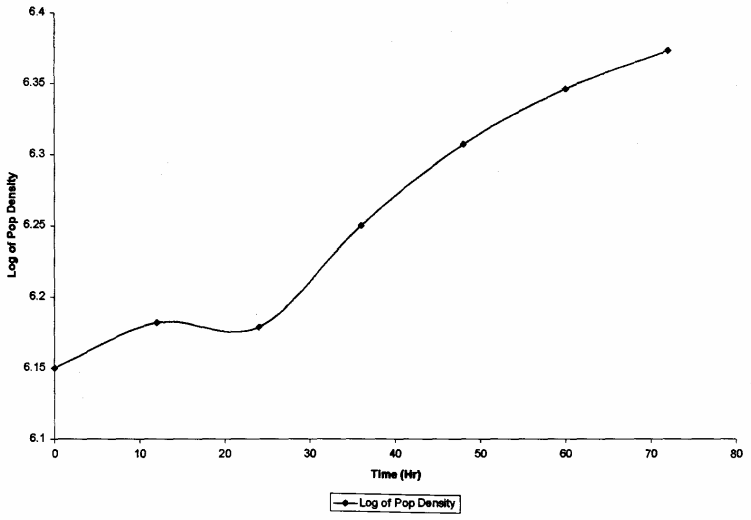

Figure 6: The Log_{10} of the population of the cells of *Desulfobacterium indolicum* -Time Profile

The population of the cells of *Desulfobacterium indolicum* increases steadily as biodesulfurization of kerosene progresses, showing that the cells use the sulfur in the fuel for metabolism leading to both growth and increase in population.

Goswami et al, (1991) in their work mentioned that two different biological mechanisms are known for the degradation of water-insoluble hydrocarbons and aromatic compounds. The first involves the case in which the microorganisms use emulsifiers to overcome poor solubility of hydrocarbons and aromatic compounds, whilst the other is to increase cell surface hydrophobicity so that adherent capacity to the hydrocarbon is enhanced. According to them, in many cases, microorganisms use both mechanisms with one mechanism acting dominantly. The dominant mechanism can be easily figured out by centrifugation of the cell broth after cultivation using hydrocarbons or oils. If the increase of cell surface hydrophobicity were the dominant mechanism, most of the cells would exist in the interface of aqueous and oil phase after centrifugation. Most of the cells would be in the bottom of the aqueous phase in the opposite case. In this work, the cells of *Desulfobacterium indolicum* were observed at the interface of the aqueous and oil phase.

The first step in the biodesulfurization of these molecules is the transfer of the molecules from the oil to the cells. It appears that these molecules are transferred directly from the oil into the cells. Many microorganisms have been shown to metabolize many insoluble molecules in this fashion. The PASHs appear to partition to the water before being brought into the cell. The enzyme responsible for the first two oxidations are to reflect the reaction it catalyzes and has been coded DszC. It catalyzes the oxidation by transferring an electron from flavin mononucleotide (FMNH2) to the organosulfur (the thiophene and 2,5- dimethyl thiophene) to produce FMN an oxidized (FMNH2) and sulfoxides of thiophene and 2,5- dimethyl thiophene and also the oxidation of sulfoxides by transferring an electron from flavin mononucleotide (FMNH2) to produce FMN an oxidized (FMNH2) and the corresponding sulfones.

The first cleavage of the C-S bonds is catalyzed by sulfone Monooxygenase (FMN hfc XO2 oxidoreductase); DszA codes this enzyme. It Transfers another electron from FMNH2 to XO2. Where X is the organosulfur.

The production of sulfite & subsequently sutfate and an intact hydrocarbon molecule is the last reaction in the pathway. This is catalyzed by a desulfinase coded by the DszB gene and leads to the release of the sulfur as sulfite and the production of the corresponding hydroxyl phenyl.

In nature, the cell has achieved its goal. It has the sulfur it needs to grow. The sulfite can be reduced to sulfide and incorporated into sulfur-containing amino acids and vitamins necessary for growth. It is worthy of note that this study focused on real fuel rather than modeled media of organosulfur compounds. This implies that the organism can survive in the fuel till it removes all the sulfur in it.

In conclusion, it has been confirmed that A could effectively desulfurize organosulfur compounds, thiophene and 2, 5 - dimethyl thiophene through a sulfur-specific degradation pathway with the selective cleavage of C-S bonds at ambient temperature and pressure conditions. Therefore, *Desulfobacterium indolicum* may be a useful desulfurizing biocatalyst possessing broad substrate specificity toward organosutfur compounds.

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A Simple Geometry for Obtaining a Gas Discharge at Low Pressure

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Abstract: A new geometry for obtaining a gas discharge has intended for the general experiments ion sources laboratories .This geometry is based on the use of a magnetic hollow cathode in which one introduces a permanent magnet in order to increase the probability of ionization gas atoms by the method of crossing electric and magnetic fields. [Nature and Science. 2009;7(1):36-38]. (ISSN: 1545-0740).

Key words: gas discharge, cold hollow cathode, ionization

Introduction:

A permanent magnet is mounted coaxially on the inside of the cathode and creates a magnetic field. The cathode has an internal open chamber or space filled with a gas and with the applying of a voltage between the cathode and anode the gas discharge starts (Pessoa et al. 2006). The ions are extracted from the gas discharge plasma through the emission opening cathode which has a fixed diameter and a variable height h i.e. a variable volume.

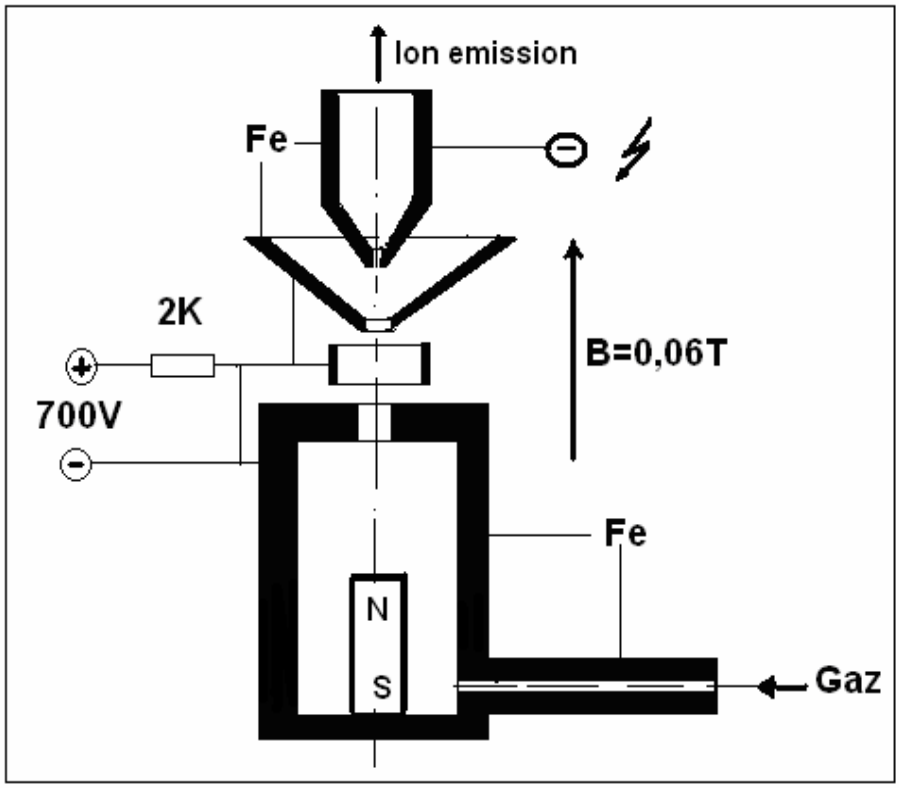

Figure 1: Arrangement of a magnetic hollow cathode

Results and Discussion

A crossed-field cold-hollow-cathode arc is stable at low working gas pressures of 10^{-2}–10^{-1} Pa, magnetic-field-and gas-dependent arcing voltages of 600-700 V, and discharge currents of 100–200 A (Boubetra et al., 2008). This is because electrons come from a cathode spot produced on the inner cathode surface by a discharge over the dielectric surface. The magnetic field influences the arcing voltage and discharge current most significantly. When the plasma conductivity in the cathode region decreases in the electric field direction, the magnetic field increases, causing the discharge current to decline and the discharge voltage to rise. The discharge is quenched when a critical magnetic field depending on the type of gas is reached. Because of the absence of heated elements, the hollow cathode remains efficient for long when an arc is initiated in both inert gases, what was already concluded in other work (Gavrilov et al., 2003)and (Goebel et al ,1992).

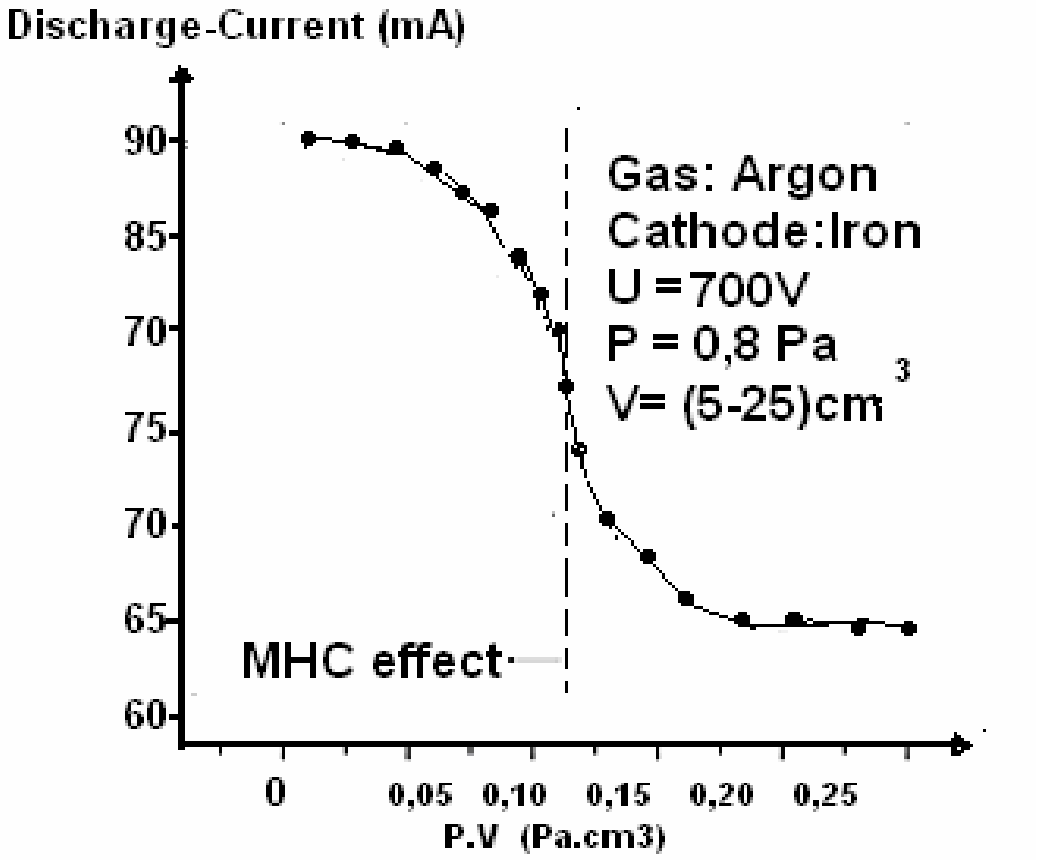

Figure 2

The MHCM discharge proprieties were obtained taking into account the effect of the gas pressure and the volumes cathode. When the volume is reduced, keeping the discharge voltage constant (700V) the discharge current is seen to rise according to figure 2 at optimized values of P.V the discharge current is higher than an other conventional sputtering discharge.

Conclusion:

According to the results obtained one can say that this geometry can be used for an arrangement magnetron to the beams extraction of plasma which could be useful like a tank of the ions and by there one of the ions beams which working with a low pressure and a small volume of hollow cathode.

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Effects Of Industrial Effluent On Quality Of Well Water Within Asa Dam Industrial Estate, Ilorin Nigeria.

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ABSTRACT

The effects of industrial effluent on the quality of ground water (well) within an Industrial Estate was studied. The quality was assessed in terms of physicochemical parameters and bacteriological parameter. Three wells within the industrial were examined in the course of the study. Results obtained showed that the turbidity varied between 1.5 to 250 NTU and colour ranged from 211 to 2519 Pt- Co. The total, suspended and dissolved solids content were high. The conductivity ranged from 161 to 731 μs, while pH ranged from 6.9 to 7.3.Calcium and Magnesium ions as well as chloride ion content of the water were high. The dissolved oxygen content ranged from 6 to 9mg/l. Bacteriological indices showed that the well water were highly contaminated having high total bacterial counts (1200- 1375 cfu/ml). The well water showed presence of feacal coliform (*E. coli*) and had high coliform counts (1600 - >1800 MPN/100ml). It was observed that the wells were negatively affected by the effluent discharged within the industrial plant. [Nature and Science. 2009;7(1):39-43]. (ISSN: 1545-0740).

Keywords: Industrial effluent, well, Ground water, Bacterial count.

INTRODUCTION

The importance of water in the control of diseases had long been recognized (Hofkes, 1981; WHO, 1996). Water is a factor of production in virtually all enterprise, including agriculture, industry and the services sector (UNESCO, 2006). The importance of safe drinking water is underlined by the assertion that: "safe drinking water is the birthright of all humankind – as much a birthright as clean air" (TWAS, 2002). It also reported that the majority of the world's population, especially in most parts of Africa and Asia, does not have access to safe drinking water and that as much as 6 million children dies daily as result of waterborne diseases linked to scarcity of safe drinking water or sanitation (TWAS, 2002). WHO (2004) pointed out that diseases related to contamination of drinking-water constitute a major burden on human health: and that interventions to improve the quality of drinking-water provide significant benefits to health.

For most communities the most secure source of safe drinking water is pipe-borne water from municipal water treatment plants. Often, most of water treatment facilities do not deliver or fail to meet the water requirements of the served community; due to corruption, lack of maintenance or increased population. The scarcity of piped water has made communities to find alternative sources of water: ground water sources being a ready source. Wells are a common ground water source readily explored to meet community water requirement or make up the short fall.

Wells are categorized based on the nature of construction: open dug wells are generally considered the worst type of groundwater sources in terms of faecal contamination and bacteriological analysis. Dug wells with windlass or hand pumped or mechanically pumped well are generally regarded to be less prone to contamination (WHO, 2004). WHO (1997) assert that open or poorly covered well heads pose the commonest risk to well-water quality; the possibility of the water being contaminated is further increased by the use of inappropriate water-lifting devices by consumers. The commonest physical defects leading to faecal contamination of dug wells are associated with damage to, or lack of, a concrete plinth, and with breaks in the parapet wall and in the drainage channel (WHO, 1997). The most serious source of pollution of well water is contamination by human waste from latrines and septic tanks resulting in increased levels of microorganisms, including pathogens. Other likely sources of contamination include runoffs, agrochemicals such as pesticides and nitrates used on farm lands and industrial effluents. Contamination of well water due to under seepage has reported in the Niger Delta area of Nigeria (Ibe and Agbamu, 1999). Seepage from effluent bearing surface water would readily contaminate wells located close to the surface water.

Arising from the drive for industrialization, parts of Ilorin town are designated industrial estate/ area to accommodate the industries. One of such industrial estate has the course of River Asa running through its whole length. The river is flows through Ilorin town almost dividing it into two halves

(Olayemi, 1994). This makes it readily prone to abuse as effluent receptacle leading to contamination. Studies have shown that the River's water quality is affected by the discharge of the effluents (Eniola and Olayemi, 1999). This is consistent with the observation of Sangodoyin (1991) that effluents discharge alters the physical, chemical and biological nature of receiving water body. Wells are a vital and common source of water in Ilorin, some of these wells are located along the course of River Asa.

In this study, the effect of the discharge of effluent into river Asa on the quality of water of wells within the immediate catchment of the river was investigated. Water samples from wells within the industrial estate were subjected to physicochemical and bacteriological investigations to ascertain the effect of the effluent on the quality of the well water.

MATERIALS AND METHODS

Open dug well with concrete apron (plinth) around the well head were involved in the study. Water samples from the wells were collected into clean sterile 250ml sampling bottles as described by WHO (1997). The pH, colours (Pt-Co), turbidity, temperature, total Hardness, calcium hardness, magnesium hardness, calcium ion magnesium ion, chloride and conductivity were determined. The suspended, dissolved and total solid contents of the water were determined as described by ASTM (1985). The total heterotrophic bacteria counts were determined using the pour plate method (APHA, 1992). The coliform counts were determined as Most Probable Number (MPN) using the multiple tube fermentation test (APHA, 1992).

RESULTS

The physicochemical characteristics of the well water are shown on Table 1. The bacteriological characteristics are shown on Figure 1. Water from the wells were found to be close to neutral (pH 6.9 to 7.3) with high bacterial count (1200- 1375 cfu/ml). The coliform count was high (1600 - >1800 MPN/100ml) and faecal coliform (*E. coli*) was isolated. The variation in the total suspended and dissolved solids contents of the wells as well as the dissolved oxygen contents of the well water are shown on Figure 2.

Table 1. Physicochemical Characteristics of the water from Wells within Asa Dam Industrial Estate, Ilorin.

Parameters measured	W_1	W_2	W_3
pH	6.9	7.3	6.9
Colour (Pt-Co)	211	2519	240
Turbidity (N.T.U)	1.5	250	4.6
Temperature (^oC)	27	28	28
Total Hardness (mg/l)	149	153	37
Calcium Hardness (mg/l)	102	96	34
Magnesium Hardness (mg/l)	46	57	4
Calcium ion (mg/l)	410	383	135
Magnesium ion(mg/l)	37	46	3
Conductivity (us)	338	731	161
Chloride (mg/l)	155	12	2

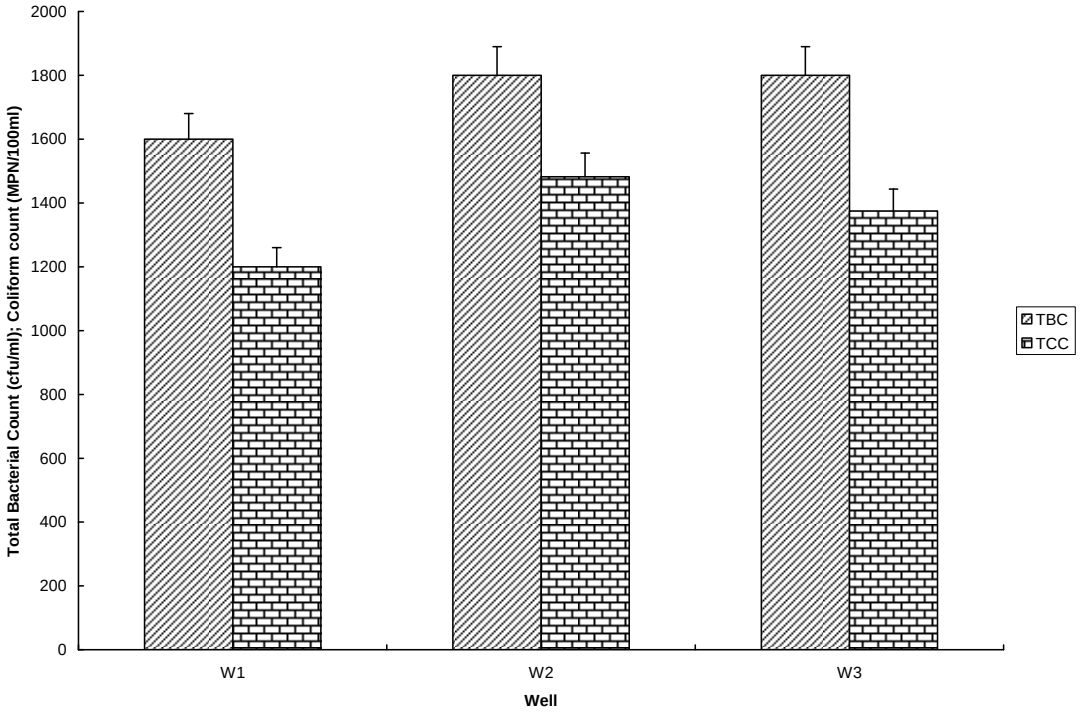

Figure 1: Bacteriological Characteristics of the Water from Wells within Asa Dam Industrial Estate, Ilorin.

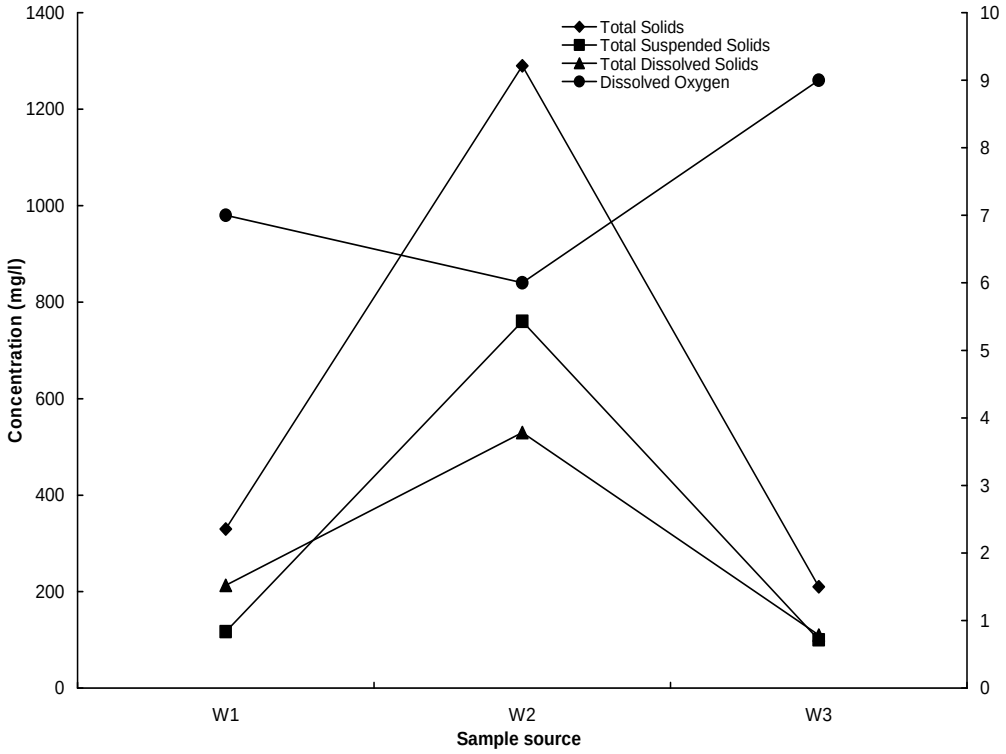

Figure 2. Variation in Total, Suspended and Dissolved Solids Contents of the Wells

Discussion

Water from the wells was observed to be coloured and turbid with the value ranges of 211- 2519 Pt-Co and 1.5 – 250mg/l respectively. Thin films of oil present on the water surface appear to make the value of the colour to be very high. The high turbidity value is as a result of increase in the type and concentration of the suspended matter released by the industry. The content of total solids, suspended and dissolved solids were also high. This is attributable to the industrial waste discharged into the surface water and suggests some of the content of the effluent have found their way into the ground water. Well water containing high total solids, total suspended solids and total dissolved solids are not fit for drinking, laundry work and livestock purpose. The high conductivity values suggest that the dissolved solids are mostly mineral salts. The high chloride is also suggestive of the use of large quantity of Chlorine or its associated compounds in activities within the industrial estate. The high bacterial count is suggestive of presence of organic matter (Gray, 1989, Olayemi, 1994). The values of dissolved oxygen obtained suggest that the water was not overtaxed by the quantity of degradable material in it and also that it was being well re-oxygenated.

Bacteriological speaking water from the wells fall short of the WHO (1997) recommended guideline standard for drinking water. It requires that water intended for drinking should not contain any pathogen or microorganisms indicative of faecal contamination. All the water samples examined contained feacal coliform (*E. coli*) and high population of heterotrophic bacteria, which is consisted with WHO (2004) report that open dug wells are contaminated, with levels of at least 100 faecal coliforms per 100 ml. This is not necessarily a result of the citing of the well along the river course but a reflection of the human activities taking place around the catchment of the wells. The unringed nature of the wells makes contamination by seepage from the soil more likely. The WHO (2004) recommends that wells are ringed and provided with an apron around the head to minimize contamination. The bacteriological quality of the wells requires that they be subjected to treatment if they are to be used for drinking and domestic purpose.

Conclusion

The results obtained showed that the water from the well were not fit for human consumption and their qualities were affected by the presence of the wells within the industrial estate and proximity to river that serves for disposal of industrial effluent.

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Biological Efficiency And Nutritional Contents Of *Pleurotus florida* (Mont.) Singer Cultivated On Different Agro-wastes

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ABSTRACT:
Pleurotus florida (Mont.) Singer was cultivated on different agro-wastes viz. soybean straw, paddy straw, wheat straw and their combination in 1:1 proportion to determine the effect of these agro waste on yield, moisture content, crude protein, total carbohydrates, fat, crude fiber, ash and minerals like Ca, P, Fe content. Soybean straw showed significantly highest yield (with 87.56% B.E.) with maximum crude protein (23.50%) and maximum phosphorus (920 mg/ 100 mg of dry mushroom) content. Maximum moisture (92.45 %) and crude fiber content (8.10%) in the fruiting bodies was recorded on Paddy straw cultivation. The combination of Soybean straw + paddy straw showed significantly highest fat (2.60%), Calcium (310 mg/ 100gm) and Iron (13.06 mg/100gm of dry mushroom) content. [Nature and Science. 2009;7(1):44-48]. ISSN: (1545-0740).

Keywords: *Pleurotus florida*, B.E. (Bio efficiency), agro-waste, fruiting body.

INTRODUCTION

Mushrooms are rich in proteins, vitamins, and minerals and popularly called as the vegetarian's meat. Mushroom proteins are considered to be intermediate between that of animals and vegetables (Kurtzman, 1976) as it contains all the nine essential amino acids required for human body (Hayes and Haddad, 1976).

Oyster mushroom (i.e. *Pleurotus spp*.) is commonly called as Dhengri in India because of its oyster like shape. Genus *Pleurotus* belongs to family Tricholomataceae and has about 40 well-recognized species, out of which 12 species are cultivated in different parts of country. *Pleurotus* is an efficient lignin-degrading mushroom and can grow well on different types of lignocellulolosic materials. Cultivation of this Mushroom is very simple and low cost production technology, which gives consistent growth with high biological efficiency. Different species of *Pleurotus* can grow well in variable temperature conditions; hence they are ideally suited for cultivation throughout the year in various regions of tropical country like India.

In the recent times, the cultivation of *Pleurotus sp*. had excelled next to *Agaricus bisporus* (Lange) Sıng. throughout the world in terms of yield and production (Erkel, 1992; Chang *et al.,* 1991). Among the *Pleurotus sp., P.sajor caju* had been widely studied for the cultivation followed by *P. ostreatus* . These studies mainly concentrated on the cultivation on wastes of forest and agricultural plants. Almost, all the available, lignocellulosic substances are likely be used as substrate for *Pleurotus sp*. Cultivation with slightly variation in the range and combination of the substrates in different part of world based on their availability in abundant and being cheaper in the respective region. (Royse, 1985; Schmidt, 1986). Most of these studies focused on the higher yield and quality of fruiting bodies of *Pleurotus sp.* with respect to cultivation times. The present study deals with the cultivation of *P. florida* on some common and abundantly available waste available for conversion in food which otherwise is left for natural degradation. The cultivation of edible mushrooms offers one of the most feasible and economic method for the bioconversion of agro-lignocellulosic wastes (Bano *et al.,* 1993; Cohen *et al.,*2002). The technology can also limit air pollution associated with burning agriculture wastes as well as to decrease environmental pollution due to unutilized agricultural wastes.

MATERIAL AND METHODS

Culture and cultivation:

The pure cultures of *Pleurotus florida* was obtained from National Collection of Industrial Micro organisms (NCIM No.1243), National Chemical Laboratory (NCL), Pune, India. The cultures were maintained on 2 % malt extract agar slants at 4º C. Sub culturing were done after every 15 days.

Spawn preparation:

Spawn was prepared in polythene packets. Sorghum whole grains were boiled in water bath for 10-15 min. at the ratio of 1:1 (Sorghum grains: water) and mixed with 4 % (w/w) $CaCO_3$ and 2 % (w/w) $CaSO_4$. Sorghum grains then packed (250g) in polythene bags (of 200x300 mm. size) and sterilized in an autoclave at 121ºC, for 30 min. After sterilization, the bags were inoculated with actively growing mycelium of the *Pleurotus* from malt extract slants and incubated (at 27 ± 2 ºC) for mycelial growth without any light for 10-15 days until the mycelium fully covered the grains.

Cultivation:

The agro waste soybean straw, wheat straw, paddy straw were collected from local farms and were used for filling the bags. Soybean straw, paddy straw, wheat straw and their combination in 1:1 proportion were used as a cultivation substrates, following the method prepared by Bano and Shrivastava (1962) with slight modifications. The substrates were chopped to 2-3 cm pieces and soaked in water over night to moisten it and excess water was drained off. After soaking, the substrate was steam sterilized at 121º C for 20 min. in an autoclave. The polythene bags of the size 35 x 45 cm were filled with sterilized substrates and multi layered technique was adopted for spawning. Each bag was filled with 1 Kg dry substrate and the spawn was added at the rate of 2 % of the wet weight basis of substrate.

After inoculation the bags were kept in house where the temperature and humidity were maintained around 25 ºC and 80 to 90% respectively with sufficient light and ventilation for 20 days. The spawn run was completed within 18 days. The polythene bags were tear-off following the spawn run. Formation of fruit bodies was evident within 3-4 days after removal of poly bags. The beds were maintained up to the harvest of the third flush, which was completed in 35 days after spawning. A small layer of substrate was scrapped off from all the side of the beds after each harvest. Each of the six treatments was replicated three times.

Yield and biological efficiency:

Total weight of all the fruiting bodies harvested from all the three pickings were measured as total yield of mushroom. The biological efficiency (yield of mushroom per kg substrate on dry wt. basis) was calculated by the following formula Chang *et al.,* (1981)

$$\text{B.E. (\%)} = \frac{\text{Fresh weight of mushroom}}{\text{Dry weight of substrate}} \times 100$$

Moisture content:

The moisture content of mushroom was also expressed in percent and calculated by the formula –

$$\text{Moisture content (\%)} = \frac{\text{Weight of fresh sample – weight of dry sample}}{\text{Weight of fresh sample}} \times 100$$

***Nutritional analysis*:**

Protein, fat, ash and total carbohydrate were determined with the procedure recommended by AOAC (1995) and Wankhede *et al.,* (1976). The crude fibers and calcium was determined with procedure recommended by Ranganna (1986). The iron and phosphorus content were estimated according to the procedure given in Laboratory manual of NIN (Anonymous, 1980). The recorded data in the present work was subjected to statistical analysis as per the procedure given by Panse and Sukhatme (1978).

RESULT AND DISCUSSION:

The results reveal the yield, B.E. and moisture content of the *P. florida* cultivated on different agro-wastes alone or in combination (Table 1). The maximum yield of *Pleurotus florida* was obtained when it was cultivated on soybean straw (875.66gm/kg straw) with 87.56% B.E., this was followed by yield on soybean + paddy straw (852.00gm/kg straw) with 85.20% B.E. while least was recorded with

wheat straw+ paddy straw (723.66gm/kg straw). The moisture content was maximum on paddy straw (92.45%) followed by soybean + wheat straw (90.23%) there was slight variation with other substrate indicating that moisture content is independent of the substrate.

Comparing the three lignocellulosic residues as substrates for the cultivation of *P. florida* shows that soybean straw supported best growth of *P. florida* as evidenced by complete and heavy colonization of substrates forming a compact white mass of mycelium within 2 weeks of inoculation. Furthermore, the quantity of fresh edible fruiting bodies (g/kg of substrate) harvest was higher in single substrate than in mixed substrate. The performance of the three substrates was also evident by their elevated biological efficiency values on Soybean straw followed by paddy straw (Table 1). The time required for harvest of the fruiting bodies on soybean straw always preceded paddy straw alone or in combination.

Reports on cultivation of the oyster mushroom on similar by-products have manifested variable levels of B.E. These variations are mainly related to spawn rate, fungal species used and supplement added to the substrate (Mane *et al.*, 2007). Some of the elevated B.E. of *Pleurotus sp.* on commonly used substrates rice straw 85.5% (Mehta *et al.* 1990), leguminous plants 103.8% (Sharma and Madan 1993).

The Protein, fat, carbohydrate, crude fibre, ash and Ca, P, and Fe contents of mature fruiting bodies of *Pleurotus florida* cultivated on different lignocellulosic substrates alone or in combination are shown in Table 2. *P. florida* fruiting bodies produced on soybean straw possessed the highest protein content of 23.5 % on a dry weight basis followed by soybean + paddy straw (22.66%). The fat content of *P. florida* was 2.60 % grown on soybean + paddy straw being the highest followed by soybean straw alone (2.50%). The % content of protein and fat content were similar as reported in earlier studies (Patil *et al.,* 2008, Patil and Dakore, 2007).

Maximum Carbohydrate content of *P. florida* was 57.80% in fruiting bodies cultivated on soybean straw whereas least was 53.87% cultivated on wheat straw + paddy straw (Patil *et al.,* 2008,). The highest crude fibre was obtained on paddy straw (8.10%) followed by soybean straw (8.02%). Other agro waste alone or in combination also yielded appreciable level of crude fibre. These results were confirmed with findings of Bonatti *et al.* (2004), Khyadagi *et al.,* (1998), (Sharma & Madan 1993). Singh *et. al.*(2003). From the present study it is evident that, *Pleurotus florida* is the suitable species for cultivation on Soybean and Soybean + paddy straw in case of productivity and nutritional contents. The protein contents of various lignocellulosic residues were compared and it was reported that the nitrogen content in fruiting bodies was higher in leguminous plant substrates than non-leguminous ones (Sharma & Madan, 1993). These results were confirmed with the findings of Kadlag *et al.,* (1998) Mandhare (2000). The protein content usually ranges between 20–30% on a dry weight basis. Substrates rich in usable nitrogen after spawn run may be a factor in enhancing the mushroom yield and quality, in addition to the mushroom species in bioconversion and bioaccumulation efficiency (Patil *et al.,*2008).

The maximum ash content of *P. florida* was found on soybean straw (8.00%) followed by mushroom grown on paddy straw (6.60%). Similar results were reported by El –Kattan *et al.,* (1991). The highest calcium content in *P. florida* was recorded when it was grown on soybean straw + paddy straw (310 mg/100gm) followed by soybean straw (305 mg/100gm) alone. The phosphorus content in *P. florida* was maximum on soybean straw (920mg/100gm) whereas least was found on soybean straw +wheat straw (800 mg/100gm). Similar amount of phosphorus was also recorded in earlier study (Caglarirmak, 2007). Highest Iron content in *P. florida* when cultivated on soybean straw+ paddy straw was 13.06 mg /100 gm while least was recorded on the soybean straw +wheat straw (11.87 mg/100gm) These results coinced with those observed by Kikuchi *et al.,* (1884), Rathor and Thakore (2004).

Commercial production of oyster mushrooms is largely determined by the availability and utilization of cheap materials of which agricultural lingo-cellulosic waste represents the ideal and most promising substrates for cultivation. The substrates used in this study can be considered practical and economically feasible due to their availability throughout the year at little or no cost in large quantities. Utilization of these agro-wastes for the production of oyster mushrooms could be more economically and ecologically practical.

Table 1: Effect of different substrate on yield, Bio efficiency (B.E.) and moisture content of *Pleurotus florida.*

Substrate	Yield (gm/kg dry straw)			Total yield	B.E. (%)	Moisture (%)
	I st picking	II nd Picking	III rd Picking			
Soybean straw	412.00	365.66	98.00	875.66	87.56	89.55
Paddy straw	376.00	318.33	140.00	834.33	83.43	92.45
Wheat straw	352.00	263.62	135.00	750.62	75.06	89.40
Soybean + paddy straw	408.00	320.00	124.00	852.00	85.20	90.17
Soybean + wheat straw	362.66	248.00	172.00	782.66	78.26	90.23
Wheat + paddy straw	310.66	275.00	138.00	723.66	72.36	89.46
S.E. ±	10.60	13.17	7.73	-	-	0.22
C.D at 5%	33.34	41.43	24.32	-	-	0.69

Table 2: Effect of different substrate on Protein, Fat, Carbohydrate, Crude fiber, Ash and Minerals like Ca,
P, Fe, content of *Pleurotus florida*.

Substrate	Protein (%)	Fat (%)	Carbohydrate (%)	Crude fiber (%)	Ash (%)	Ca*	P*	Fe*
Soybean straw	23.50	2.50	57.80	8.02	8.00	305	920	12.38
Paddy straw	22.40	2.28	55.50	8.10	6.60	292	860	12.81
Wheat straw	21.33	2.30	56.00	7.80	6.40	287	820	12.28
Soybean + Paddy straw	22.66	2.60	54.90	7.56	6.50	310	840	13.06
Soybean + Wheat straw	22.40	2.30	57.10	7.50	6.35	275	800	11.87
Wheat + Paddy straw	20.25	2.28	53.87	7.40	6.50	304	830	12.92
S.E. ±	0.27	0.03	0.36	0.17	0.06	8.09	15.05	0.11
C.D. at 5%	0.87	0.11	1.16	0.54	0.19	25.46	47.36	0.35

*mg/100gm dry mushroom

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Asian Elephant's *Elephas maximus* Behaviour in the Rajaji National Park, North-West India: Eight Years with Asian Elephant

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Abstract: In order to generate scientific knowledge on behaviour of wild Asian elephant *Elephas maximus*, field study was conducted from 1999 to 2007. The data were derived from a novel combination of self field observations (direct and indirect methods), traditional knowledge of various local communities, and available literature on elephant studies. During the course of study 19 different behaviours of elephant were documented from Rajaji National Park, north-west India. Despite, the status, movement pattern, habitat utilization, feeding behaviour and human-elephant conflict of Asian elephant, extremely rare research work has been carried out on its behaviour in the wild. My review of available evidences suggested that wild elephants also used to perform various usual and unusual behaviours, which are directly linked with their management and conservation. The elephants in Rajaji are emerge out in the open areas in evening hours. The movement of elephants was entirely seasonal and they also utilize the adjoining protected habitats. Fodder requirements are quite variable in groups and bulls and breeding season seems maximum to extent from May to November. Locomotion, social organization, drinking and bathing, resting and sleeping, defecation and urination, recognition, male-male aggression, syampathy and cooperation, play behaviour, association with other wild animals, parental care and elephant communication were another major behaviours observed in elephants. Besides, four unusual behaviours were also studied during this period. This is the first documented study, which could be helpful in generating more biological information about the activities of wild Asian elephants. All of these findings may have wider implications for developing predictive models of human – elephant interactions. [Nature and Science. 2009;7(1):49-77]. (ISSN: 1545-0740).

Keywords: Asian elephant; *Elephas maximus*; behaviour; Rajaji National Park; Conservation; India

Introduction

India has between 21,000 and 25,000 Asian elephants (*Elephas maximus*) in the wild and among them Uttarakhand state harbours 1346 elephants distributed within 14 protected areas. India currently has the largest surviving population of the Asian elephant, approximately 50 % of the total world population of the species (Daniel, 1996). Historically, elephants were known to raid crops but during the recent past because of increasing human population and decreasing forest cover, the conflicts between man and elephant have escalated. A number of wildlife habitats have undergone or are being threatened with fragmentation due to various anthropogenic factors and this has adversely affected the large mammal populations residing in them (Johnsingh et al., 1990). Recently, developmental activities and habitat destruction have caused major decline in the abundance of the terrestrial megafauna. As most of the wild animals are presently categorized under threatened category therefore, there is increasing concern that the area-wise decline of the elephant will have unexpected and grave consequences for the long-term viability of the terrestrial ecosystems.

The Rajaji National Park was established to enhance the long-term survival of the Asian elephant in a sub tropical moist deciduous forest in India (Figure 1). But during the recent past natural continuous forest ranges of India has been broken up into many parts due to agriculture, urbanization, increasing road traffic and development related activities as well as other anthropogenic activities. This situation creates many problems for various organisms living in forests especially for large size mammals like elephant. Genetic isolation, limitation of dispersal, migration and the decline of populations of animals requiring large territories are the most common problems connected with fragmentation of forests and other components of the environment. Shivalik landscape (lesser Himalayan zone) is one of the last few places in the world where elephants exist and offers urgent need for conservation. From conservation point of view Rajaji National Park appears to be India's one of the most successful national park and its development has helped to boost the population of Asian elephant in their natural habitat.

Traditionally wild animals used to perform different unique behaviours to fulfill their life requirements like social calls, alarming calls, threat calls, greetings, matriarchy and hierarchy, pseudo fights, play, association with others and communication and at the same duration they perform different behaviours along with body contexts. This aspect has remained neglected during the past, which is highly required to be documented and will be helpful in management of any wild species. Knowing behaviour of wild animal is a valuable conservation tool, which can help in management and conservation of any threatened species.

Presently human beings have been fascinated towards different behaviour of animals particularly in wild form. The behaviour of wild animals is a subject matter of great interest leading to understanding about the nature of various wild animals, which may be helpful in their management and conservation. Behaviour of Asian elephants (*Elephas maximus*) particularly in circus and domesticated form provide lot of recreation to the human beings and easy to watch and enjoy. However, the behaviour of wild elephants is a tough task to be observed and studied in wild especially in foothills dominant areas. The elephant being a flagship species in this area plays an important role in the structuring of plant and animal communities. Several research studies on the behavioural biology of African elephant (*Loxodonta africana*) were conducted during the recent past but only few studies has been carried out on the behavioural biology of wild Asian elephants. In recent years, due to dwindling of forest areas only few protected areas are available for wild elephants where they can be observed in their natural habitat performing their usual activities and different behaviours.

Methods

Study Area

Rajaji National Park [29^0 15' to 30^0 31' North Latitude, 77^0 52' to 78^0 22' East Longitude] is spread over an area of 820.42 Km^2 in and around the Shivalik foothills, which lies in the lesser Himalayas and the upper Gangetic plains (Figure 2). Rajaji National Park (RNP) was notified in 1983 by amalgamating three erstwhile wildlife sanctuaries namely, Rajaji, Chilla and Motichur. Spread across Hardwar, Dehradun and Pauri districts of Uttarakhand state, Rajaji National Park has been designated as a reserved area for the "Project Elephant" by the Ministry of Environment and Forests, Government of India with the sole 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 has cut across these hills at Hardwar. The Chilla forest area of the RNP lies in the east of the river Ganges and is attached by the Garhwal Forest Division. The study was conducted in Hardwar (District-Hardwar), Chilla (District-Pauri), Motichur (District-Dehradun) and Kansrao (District-Dehradun) forest ranges of the RNP. Besides, Laldhang forest range (Lansdowne forest division), Shyampur and Chiriapur forest ranges (Hardwar forest division) were also included in this study. The altitude lies between 302-1000 m asl. 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 phillipinensis* (Rohini), *Acacia catechu* (Khair), *Adina cordifolia* (Haldu), *Terminalia bellirica* (Bahera), *Ficus bengalensis* (Bar) and *Dalbergia sissoo* (Shisham) in its premise besides many other important fodder plant species. This entire belt is natural home of 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), *Cervous unicolor* (Sambhar), *Sus scrofa* (Wild boar) and *Ophiophagus hannah* (King cobra).

Data Collection

All the field observations were made during 1999 to 2007. It is not easy to sight elephants in dense forest habitats due to thickness of the undergrowth and foggy climate especially during the monsoon and winter period. Also there are chances of any casualty. In few of the forest pockets it was difficult to observe directly the elephants in RNP because of dense sub-tropical vegetation and presence of undulating foothills with bushes taller than the animal. Thus the study incorporated both direct as well as indirect methods.

Direct Method

For studying the behavioural biology of elephant's four forest ranges (Chilla, Hardwar, Motichur and Kansrao) of the RNP and few adjoining forests (Shyampur forest range, Hardwar forest division and Laldhang forest range, Lansdowne forest division) were selected and in-depthly surveyed. It was not possible to observe the elephants during monsoon as the areas are dominated with tall grasses and dry

period was the best time to observe the elephants especially near to water sources. The study area was visited at weekly intervals during which observations on elephants were made along the motorable forest track, present in between different forest habitats. Few other connected rough routes, which link the grassland habitat with motorable road were also used during the course of study. As few forest beats of the study area does not comprises of any road, therefore, study was made on foot. Although some animals were observed up to a maximum distance of 100 meter, most of the observations fell within 50 meter. Besides, all the potential habitats (water dominant areas, cool shaded areas, fodder enriched areas and rough forest routes) were also investigated on foot during early morning, mid-day and evening hours. Cool shaded trees like *Ficus bengalensis*, *Adina cordifolia* and *Ficus glomerata* and dense forest of *Mallotus phillipinensis* and other favourite fodder species were examined mostly during mid day (March-June) hours as elephants generally take rest under these cover. Whereas all the water sources (perennial/annual) were investigated alternatively during evening hours.

As the elephants in RNP have been known to emerge from the forest predominantly during evenings, all sightings of elephants were made between 1500 hours and 1900 hours. All the natural behaviours of the animal were observed and recorded directly. Different forest blocks of concerned forest ranges were selected one after another sequentially and searched for elephants for about 10 – 12 hours (depending upon weather conditions) in a single day search. 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. The data collected was as part of the animal monitoring activities and the daily record was based on direct sighting of animals, indirect evidences like feeding sign, footprints impression time and fresh dung piles. The direct sighting were noted in duly prepared proforma, recording the group composition, age and sex, if observed in groups and also the place of sighting, time and vegetation type. 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 on this problem, were also interviewed. Field binocular was also used for observing their movement behaviour without disturbing the animal from an adequate and safe distance.

Movement behaviour

Different groups and solitary adult male elephants were selected and followed in Chilla, Hardwar, Motichur and Kansrao forests of the RNP alternatively. Their activities along with movements were duly plotted on map. For census purpose, the four categories recognized by Eisenberg and Lockhart (1972) – namely adult, sub adult, juvenile and calf were adopted. Photographs of the groups and bulls were also taken for confirmation upon repeated sightings. Whenever any marked groups or solitary males were encountered during early morning circuit, their location was noted along with ongoing activity. An attempt was again made later in the evening hours to relocate the animal that had been observed in the morning. Video camera was also used to cover small footages of group composition along with different behaviours of recognized groups. Whenever herds were encountered, they were observed until they disappeared from sight or until darkness made further observations impossible. As elephant movement was restricted in between Chilla – Motichur and Rajaji – Corbett corridors, therefore, it has made us easy to follow and observe these recognized groups and bulls those were performed their movements in eastern part of river Ganges.

Feeding behaviour

For studying the feeding behaviour of elephants, all the plants species 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.

Identification of the elephants was important to verify their movement as in the same area there was 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.

Indirect Method

In few of the places the indirect count method was followed for checking their number in study area (Dawson and Dekker, 1992; Ramakrishanan et al., 1991 and Santiapillai and Suprahman, 1986). This involves path counts and frequency of elephant signs. For conducting the study on elephant's presence, all the observations were made from a vehicle and through adopting the road-strip count method (Hirst, 1969; Santiapillai et al., 2003) to monitor the fluctuations in elephant numbers.

Results

1) Movement and Migration Behaviour

Movement is one of the most important ecological factors that represent the home range as well as habitat utilization of an animal. Both movement and migration depends upon the availability of natural food and water (Figure 3). Changes in season and scarcity of water and natural fodder species force wild animals to leave a place for few months and reached to new feeding grounds for fulfilling their feeding, water and other routine requirements. There are seasonal variations in fodder species as RNP area falls under sub-tropical moist deciduous forest vegetation type. Elephants use whole of the park area as their natural habitat but mostly they leave some of the areas having less vegetation cover and water for few months and move towards other ranges richer in fodder species and natural water. Although at that time few of them (mostly solitary bulls) use the same feeding grounds or move frequently in all the forest beats of the park as a general rule of migration of any species. Selected range wise movement pattern of the Elephants is described below.

(i) Hardwar Forest Range

On the onset of winter from the month of mid-October, when there is slight scarcity of fodder species elephants move towards the Dholkhand forest range, which is situated towards north-west direction and towards Kansrao forest range, situated north through crossing Motichur forest range. Study revealed that elephant's move from Hardwar forest range to the adjoining ranges on the arrival of winter and also at the onset of summer period especially from the month of March to June, which were also known as the forest fire months. But the movement of few of the solitary bulls and occasionally group (very rare) have been observed in whole of the range. Most of these movements are obviously being restricted by various villages, temples, railway track and national highways those are present in the vicinity or in between the park area. Hardwar forest range is partially covered on one side by villages (Nai Basti-Bhimgoda, Lodha Mandi, Ravli-Mehdood, Roshnabad, Aehtampur Aanaeki and Aurangabad), therefore, instances of man-elephant conflicts are relatively more in this area, than in other ranges. These conflicts may be in any form viz. crop raiding and manslaughter. Dudhia forest beat due to its closeness to the Haripur Kala village and river Ganges is one of the most sensitive area as far as elephants' casualties are concerned.

During the study period occasionally, the movement of only solo bulls was observed in this part of the park. Despite the fact that Dudhia area is rich in *Dalbergia sissoo* (Shisham) and *Acacia catechu* (Khair) forest, being the preferred food item of the elephants. Group movement was almost restricted in this forest pocket due to most active nature of anthropogenic and developmental activities. Generally, the solo adult bulls follow the city route to reach the Dudhia forest and river Ganges by crossing the railway track and Hardwar–Dehradun National Highway. Elephants enter to the city from northern Kharkhari forest beat and moves towards Chilla area after the sunset and re-enter to the northern Kharkhari forest beat before dawn. During this long journey of about 2 kilometers elephants crossed many of the minor routes along with various colonies. Besides, solo bulls from Chilla forest also enter in this forest beat after passing through the islands situated in between river Ganges. This track falls under Chilla – Motichur corridor and is one of the important habitat as far the elephants' conservation is concerned.

During the field observations it was observed that this pocket of the area is very sensitive for the movement of the elephant mainly due to huge scale anthropogenic activities. During 1999 to 2002 few cottages have been constructed in this area inhabited by about two dozens of sadhus and likes; on the other hand this pocket has been part of the traditional route for the elephants to interchange the forest. Rapid developmental and construction activities in Haripur Kala village situated peripheral to the island and in adjoining areas; the elephant's movement has been disturbed. The residents of the area revealed that seven years ago an adult tusker was killed by electrocution by a villager, however no human casualty has been reported till now.

(ii) Chilla Forest Range

Elephant use this forest range round the year because of altitude wise variation of rich fodder species. On the arrival of winter elephant's movement is towards lower areas like Chilla, Mundal and Khara forest beats. At the same time elephants also utilize the adjoining forest of river Ganges, which is spread up to Rishikesh along the river. On the arrival of rainy season elephants migrate towards upper areas like in Luni, Pulani, Rawasan and Kasaan forest beats and that was the time when elephants start their long term migration towards Lansdowne forest division. Many of the groups and solitary bulls use all of the forest beats for their local movement. During the summer elephants also use the Gohri forest range, which is in the north of the Chilla forest range.

Elephants also use the Ghasiram water stream for visiting to river Ganges especially when their movement was frequent in and adjoining forest beats, which were attached to river Ganges along with few bridges, which are situated in Ganga canal of Chilla hydro electric power plant. Few of the groups were also observed to use the Shyampur and Chiriapur forest ranges of Hardwar forest division during rainy season because of availability of Ganga canal water. At the same place elephants perform their movements towards river Ganges through crossing the Hardwar-Bijnor National Highway. Currently only bull elephants are utilizing this track whereas no groups were reported during last three years. As per last 4-5 years data, groups of the elephants were observed in the same area but rapid developmental activities has restricted the frequent movement of elephant's group towards river Ganges.

Few of the main reasons affecting local movement of the Elephants in rainy season are:

a) During rainy season the elephants were seen moving towards upper areas of the park. This is because the low lying areas become swampy and unfit for free movement of the Elephants.
b) Another major factor contributing to their upward movement is the abundance of a blood sucking fly locally called as "daans" in low lying areas which irritates these elephants by hovering around their ears and trunk. This fly is commonly found affecting the cattle stock of Gujjars.
c) Forest fire is also one a factor to force the Elephants movement to a separate area where fire had not been so extensive. This fire if spread extensively then the movement of such a large animal also restricts to the same area for some time.

(iii) Motichur Forest Range

Elephants in summer use this forest range extensively as compared to winter and on the arrival of winter their movement is towards Kansrao forest range (north-west) and towards Beribara and Dholkhand forest ranges. In summer, elephants were observed more around the natural water sources (Koyalpura west, Kalakund, Jamunkhatta and Danda) besides the fact that this forest comprises of dense vegetation cover. Few of the group and solitary bulls use whole of the range for their movement. Elephants frequently use the Motichur rau (seasonal water stream) as a corridor for going to river Ganges. Occasionally they also follow the forest route between Motichur Forest Range Office and Raiwala area for going to river Ganges by crossing the Hardwar – Dehradun National Highway and railway track situated in between the protected areas. Establishment of Satyanarayan area, Raiwala area and Khand village has created a permanent barrier to frequent movement of elephants to different forest ranges. In this way they are forced to restrict themselves in low land areas as all of these areas lie in between the park area.

This is again one of the important and long stretched crucial corridor for elephant movement from Rajaji to Corbett National Park. This corridor is known as Chilla – Motichur corridor. River Song and Suswa flow through this range and elephants from Motichur and Kansrao forest ranges utilize the thick vegetation cover near to river water especially during dry season. Elephants, which move from Kharkhari forest beat to Motichur and Kansrao forest represent their seasonal movement as they leave the Kharkhari forest for few months mainly due to scarcity of water sources. During the recent past rapid developmental activities has restricted the frequent movement of elephants in this forest stretch and therefore, caused irregularity in the confirmed movement pattern of elephants within their home range (Joshi and Singh, 2007).

(iv) Kansrao Forest Range

Kansrao forest represents one of the best habitat where elephants can be observed in large groups, while performing their seasonal movement. On the arrival of summer season elephants from Motichur and Dholkhand forest move towards Kansrao forest (north-west, north-axis) as this forest is blessed with several water sources and Song river. Besides, cool shaded forest canopy also promotes the large scale

movements of elephants. Jamunkhatta and Bahera forest blocks are again the best resources where elephants stay for longer duration. But at the onset of winter elephants migrate from Kansrao and at that time their movement is towards south-west axis. During hot periods elephants also use Beribara ghata forest as this forest stretch was quite cool and dense forested habitat. During dry periods elephants used to cross the railway track regularly to visit river Song, which flows on northern axis and on the edge of the park boundary.

2) Feeding Behaviour

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. Sometimes elephants were observed 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 mostly fed on the tree species like *Mallotus phillipinensis* (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 use various grasses and shrubs as their food, 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* (Bichla Grass). 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.

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. Elephant prefer 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. Only bark of these trees is being utilized by elephants they spent even whole of their day to feed on these species (Figure 4). 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 ocassionally. Generally bark and soft twigs (without leaves) were consumed as food especially

during dry periods (April-June). This study 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. In Shyampur (Hardwar forest division) and Chilla forest range (RNP) elephants are currently utilizing *Eucalyptus* spp. (Eucalyptus) as their food especially during dry months. It was observed during the course of investigation that elephants start to feed on this resource after August 2007 whereas before 2007 elephants have not utilized the said species as their food. Observations indicated that only soft bark of this plant species is being utilized by elephants and only four identified adult bull elephants were observed to feed on, whereas till today no single group was utilizing Eucalyptus species as their food. This observation is a new record from north-west elephant range of India regarding to elephants' feeding behaviour.

Elephants sometimes spent long time to feed on some particular plant species like *Dendrocalamus strictus* (Bamboo), *Mallotus phillipinensis* (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. 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).

3) Reproductive Behaviour

Elephant is a highly social animal with group instinct and generally one group consists of 5 to 15 individuals, which comprises of adult cows, sub adult cows, juveniles and calves (newly born). Adult bull elephants are rarely seen within any group, they were observed to join the groups for only breeding purposes. Adult bull elephants prefer solitary life and they utilize wide range of feeding grounds and move more as compared to groups. As RNP area falls under sub tropical moist deciduous forest type; therefore, feeding and breeding parameters are dependent on availability of natural food and water. Several studies have conducted on the breeding habits of elephants but still little is known about the actual breeding biology of Asian elephants in the wild.

Like many other animals elephants also show the phenomenon of love play, which includes mixing of bull elephants in the groups, selecting prospective partner to mate, smelling of genital organs, sniffing the urine and dung, touching the trunk especially temporal gland and discharge of urine. The whole process was observed to happen within 15-20 days but sometimes it also took one month if environmental conditions are unfavourable (scarcity of fodder and water and high rate of movements). Sometime it was also observed that the adult cow within a group does not accept the bulls in their groups, at that time groups produce loud noise and reflects their weirdness. Long duration of love play and secretion of urine are important factors, which promotes the successful mating. The males are capable of making a second crossing after about 45 minutes and may do the copulation act 4 to 5 times a day (Iyppu, 1990). The duration of coitus was observed to be 3-4 minutes depending upon presence of group members and cooperation of the cow elephant.

The contact promoting was important factor, which includes separation from groups and mounting of bull elephant but several times false mating were also occurred, which expands the conception duration. The total time required for love play and actual act may take one or two hours. In younger bulls, which are afraid of grown up leaders, the whole act may be brisk and is completed in a short period (Iyppu, 1990). When cow comes under heat, exhibits her approval for the conception and at the same time both of them respond equally towards each other and the process of smelling of genital organ by male was observed to be quite frequent. As per the observations of the study it was not compulsory that the bulls, which are in musth condition only exhibits healthy sexual contacts, mating bulls are often observed to have no signs of

musth phenomenon, besides the fact that the bulls those are in musth are quite aggressive then those who do not exhibits the phenomenon of musth and can dominate over other males.

Mating was observed mostly inside the dense forest zones especially in those areas, which are enriched with water. Whenever the mating process was completed (10 to 15 days) bull elephants leave the group and starts to live solitary life. Longer stay of bull elephants with a group was also observed during the study period but at the same time they also leaved the group for a short period of time to perform movement on a wide range as the group movement was always restricted to some extent (03 kilometers). Examinations of genital organ with trunk, hugging, touching of temporal region of males and sniffing the dung are important pre-contact promoting behaviours of male and female elephants (Nair, 1990).

Most of the newborn calves were observed during January to May, which corresponds to the wet as well as dry season. The gestation period in Asian elephants varies from 18 to 23 months and if an average value of twenty months is taken as the gestation period, then the breeding season seems to be maximum to extend from May to November, which through embraces the hot, rainy and beginning of cold seasons, but can be taken up by and large as - warm period. On the basis of these observations, it would appear that there are some particular months of breeding. The important ultimate factors that influence the animal's reproductive cycle are probably the seasonal availability of food and water (Laws et al., 1975). Since, there is never any extreme shortage of food in RNP area therefore; the breeding season of elephant's was never so pronounced and drastic. Numerous perennial and seasonal rivers and streams further ensure the yearlong availability of fresh water.

4) Locomotion

The elephants usually move at a slow pace, but are capable of moving very fast. Movements were quite dependent on the activity they perform. If the animal is feeding, the slow walk is generally used and if the animal is moving towards water sources and crossing the forest road their walk became fast. In both the slow and fast walk, the position of the trunk and tail are quite variable. The movements of calves and juveniles are fast mainly due to their playing nature, chasing each other and to catch their mothers while changing the track and at the same time they upraise their trunk and tail for a short period of time. Fast movement by adult animals was observed only in case of flight and attack.

During the course of study several times I encountered the charging behaviour of elephants either of group or solitary bulls (Figure 5). It was observed that solitary adult bull elephant can run fast than cow elephants. Although charging by group leader cow or by whole group is very rare phenomenon whereas charging by adult bulls was noted to be more common. Charging by group or cow was measured to approximately18 kilometer per hour and by bull elephant was approximately 30 kilometer per hour. Besides, bull elephant can run fast for a longer period of time but cow was observed to run fast for a short distance mainly due to group constraints. During the charge tail is generally arched upward and trunk is curled in between the fore legs.

Faster walk was also observed during when the elephants performs their journey adjoining to human habitation areas mainly due to anthropogenic disturbances and threat. Group movement was always slow and restricted to a shorter distances whereas movement of solitary bull was quite vast and bulls make a large seasonal territory as compared to groups and herds. This also depends upon the musth phenomenon in males during which period, their movements became expand to join groups and in search of prospective females for mating needs. During the study period el;ephant movement was observed more during night and during the day they travel shorter distances and their walk was also observed to be slow.

Swimming

Elephants are well capable of swimming even under high flow of water. Several times I have observed the elephants during swimming in river Ganges. When swimming, their bodies remained sub-merged in river water, only the tops of the head and trunk tips being above the water. It was observed during the present investigation that elephants swim 100 meter wide river in 35 seconds (Figure 6). They sometimes also lie down in the water for few minutes to cool down their bodies especially during hot periods. When elephants are in groups, they swim serially and during the said period juvenile elephants are completely protected by their elder ones. The groups or herds with baby elephant (calves, smaller than 2 years) were only observed to drink and play in river bank area; they completely avoid swimming in the river water. As RNP and its adjoining protected habitats consist of several islands situated in between river Ganges, therefore, swimming in elephants is apparently common. Besides, several corridors are also

present in between different forest stretches and elephants traditionally use these corridors for interchanging the different forest zones.

5) Social Organisation

Social organisation is highly developed in elephants. A group of elephants is in general called herd, which is a large family unit. These herds move separately and maintain their kindship in terms of acoustic communications and through intra-mixing of various groups time to time. On several occasions (long-term migration and when elephants' movement was nearer to human habitation areas) several smaller groups may join to make a large herd, but this large group formation was restricted to the herds of same kindship. Social factors such as home range, average group size and seasonality of breeding have a profound influence on the population dynamics of many large mammals and are also important in their management.

During the study period inter-mixing of groups was mostly observed during their long-term migration. Besides, intra-mixing was also observed during their local movements especially when elephants inter change the forests through internal corridors. Whenever two elephants were in the same vicinity, the first indication that one has become aware of the presence of the other was generally the extension of the trunk by one or both in the direction of the other. When one elephant approaches another, the trunk was generally extended forward towards the animal being approached. Initial contact between two animals generally involves mutual examination by both animals; the trunk tip was extended towards the other individual, the most frequent areas of contact being the ear, mouth, eyes, temporal gland point, tail, anus, feet and genitalia (Mc Kay, 1973).

The oldest cow usually leads the herd however, this was a disputable point whether bull form the part of the herd or not. Bulls are observed to live in the groups for a short period of time and during the same period they randomly leave and join the groups. During the course of musth in males their movement was enhanced and they perform longer distances as group movement was restricted to shorter ranges. When a group was on move, the group forms a formation in such a way that young ones are protected in all manners. Usually, the oldest cow elephant [leader] heads the groups, the bull move on the periphery while the young ones are kept inside. Some bulls live a truly solitary life, sometimes accompanied by a companion who may be of around equal age. Generally solitary bulls branded as rouges though many of them may be inoffensive and peace loving. The extensive generation overlap leads to the establishment of linear dominance hierarchy among the females, and the leadership often falls on the oldest female in the group (Santiapillai and Suprahman, 1986). The mixing and separating of the different groups was another factor, which shows their social organisation.

The most cohesive social organisation in elephants was that of the adult female and her offspring, which constitutes a family unit. A number of family units join to form a clan and such clans consist of closely related animals of all classes excluding the adult males. Lactating cows with attendant young may aggregate together to form nursery units. Young pubertal males also mix with the herd to form loose associations for their reproductive activity. Since, the elephant calf is vulnerable to predation, therefore, the social organisation of the matriarchal groups serves as a protective device and at the same time, creates a social behave within which the young can mature and learn the template of their environment. It is therefore inevitable that an animal, which remains a member of a family unit for over 20 years should develop strong social ties with its mother and siblings (Eisenberg, 1981).

6) Drinking and Bathing

Water is the most important environmental factor that determines the elephant's distribution in any area. The elephant's home range must contain at least one river system in a general view. In the RNP, elephants were observed to drink between 15.00 and 19.00 hours. They were also observed to drink water during early morning hours (3 am to 7 am) and very occasionally during midday hours as this will quite dependent upon the presence of calves within any group. It was observed that drinking activity was quite frequent when the calves are in the group and at that time elephants allow themselves to drink water as per the requirements of their young ones. This factor was also dependent on the temperature, humidity, season and the amount of intake of food diet and water. Elephants are quite selective for certain specific locations along the river, this indicates that the drinking or bathing sites could be traditional and perhaps memory or habit based. These fixed points facilitate regrouping by members of a herd who have been foraging alone or as small units. Water holes appear to be especially important in this regard.

Elephants appeared to have traditional drinking spots, both water holes and sandy stream beds. While drinking the water they also spray the water on to the body. Besides, bathing with water, elephants also frequently cover the body with mud or dry soil (Figure 10). This helps them to cool their body for long period of time and get rid of the tiny flies, which irritates the elephants during their movements especially during monsoon period. After bathing with water or mud elephants rub their body against trees and rocks.

Presently, within few of the areas (where Gujjars still exist) their natural time for the same activity appears to be changing mainly due to anthropogenic pressure. In such conditions, when their movement was towards water source during day hours or in early evening hours, they swallow the water immediately and return back to forest area. On the other hand when they reached the water source after sunset or late evening hours they spent more time in the same activity. Sometimes it was also observed that an adult bull (occasionally groups also) spent whole of the day near to water source and feed on vegetation of adjoining areas. In RNP Gujjar rehabilitation programme has provided the better opportunity for livelihood to pastoral Gujjars and on the other hand it has promoted the regeneration of forest wealth along with movement related activities of wildlife (Joshi and Pande, 2007).

In Chilla the elephants after rains when move towards lower areas get their water supply from river Ganges by crossing the Hardwar-Chilla-Rishikesh road, which passes through the park area. They spent whole of the night in the adjoining forest of river Ganges and return back towards eastern direction in early morning hours. When any herd starts bathing, firstly the leader adult cow enters in the water, after that sub adults with calves enter the water body. During the said period adult elephants continuously sense the area and if they don't recognised any threat, only then other fellows of the herd enter to the water and at that time adult cow always remains alert towards her young ones. Sometimes it was also observed that elephants do not drink water if sense human presence and in such situations they returned back to adjoining forest upto the sunset.

Sometimes it was also observed that when elephants were drinking water and any incident of human presence will occurred, the adult bull (if present within group) or cow might suddenly charge the object. The most interesting thing was that they do not make any sound when they are entering the water and after entering, they cautiously try to listen to any disturbing or threat sound. When they observe that no interruption signal was around, they start bathing and sprinkle water over their body. During summer, they sometimes also observed to stand or laid down for few hours in the water. Sometimes elephants rub their bodies with trees just after taking swampy bath and these rubbing scratches were usually observed nearer to water points especially in areas those are continuously utilized by elephants. The cattle's and deer's also utilizes trees for the same activity but the identity of the species using the tree can easily be remarkable from the height of mark.

In Asian elephants the bouts of drinking and bathing are predominantly during the mornings and evenings hours (Seidensticker, 1984) whereas in the Wankie National Park (Africa) peak drinking activity of elephants was observed during the night (Weir and Davison, 1965). In the Way Kambas Game Reserve, elephants were observed to drink between 17.00 and 18.00 hrs. and the animals seem to prefer certain specific sites along the rivers (Santiapillai and Suprahman,1986). Elephants generally do not drink at any point along the stream or channel but have particular drinking spots, which they visit regularly as well as particular crossing points (Mckay, 1973).

7) Resting and Sleeping

Elephants often rest for period of several hours under shade in the immediate vicinity of their feeding areas especially during hot periods. The highest number of elephants was encountered regarding to this observation between 11.00 and 15.00 hours. The resting places are not specified as far migration was concerned but during local movement their resting places are generally fixed. Standing for a long duration in cool shaded trees represents the resting and sleeping in elephants but sometimes they lying down under dense canopy of trees and take rest. Elephants often use the same areas to rest and sleep. Such elephant "resting places" have been encountered in all the forest ranges. They measure about 50-100 m^2 and are characterized by the places where dense vegetation covers like *Ficus bengalensis, Butea frondosa, Mallotus phillipinensis* and *Adina cordifolia* were present. During the period of rest occasionally, branches of various fodder species are also plucked out for feeding purpose. Sometimes elephants also break down the twigs of trees and heave it to their body to get rid of the small insects and flies. It was observed that when elephants were on rest, calves lying down over to mud under the legs / belly of their mothers. Sometimes play behaviour was also observed among calves during the course of rest.

Within the study areas many "rest rooms" of this type are regular encountered during the hot period (mid day hours). Evidence of regular utilization of such areas was further identified through numerous dung piles that lie within the resting areas. Sometimes they also prefer to take rest between the ridges of foothills where water is present in adequate amount, besides the fact that these areas are quite cool during summer. Elephant 'rest rooms' have been encountered both within the Way Kambas Game Reserve and elsewhere in Sumatra (Santiapillai and Suprahman, 1984a). They measure 50-100m^2 and are characterized by the sparse or total lack of ground vegetation and scrub. Rest rooms are usually situated at the intersection of two game trails (Mckay, 1973). Asian elephants suffer more than the African Bush elephants from heat dissipation and therefore need regular access to shade during the day (Santiapillai and Suprahman, 1986). The resting follows maximum during the dry season.

8) Defecation and Urination

As a consequence of prolonged feeding activity the urination and defecation are regular phenomenon in elephants. Being a non-ruminant, the rate of passage of food through the alimentary canal is much faster than in ruminants (Santiapillai and Suprahman, 1986). In the present study defecation in elephants was observed maximum in early hours between 6.00 - 10.00 hours and between 15.00 - 18.00 hours, which was also the time to drink. During summer, they were observed to defecate during resting period under a dense tree (between 12.00-14.00 hours). It was observed that the defecating rates were similar in different age-classes.

The maximum number of times they defecates was noted in shady portion of small water bodies where sufficient amount of vegetation was available, under the dense trees like *Ficus bengalensis*, *Butea frondosa*, *Adina cordifolia* and *Mallotus phillipinensis* in the area where fodder species were abundant frequently and near to the natural water source. Sometimes they defecate in rough routes in foothills and in the forest roads. Water is another major factor, which influence defecation in elephants. Study revealed that elephants defecate mostly near to the water source and just after drinking the water. Defecation in elephants was also observed during such conditions when there was anthropogenic disturbance and they got fear of that. The elephants have the tendency to defecate, whenever they crossed a forest road (Laws et al., 1975). A bimodal distribution was observed in the defecation of African elephants with peaks between 09.00 hours and 12.00 hours and between 15.00 hours and 18.00 hours (Wyatt and Eltringham, 1974), while as per the observations of another study several peaks with maximum at 09.00 - 10.00 hours and 16.00 - 17.00 hours were observed in the defecation rate of the elephants and the defecation rates are to be similar in different age classes (Coe, 1972).

9) Recognition

There is no parallel to the memory and intelligence of the elephants in the animal kingdom. Since time immemorial many stories and incidents happens, which reflects the strong memory and high intelligence of this largest terrestrial animal. They also follow a fixed pattern for their various routine activities as other wildlife do. Elephants trumped loudly whenever they are excited or aggressive. Trumpeting occurs when elephant attack, when they are surprised and when an individual has gone the wrong way and feels that it has lost contact with the herd (Oberoi, 1980). Generally groups of elephants produce these rumbling sounds, indicating that it was used in maintaining group cohesion or as a greeting sound when elephants approach each other. Most of this type of trumpeting was observed in the study areas especially in the early morning hours when they are in cheerful humor (mostly by solitary bulls), when group was feeding, if they feel any threat and when their any fellow dies.

It was also observed that elephants by producing loud noise call their other fellows. Cow elephants call their young ones by slapping their ears against the head and when companions meet, they softly peep and rumble. When they feel threatened, they often upraise their tail, stop flapping ears, beat their trunks against the ground; spread mud and produce a sound like that of a tiger. That was only the moment when they are completely ready to charge anyone.

Few of the investigations indicating rather good vision are supported by the ability of elephants to recognize objects they have seen even after a long lapse of time. Few of the workers also observed that the members of the herd of wild elephants numbering 50 to 100 individuals recognized each other individually. They also know their exact paths, even though many of these paths are used only every few months (Oberoi, 1980). During the study period it was observed that when they move outside from the protected area regarding to crop raiding and crossing the forests through human habitation, they follow a fixed route and sometimes that route even may be longer than other choices by about 2-3 kilometers but they only re-

enter to the forest area from the route they have been using. It was also observed that during the course of inter-mixing and intra-mixing between various groups', elephants trumpet loudly and produces several types of greetings and recognition sounds. Vocalization among elephants can also be noted during the pre-mating processes, when some bull elephants are associated with the group and represents their dominance ness and the process continues for about one to two months.

10) Pseudofights / Male-Male Aggression

Young elephants display "Pseudofights" or "play fights". During these fights young bull face each other at a distance of 5 to 10 metres. Then they raise their heads, swing their trunks over their forehead, spread the ears and rush at each other until the bases of their trunks meet. Shortly before they collide, they wrap the trunk around the partner's head or entwine it with the other's trunk. Their stiffened legs push the body forward. If neither elephant can show the other back, they stop the motion, retreat and charge again after few minutes (Oberoi, 1980).

Serious fights, which are a rare occurrence, are conducted with tremendous vigour. These fights consist of a series of head rams where by the tusks clash and the trunks wrap around each other. This type of incidence occurred in year 1999 in the month of November in Rishikesh-Dehradun national highway in which two adult bulls (one solitary bull-tusker and a bull from a herd) was involved in the fight. Another such kind of fight was observed in year 2004 in Shyampur forest range of Hardwar forest division, which was peripheral to the Chilla forest range of the RNP. During the course of this type of fights elephants trumpet loudly and represents their effectiveness for joining the groups for mating purpose. Sometimes a tusk can penetrate one of the combatants in a vital spot and kill it. Many of the workers observed this fight could be continues till death of anyone of them or until one of them ran away. Tusks are often broken during elephant fight, revealing that how much energy is involved. Male-male aggression were also denoted by several workers and called them as "pseudo fights". The aggression is basically for the mating purpose, besides few other biological requirements, like joining of group, feeding and during the course of migration.

Several times these fights were observed during the long course of study. Serious fights were accounted highest for Chilla (2002) followed by Shyampur (2004) and Barkot (2006) whereas two major fights, one in 1999 and another in 2007 were also observed during the field observations but both of these were result less. Latest serious fight was observed in April 2008 in which one sub adult bull aged about 15 years was died. Tremendous trumpeting was observed during such incidences and sometimes tusks have played the major role in killing one of the combatants through penetrating the elephant. Elephants' fight could be continues till death of anyone of them or until one of them ran away. Tusks are often broken during elephant fight, revealing how much energy was involved. Male-male aggression were also denoted by several workers and called them as "pseudo fights". The aggression is basically for the mating purpose, besides few other biological requirements like joining of group and feeding.

11) Sympathy and Co-operation

There are many observations on elephants' attempting to help injured companions and to support them and even to attempt to hold up their dead one of their group. When their any group member was injured, the entire group member attempts that by lifting up their injured comrade and by helping in various ways. In one such case in Chilla forest, a hind leg of an adult cow got injured and swelled and she moves very slowly along with friction. The most wonderful thing I observed was that about 20 elephants including calves were present there and help her in various manners like helping her in reaching the water source and other activities like feeding. They support her with the help of their trunk by pushing her and lifting her injured hind leg.

Elephants also feel grief and mourn when anyone of their fellow dies. This behavioural aspect is one of the major, which shows their cooperativeness. I once during my study period observed them in this very grievous, unique, woeful behavioural aspect, which I had never seen earlier. I was shocked to see them. It was a train accidental death of a male juvenile. When I reached the spot I saw that three cow elephant were crying and roaming continuously around 50 meter from injured fellow. When elephants saw us (I and a forest guard), trumped loudly and suddenly five other elephants came there from nearby forest and constituted a group. All of the elephants were roaming continuously till evening. As per my observation they remained without feeding for 18 hours and not letting anybody reaching near the injured fellow.

A cow giving birth was often surrounded by other cows and was protected by them. During my study period, I once saw another such type of co-operative behave among them. When cow gives birth, all the group members were responsible for caring of young one. Another interesting thing was that the mother elephant do not leave the newly born calf even she may not charge any one. For few months, I kept under my observation a cow elephant that was debilitated because of an injured trunk. The trunk although was cut partially but was still hanging from its base. There I saw that her fellow elephants help her in many ways, especially in feeding. This show, how the sympathetic, helpful as well as cooperative elephants are. During the course of this study sympathetic and co-operational behaviour was quite frequently and conspicuously observed among the elephants. There are many observations of elephants attempting to help injured companions and to support them. During many elephant hunts, an entire herd by trumpeting and screaming has approached and attempting to lift up their wounded comrade.

In year 2001, a calf was pushed over by a train and was seriously injured. The mother of that calf was continuously roaming and roaring around her baby and remained with it for one day and not letting anybody reaching near her calf. When park officials with the help of tranquillizing gun and domesticated elephant trying to uplift her calf for treatment, the cow made it difficult to carry out rescue operation, as cow continuously charges the object which came nearer to her calf whether that was domesticated elephant or motor van of the wildlife specialists, which were trying to tranquillize her. She was continuously crying with tears in eyes and trumpeting loudly and was really in big trouble, as her any effort cannot do anything for saving her baby. At that time, tranquillizing gun (which was used for making the cow unconscious) and domesticated elephant are no effective to control that cow. In general, she with her injured baby facing to about 50-60 persons watching towards both of them and I was amused to see such type of elephant–human interactions. After 24 hours (next day), when cow elephant sensed that her calf was died, she finally sorrowfully left her baby nearer to the people and go towards forest area with huge of the tears in her eyes.

In one such case in West Bengal, elephant while crossing a nallah, fell into it. It was quite in trouble and all efforts were of no use to come out. The fellow elephants moving along with this elephant came for its rescue and tried their level best to take it out, but without success. The elephants remained with it for three days and not letting anybody reaching near her. When the local people informed the forest department, officials came, and studied the situation. But elephants standing nearby made it difficult to carry out rescue operation. When elephants sensed that they have come to help out their companion, they left the spot and stood about 100 meter away, watching foresters taking out the poor creature. When the elephant finally, was taken out, each and every individual, fellow elephant came to it and greeted her (Oberoi, 1980).

12) Play Behaviour

Play behaviour was also observed on several occasions among the elephant groups. This behavioural aspect was most frequent during evening hours and especially during dry periods. The calves within the group are usually more playful than adults. When juvenile elephants are in water point they starts splash dry mud and water here and there. Sometimes they also run suddenly to few feet and quickly come back to their mothers. A group when rests under tree during hot period, they play with each other by entangling their trunks. They were also seen pushing each other with their trunks. Another interesting aspect in the study of their play behaviour is that they were seen sliding down the muddy slopes with their front legs bent back. In such a case the adult cow elephant were seen guarding the movement of their calves. They support them by bringing their trunk in their front.

It was observed during the study period that sometimes-adult cows from a group were also included in the play. Once during the study period a cow was encountered who was playing with three calves of a group. All the three calves were playing with each other through entangling their trunk and through pushing back each other and at that time cow elephant was looking after their playing activities and regularly separates each other at an interval of every 2 minutes. This type of playing behaviour was generally encountered nearer to the water stream and in evening hours.

Play behaviour among elephants mostly occurred within the calves of the herd. The adults would kick the infant, often sending him sprawling onto the ground. When the mother is feeding on grass, at least, quite often the infants are allowed to take food from the handful that the mother has collected in playful manner (McKay, 1973).

13) Association with other wild animals and affects on the Habitat

The elephant produces several effects on the habitat, which besides affecting the plant communities themselves can affect other animals leaving in the same area. On several occasions the elephant during feeding were associated with *Axis axis* (spotted deer) and *Cervus unicolor* (sambhar). Co-movements of herbivores and elephants were also observed during the study period. Elephants some times break the twigs of the trees like *Ficus bangalensis, Ficus glomerata, Bombax ceiba, Terminalia tomentosa, Syzygium cumini, Bauhinia variegata, Zizyphus mauritiana* and *Embelica officinalis* as part of their habit. The leafy portion of the twigs was consumed by ungulates. Elephants were also seen digging holes in dry riverbeds mostly during the dry season to uplift the fresh water for drinking needs. Besides, the fact that elephant do not prefer to drink impure water and for that they dig out the water from sandy area of river beds. These small water points are further utilizes by spotted deer, sambhar, mongoose, jungle fox, jackal, wild boar and birds.

Terrestrial mammals such as tiger, leopard, spotted deer, sambhar, wild boar and barking deer besides the human beings regularly use trails, which were used and maintained primarily by elephants. In general an elephant when feeding on a tree or shrub does not tear down the entire plant; rather it tends to remove small twigs and for certain species entire branches. They sometimes fell down entire part of the plant to feed on (*Dendrocalamus strictus*) and sometimes also push and twist the trees full of leaves, but are out of their range, apparently to bring to their reach. Elephants have poorly developed digestive system and therefore, raw material was removed along with dung piles, which was sometimes use by jungle fowl, peacock and termites. Another important point is that elephant play a major role in dispersal of seeds of large trees like *Dalbergia sissoo, Shyzgium cumini* and *Ficus glomerata* through defecating them in different parts of the forest. Elephants are having association with other wild animals, which benefits both of them - the elephant itself and the other animal in various ways like feeding and drinking (McKay, 1973).

14) Aggressive and Defensive Behaviour

Normally, elephants are not aggressive by nature while in wild but presently fragmentation of wildlife habitats has forced them towards changing their behaviour from social to aggressive. It was also pointed out that adult males are more aggressive in nature than females. The lead cow flaps her ears signaling to fellows to which the other responds by gathering together (Figure 7). Under her guidance other adults and sub-adults form a closed or semi-circular defensive formation, with the calves between them (Figure 8). Elephants also show the phenomenon of "re-directed aggression", which is usually exhibited by an object that simultaneously, evolves fear (Oberoi, 1980).

Elephant groups generally do not fear of any danger and if they feel and foresee any anthropogenic disturbance, they firstly change their way. When their calves are with them they gather together and attempt the condition. The lead cow flaps her ears and trumpet loudly signaling to fellows to which the other responds by gathering together. Under her guidance other adults and sub-adults form a closed or semi-circular defensive formation, with the calves between them. Elephants also show the phenomenon of "re-directed aggression" and this has usually exhibited by an object that simultaneously evolves fear.

Another distinct pattern of behaviour was the twiddling of the trunk, the swinging of the one of the front leg to and fro, throws mud in air and trumpet, when an elephant appears to be deciding between attack and retreat. In year 2000 a group killed a leopard, which had come suddenly on their way. Once when I was returning back from the jungle, in the evening a very surprising incident occurred in Kharkhari forest beat of the Hardwar forest range. A juvenile elephant suddenly attacked a pig. Elephant lifted the pig and threw it to the ground, and then it trampled the pig under its feet and killed it almost instantly. During the course of study elephants had charged me several times inside the forest area (during day hours) and twice outside the park area (during night study). On sensing some danger the elephants produce a band like light sound. On hearing it the whole group becomes alert and they stop all their activities to sense the impending danger. Many other incidents of killing the villagers by the solitary bulls or a group within the park area also occurred during the study period.

Defensive behaviour among elephant is quite restricted to the condition when the calves are with the herd. Solitary bull sometimes also shows the behaviour of defense. Most of the wildlife biologists observed this defensive behaviour only due to natural phenomenon, large scale of loss of their habitat by human beings and their unnatural deaths. In the present study defensive behaviour among elephants was seen only due to the interference in their any activity, especially during their movements. Indian elephants have got its own customary habits and it is a social animal with sense of survival (Oberoi, 1980). They exhibit behaviour like that of human beings, but the behaviour originates when they feel any fear and danger towards their calves and themselves too.

Typical death of a woman by an elephant

Normally, the elephants kill human beings by holding in its proboscis and crushing under its legs. However, a heart breaking scene was observed in Tibri forest beat of the Hardwar forest range. It was a case during February, 2007 when three women are cutting fuelwood inside the forest and were killed by elephants. The death of one of the women was very unusual and breath-taking. The elephant grabbed the women and broken one of the leg and hand of the women. The broken leg and hand were thrown away at a distance of 20 meter away from the body. Then, head of the women was crushed by the elephant under its leg. The skull was totally damaged and in this process the brain portion came out of head region and was thrown away at a distance of 10 meter and fell on a stone giving an impression is if it was taken out manually by some one and kept on a stone carefully. The elephant had also pulled the hairs from the head of the women and thrown away. Thereafter, the elephant crushed the chest portion of the women under its leg. The chest and skull portion were so heavily crushed and damaged that the crushed portion of the body had formed a very thin layer on the earth. It was a very heart-breaking incident and normally we do not hear or see such type of killing by an elephant. Normally, elephants just kill a person either by piercing its tusk inside the body or thrashing the person and leave the body. This particular incident indicated highly aggressive and unusual nature of an elephant.

15) Acrobatic Behaviour

Elephants are well capable to perform their body posture in acrobatic modes as circus elephants perform (Figure 9). During field observations this type of unusual behaviour of elephant was observed especially in bulls. It was on 30th April, 2007 when I was inside the forest area regarding to my research study. Suddenly, I came across a group of elephants consisting of several adult females, sub-adults and juvenile and one adult male elephant. These elephants were crossing from one compartment of the forests to another compartment. The adult male elephant was following the group at few yards distance. The adult male elephant had seen us and started running towards us up to a few meters then suddenly the adult male elephant stopped at one particular point. The incident, which I saw at this point, was quite amazing and rare; typical kind of behaviour of an elephant was observed. I saw that the elephant first touched the ground with its proboscis and then started bending on its trunk. In next scene the elephant raised one of its hind leg in the air followed by another hind leg and then right front leg. At one point of time all the three legs of the elephant were in the air and the elephant was bending on its trunk and left front leg. The incidence continued for a minute. I did not believe my eyes, seeing such a unique kind of acrobatic behaviour of an adult male elephant. I had enough time to click photographs. In fact nobody can believe that a wild elephant can do such type of activity.

Of-course, domestic elephants used for circus purposes are expected to behave in such a manner that too after a long training. After sometime the elephant came to its normal position and left the spot to join its group. I had apprehension as if there was something special in the soil at that point. Therefore, I took the soil from that spot and tasted, I did not find any special taste in the soil which could indicate that the elephant might have bent on that spot to lick the soil which could be salty in nature as elephants are known to lick the salt. It was difficult to imagine what led elephant to perform acrobatics. It leads us to conclude that this was a very typical kind of behaviour of an adult male elephant.

16) Climbing and Sliding Behaviour

An interesting feature related to unusual movement of the elephants was observed during the study period. The elephants were seen mounting easily on foothills and slide down from there (Figure 11). They sometimes use sharp slopes for their movement in which human beings can't slide down easily. During the study period on many occasions their movement on foothills and slopes were observed. The movement of the elephants was also confirmed by examining signs and impressions like: presence of dung piles, footprints, damage of the vegetation etc. This kind of movement was seen to be exercised even by the juvenile as they can also mount on foothills and use the sloppy areas of the forest. This type of movement behaviour of the elephants sometimes may prove fatal to them as there have been reports of death of the elephants especially calves due to falling down from these foothills.

During the course of study several times elephants' frequent movement on top of the hills was observed, which seems to be quite hazardous, dangerous and uncomfortable for such a huge animal. It was not easy for human beings to climb on the top of such hills. Similarly, movement of elephants, most probably of adult male elephant, in very narrow passages and deep slope was also observed during present

investigation. At one spot in Mundal forest beat (Chilla forest range) I found footprints and feeding signs of elephant movement in a very narrow passage at a height of about 30 feet. I could see that there was a climber - *Bauhinia vahlii* (Maljhan) at that spot, the favourite food item of elephant. The elephant had mounted up to that height just to feed on this fodder species. Study revealed that the wild elephants are well capable of taking any risk while exploring the new areas. It may be mentioned that the adult male elephant are more interested in such type of activities rather than a group of females with young ones and juveniles. This behavioural aspect also indicated that adult male elephants are more confident and brave enough to explore new as well as dangerous routes as also have the benefit of enjoying feeding on variety of fodder species. This kind of movement was seen to be exercised even by the juvenile as they can also mount on foothills and use the sloppy areas of the forest. This type of behaviour of the elephants sometimes may prove fatal to them as there have been reports of death of the elephants especially calves due to falling down from these foothills.

17) Long-term Bulls Association

Generally it was said that bull elephant prefers to live solitary life after attaining age of 16 years. Male elephants were observed to join groups during breeding periods; sometimes their solo movement was also observed near to groups for short duration. During the last three years, I have continuously observing six adult bull elephants (recognized bulls) living together and performing their movement inside and outside the protected area. In 2007, four of them were separated from each other for a short period during summer (March - June) either they can be seen moving together throughout the year. Study revealed that all of these have a permanent and very close association round the year. A adult bull elephant (about 50 year old) was always observed to lead the group, while they performs their movement towards human habitation areas as in few of the places villages are situated in between the different forest pockets. Several times, they were also observed in resting position under cool shaded trees like *Ficus bengalensis* and *Adina cordifolia*. Besides, their play with each other was also studied deeply during the course of study. Looking into the situation, it could be revealed that all of them prefers to live associational life and their behaviour was observed to be very faithful for each other.

Annual movements of these bulls were also traced during the study period and it was observed that during the last three years, they are utilizing only the Chilla (RNP), Shyampur and Chiriapur (Hardwar forest division) and Laldhang (Lansdowne forest division) forest ranges for their movement related activities. Any serious fight of bull elephants for mating was not observed during few previous years in this forest stretch whereas these serious fights were occasionally observed in few other forest compartments during the same period. This is one of the unusual behaviour of bull elephants, which reflects their very close and permanent associational behaviour.

18) Parental Care

Elephants are highly social animals and show parental care behaviour. Newly born babies are kept under high care by their elder ones for about 10 to 15 years and the mother nurses and suckle the calf beyond 2 to 3 years. At the same time calf started to feed on smooth vegetation under the care of their elders. When the male elephant has attained the age of 16, they prefer solitary life and separates from the herd. All the members within a group exhibit an equal responsibility to care the newly born infants but adult cow elephants are more careful towards calves as compared to bull elephants. Once an elephant herd marched to the lake with a baby elephant, which was almost motionless, virtually lifted by the mother and another cow elephant. There are many incidents of such type, which were observed during the study period. It was also observed that any herd when feeding, spreads within 50-100 meters (depending on herd size) and at that time adult bull separates from herd but the calves move with their elder one (adult cows or sub-adult cows). Calves also sometimes show play behaviour, when they were in water, they splash mud and play by entangling their trunk with other. Similarly, when calves fed or bath and when they are crossing any water stream, their elder ones are too much careful of them.

19) Elephant Communication

Most animals communicate with one another to a greater or lesser degree, and the more social a species, the more communicative it tends to be. Communication come in many forms – it may be acoustic, visual, chemical or tactile and, depending upon the message and the medium, different types of communication may be more or less effective. While there aren't any species that can compete with human language in terms of its richness and complexity, some species have relatively large vocal repertoires and

extensive communication networks. Elephants are one such species. Elephants also communicate with one another using intricate chemical and tactile signals and visual displays. Like all highly social mammals elephants have a well-developed system of communication that makes use of all of their senses – hearing, smell, vision and touch. This includes an exceptional ability to detect vibrations.

Studies on African elephants (*Loxodonta africana*) have established the fact that elephants communicate over a variety of distances from touching to perhaps 10 kilometers or more apart and they convey information about their physiological (e.g. sexual, hormonal, body condition, identity) and emotional (e.g. fearful, playful, joyful, angry, excited) state as well as communicating specific state of their intentions or desires. Elephants live in a complex society bound together by different layers of communication. Individual elephants use a combination of vocalizations, visual, tactile and chemical signals to communicate different behavioural contexts. Male and female elephants live in two rather dissimilar social worlds, and the manner in which they use their communication skills reflects their different ways of life. The survival of females and their offspring depends upon the cohesion and co-ordination of the extended family, and on their ability to compete with other groups for access to scarce resources. Their use of signals underlines the importance of the unit. They use active communication to reinforce bonds between relatives, reassure youngsters, reconcile differences between family and friends, form coalitions against aggressors, and keep in contact over long distances. Males live a more solitary life where reproductive success and survival depend to a large degree upon an individual's ability to passively detect sounds and scents of the prospective female.

Vibration through the substrate has likely been important to animals as a channel of communication for millions of years, but our awareness of vibration as biologically relevant information has history of only the last 30 years. Elephants live in a complex society in which both long-and short-distance communication plays an important role in the ability to locate mates and to maintain intra-and inter-group cohesion. Elephants use a variety of sensory channels in ways both complementary and redundant to achieve this communication, as well as to advertise physiological states, allow reliable assessment of intent, and engage in other behaviours of group living. The majority of long-distance communication is probably via infrasonic vocalizations and chemical signals, whereas usual vocalizations, chemical signals, visual and tactile displays all play a role in short-distance interactions. Wild elephants organize socially around a matrilineal family unit, composed of closely related females and their offspring. These family units have well-developed communication systems to coordinate their movements over large areas.

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 14). 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 (0.4 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.

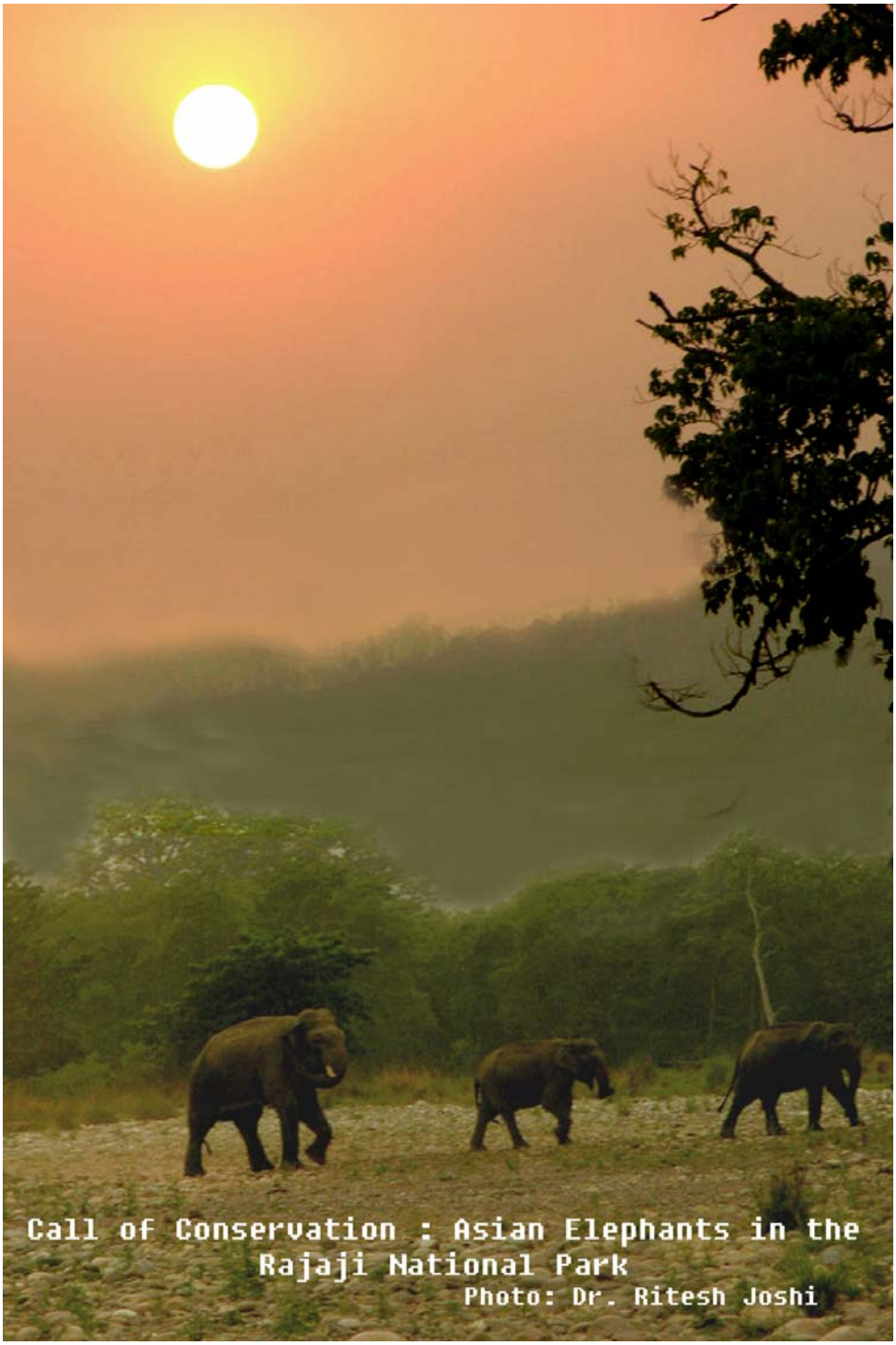

Figure 1. Elephants during the sunset in the Rajaji National Park.

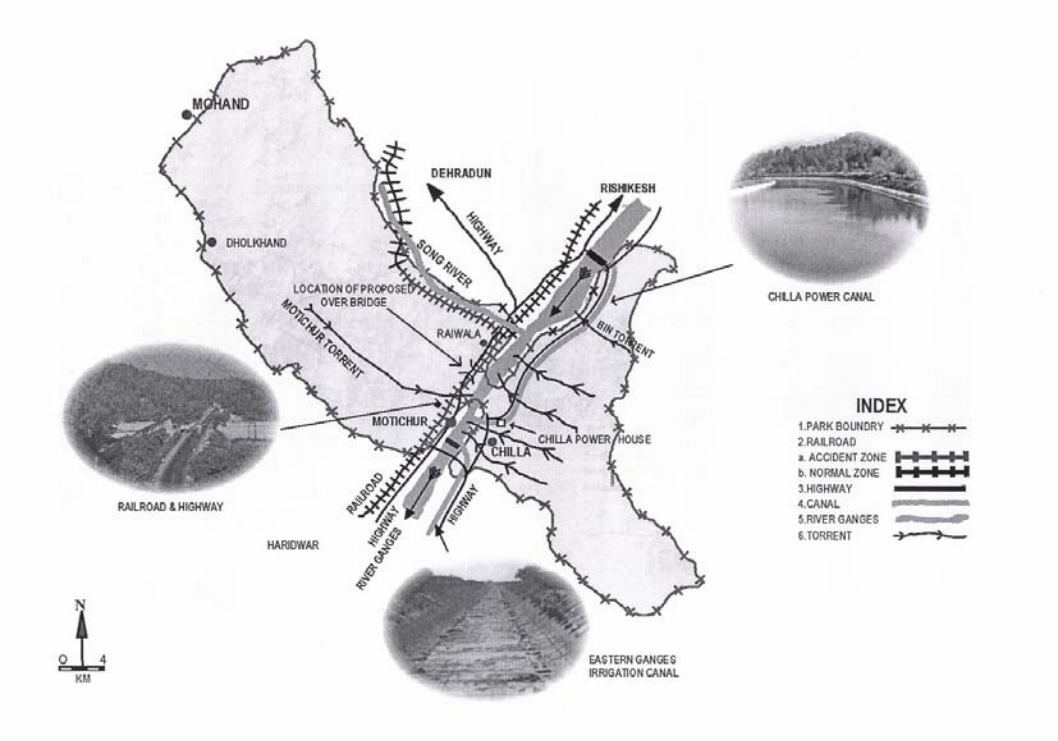

Figure 2. Map of the study area.

Figure 3. Elephants during migration.

Figure 4. Elephant calf feeding on *Tectona grandis*.

Figure 5. Charging by an adult cow.

Figure 6. Elephant is swimming in river Ganges.

Figure 7. Elephants sensing threat.

Figure 8. A seven days old calf under protection of her mother.

Figure 9. Acrobatic behaviour of an adult male elephant in the Rajaji National Park

Figure 10. Bull taking mud bath.

Figure 11. Bull standing on top of hillock

Figure 12. Recording the geo-positioning of bull elephant during his walk at highway.

Figure 13. Knowing the behaviour of wild elephant calf after a rescue.

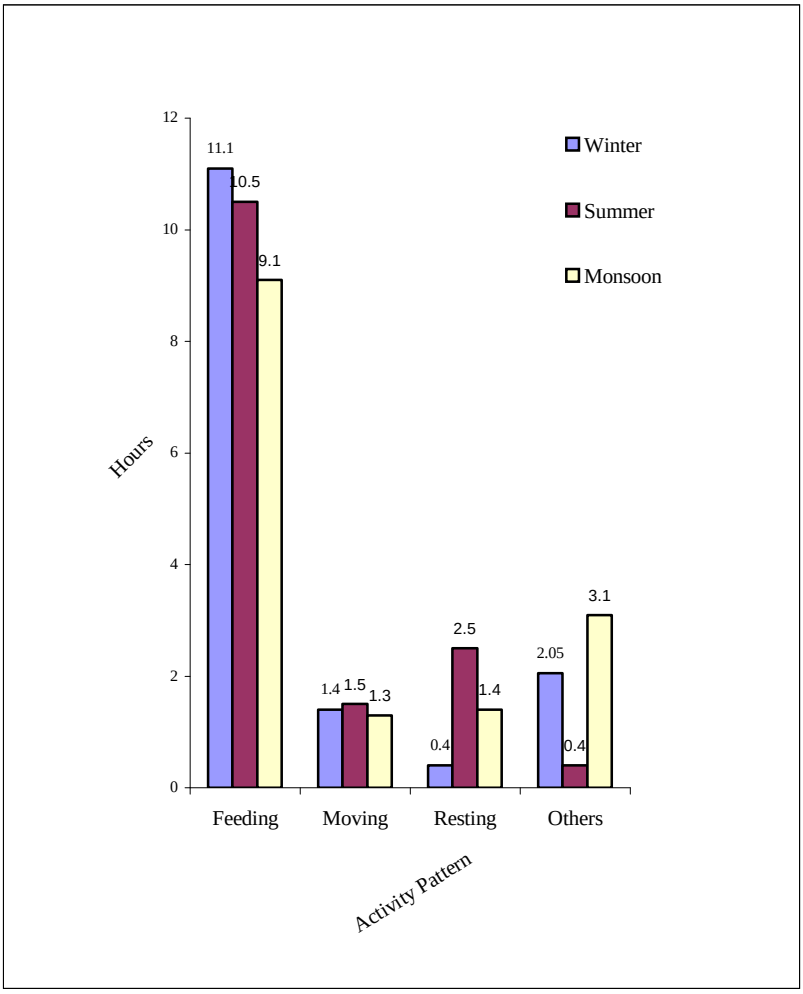

Figure 14. Comparative time-activity budget for different activities in different seasons

Discussion

Elephants are well known for their various types of behaviours such as playing behaviour, aggressiveness, social organization, parental care, pseudofights, sympathy and co-operation, recognition and communication and associational behaviour. During the recent past extensive work has been carried out on the movement pattern, habitat utilization and feeding biology of Asian elephants (Daniel, 1996; Sukumar, 1989; Santiapillai and Suprahman, 1986; Joshi et al., 2001; Williams, 2002; Joshi, 2002; Joshi et al., 2007) but the work on its behavioural biology has remained neglected. Therefore, less information is available in relation to behavioural pattern of elephants in the wild. Wild animals are closely linked to their environment and both living and non living components influence their live. During the recent past wildlife biologists has explored new conservation tools regarding to long term survival of endangered wild species but currently there is a need to obtain more and more biological information about various endangered wild species to enhance the status of their habitat and population in forested habitats.

The same populations of elephants used to perform their movements in Gohri, Chilla, Laldhang, Kotdwar, Shyampur, Chiriapur and Sonanadi forest whereas group movement was almost restricted towards Corbett National Park area as both of the forest zones are disconnected mainly due to huge amount of anthropogenic and developmental activities. It was also observed during the study period that in few of the places, elephants utilize the same feeding grounds round the year (recognised groups). Elephants inter-

change the forests of Rajaji and Corbett National Park as their part of traditional migration. But presently in few of the areas their traditional feeding grounds and corridors are denied to them, which have causes man–elephant conflict. The long-term effects will include genetic isolation, habitat fragmentation within the same forest and enhancement in the human-elephant conflict in adjoining areas. Genetic isolation of elephant populations may also increase the chances of replacement of interbreeding to intrabreeding, and thereby reduce the population persistence even for wide ranging wildlife species (Joshi and Singh, 2008a).

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 by the side of road and the presence of human population along the entire corridor area have almost restricted the migration of elephants using this corridor (Johnsingh and Williams, 1999). The motor roads, which are adjacent to the forests like Hardwar-Dehradun National Highway and BHEL roads 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).

A large mammal like the elephant could be expected to move more considerable distances even with a short period and families of a clan seemed broadly coordinated in their seasonal movements (Sukumar, 1989). In the dry months i.e. from January to April, when no rainfalls occur, the groups seek the neighbourhood of streams and shady forests. From the month of July, after the first shower, they start roaming and feed on the fresh grass. This grass in hill tracts become long and course by July and August, the elephants then shows their upward movements. The reason for the elephants and other animal's migration is the high lands, continuous and uninterrupted hilly terrain for grazing, assured food, ideal breeding ground and thick population (Sinha, 1981).

During the last 4-5 years, state Government has constructed about four flyovers in Hardwar – Bijnor National Highway. As a result of anthropogenic activities about 18 kilometers forest stretch existing on both the sides of highway has got disturbed. Besides, agricultural expansion near river Ganges has lead to the loss of forest wealth, which has also hindered the traditional movement of elephants. This forest stretch is one of the major corridor for elephant movement and presently has got disturbed mainly due to habitat loss around the national highway. Sometimes few of the male elephants associate to enter the forest near to river Ganges through this route. Elephants cross the national highway in the evening hours and come back to the forest area in early morning hours. Besides, elephants also utilize the Gaziwali bridge, Shyampurwali bridge and Pili bridge situated peripheral to the canal road in Ganga canal for their outside movement and to feed on the cultivated crops in nearby villages. During the study period all the villages suffering from crop raiding have been investigated. The affected villages are Jagjeetpur, Mishrpur, Panjneri, Ajeetpur and Jaipota in the western side of the conservation area and all these villages are situated peripheral to river Ganges. Villages Kangri, Ghaziwali, Shyampur, Sajanpura, Pili and Rasiabad are located peripheral to forest area and national highway whereas villages Gaindikhata, Lahadpur, Chiriapur, Vasuchandpur and Naurangabad are also situated adjacent to the forest area and national highway on south western direction of the conservation area.

The villages along the river Ganges are situated on land that was once part of the elephant's home range. Therefore, the increasing elephant – man conflict is unfortunate but inevitable. The electric fence erected along with these villages and river Ganges has presently got damaged due to lack of proper maintenance. It was observed that elephants are utilizing their traditional feeding grounds in few of the areas, which are presently denied to them and are replaced by human settlements.

The present study reveals that elephants utilizes whole of the park area for their movement, but mostly they leave some of the areas for few months, as part of their seasonal migrational activities. The local movement and long term migration of elephants within the RNP shows a definite range use pattern. After the isolation of Chilla forest and Motichur forests the elephants population of the RNP has divided into two parts. Presently, elephants of Chilla and adjoining areas in the eastern part of river Ganges show the better migration between the Chilla area and Kotdwar (Lansdowne forest division) whereas the elephant populations of Hardwar, Motichur, Kansrao and Dholkhand has been isolated. Again due to large scale developmental activities inside the Dogadda forest area has caused the hindrance in their corridor area. Slowly seasonal movements and migratory routes have also undergone to minor changes. Elephants in North Bengal are pocketed but these pockets have increased in number and also changed their locations

with the passage of time. Elephants are trying to adopt themselves to the changing environment by changing their ranges, moving on to new areas and by adopting new routes (Barua and Bist, 1996).

The reasons for migration of elephants can be annual fire, drought, non-availability of fodder, paucity of drinking water and absence of cool green shades in their respective areas (Ramachandran, 1990). In Chilla, the elephants, which were deep in the hilly terrain of north in the rainy season, gradually start moving towards the south due to scarcity of water winter season in the hilly areas. The study further reveals that the animals are directly affected by water availability and availability of fodder species inside the park area. Presence of river Ganges in Chilla area further ensures the migration of animals at the onset of summer.

Group generally comprises of adult cows, sub adult cows, infants (both sexes) and occasionally sometimes a matured bull was also seen within a group. Different groups generally do not mix up except during large scale migration. Members of a group during their feeding are usually spread within an area of 50 - 100 meters. However, the calves are always under direct touch and close to their mother. The young bulls on reaching the age of 12-14 years tend to prefer the solitary life, but at times two adult bulls may associate temporarily for their mutual understanding such as feeding together and crossing of their traditional corridors now converted into high traffic zone.

Elephants are known to feed on a wide variety of plant species. Elephants utilize 50 fodder plant species round the year in the Rajaji National Park and their movement was dependent on the seasonal availability of water and fodder species (Joshi and Singh, 2008b). 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 (IUCN 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).

India's elephant populations are currently threatened by habitat deterioration, developmental activities, anthropogenic pressure inside the deeper forest regime and unregulated exploitation of natural resources. Biogeographically, the RNP is part of the Shivalik foothills (lesser Himalayas), north-west India. Scientifically it belongs to a habitat type of important conservation value about long-term survival of Asian elephant. Knowing behaviour of any species is a valuable conservation tool, which can be helpful in management and conservation of any species. Relatively little work has been carried out on the behaviour of wild elephants in India. To understand the behavioural aspects, continuous ecological monitoring was done in and around the RNP area and 19 different behaviours of elephants were observed (Figure 12 & 13). During the recent past large habitats of elephants has been fragmented mainly due to developmental activities. Human encroachments into the deeper forest regime are another major factor, which has caused hindrance in their routine activities.

The behaviour of elephants in Rajaji shows a great variance with respect to the seasons, availability of natural water and traditional movements. Currently, natural continuous forest ranges of Shivalik region has been broken up into many parts due to agriculture, urbanization, increasing road traffic and development related activities as well as other anthropogenic activities. This situation creates many problems for various organisms living in forests. Genetic isolation, limitation of dispersal and migration and the decline of populations of animals requiring large territories are the most common problems connected with fragmentation of forests and other components of the environment. RNP is supposed to play an important role in maintaining the elephant population for last few years. However, few of the activities mainly of anthropogenic origin are responsible for the erratic patterns observed in the behaviour of its population. Study indicated that elephants of RNP used to perform usual as well as unusual behaviours but it has been suggested that more studies on the behaviour of elephants are required through which database management approaches could be generated, which will ensures the long-term survival and conservation of Asian elephants.

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In vitro Conidial Production of Aquatic Hyphomycetes on Submerged Leaf Litter

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ABSTRACT: Submerged leaf litter of three forest plant species viz., *Acer oblongum*, *Lyonia ovalifolia* and *Pinus roxburghii* were sampled to study the in vitro conidial production of aquatic hyphomycetes. Out of 25 species encountered, only12 species were found common to all host plant but the rate of conidial production was quite variable. Of these 12 species *Acer oblongum* supported maximum conidial production for *Triscelophorus monosporus* and *Lyonia ovalifolia* supported maximum conidial production for *Tetrachaetum elegans* whereas *Pinus roxburghii* supported maximum conidial production for *Flagellospora penicillioides*. Coniferous leaf litter i.e. *Pinus roxburghii* was found with maximum conidial production (92487 conidia/ cm ^{2}/ litre) as compared to the leaf litter of other studied forest plants (51506 and 42144 conidia/cm^2/litre on *Acer oblongum* and *Lyonia ovalifolia* respectively). It is interesting to note that Lyonia ovalfolia was colonized by highest number of species, whereas *Pinus roxburghii* had the least species diversity. The maximum number of conidial production was found during winter and spring months while the maximum number of species variation was observed during rainy and autumn months. [Nature and Science. 2009;7(1):78-83]. (ISSN: 1545-0740).

Keywords: submerged leaf litter, conidial production, aquatic hyphomycetes

INTRODUCTION

Aquatic hyphomycetes, ramifying on decaying leaves especially skeletonized or decorticated petioles occur throughout the year in any fast flowing stream, however, their abundance relates to availability of leaf litter in stream (Webster and Descals, 1981). Though these fungi are being well studied with the reference of their qualitative point of view i.e. occurrence, seasonal periodicity, variation in their species composition by many mycologists (Triska, 1970; Gonczol, 1975; Sander and Webster, 1978), but little is known for their quantitative studies (Willoughby and Archer, 1973; Muller Haeckel and Marvanova, 1979). Iqbal and Webster (1973) took the initial step to understand the rate of conidial concentration in a water body by filtering the water. Recently, Sati and Tiwari (1992, 1995) developed a simple technique to determine the rate of conidial production by modifying the method of Webster and Towfic (1972). In the present investigation an attempt has been made to study the fallen leaves of three forested plant species were studied for the production of conidia in unit area substrate per litre of water in captivity.

METHODOLOGY

To determine the rate of conidial production in per unit area of the substrate in per liter of water, Sati and Tiwari (1995) was followed. Submerged leaf litter of known three forest trees i.e. *Acer oblongum*, *Lyonia ovalifolia* and *Pinus roxburghii* were incubated in the sterile petri dishes containing 20 ml of sterile water at monthly intervals. Prior placing the leaf litter for incubation, the area of each piece of leaf litter was determined with the help of graph paper. After 2-3 days, the incubated dishes containing leaf litter were gently shaken to homogenize the fungal conidia produced in water. The drops of 0.01 ml conidial suspension were pipetted out on glass slides for screening. The counting of conidia was made directly under the low power of microscope and conidial number was recorded individually to each species. Finally the rate of conidial production for each species occurred and total species in unit area (1 cm^2) were calculated using the following formula –

$$RCP = \frac{2000\ n}{a} \text{ Conidia/ cm}^2\text{ / litre}$$

Where, RCP = Rate of conidial production
n = No. of conidia present in .01 ml of conidial suspension used
a = area of leaf litter substrate incubated (cm^2)
(2000 is used if 20 ml sterile water is supplied to the incubated substrate in dish)

RESULTS

The results of monthly variation in conidial production of aquatic hyphomycetes per litre per unit area of substrate i.e. leaf litter are summarized in Table 1-3. Altogether 25 species of water borne conidial fungi were encountered on the incubated leaf litter of *Acer oblongum*, *Lyonia ovalifolia* and *Pinus roxburghii*. The colonization pattern of these species on three different host plants as well as the rate of conidial production per cm^2 area in unit volume of water is tabulated in table 4.

Acer oblongum Wall. ex DC.

19 species of water borne conidial fungi belonging to *Alatospora, Anguillospora, Articulospora, Camposporium, Clavariopsis, Dimorphospora, Flagellospora, Heliscus, Lemonniera, Lunulospora, Tetrachaetum, Tetracladium, Tricladium* and *Triscelophorus* was found colonizing on incubated leaves of *Acer oblongum* (Table 5.1). This substrate was abundantly colonized by *Triscelophorus monosporus.* Remaining species were found as moderately and least abundant. The average conidial production in *Acer oblongum* was 51506 conidia/cm^2/litre. *Triscelophorus monosporus* accounted a maximum number of conidia i.e., 7953 conidia/cm^2/litre where as *Lemonniera terrestris* accounted only 165 conidia/cm^2/litre.

The maximum number of conidial production was analyzed during September while minimum number of conidia were analyzed during April (112060 and 13240 conidia/cm^2/litre respectively).

Lyonia ovalifolia (Wall.)Drude

The leaves of *Lyonia ovalifolia* were colonized by 21 species of water borne conidial fungi (Table 2). The total average conidial production was 42144 conidia in unit area of substrate per litre. *Tetrachaetum elegans* was occurred with maximum number of conidia i.e. 6085 conidia/cm^2/litre in average. A least number of conidia were produced by *Lemonniera terrestris* 188 conidia/cm^2/litre in average.

Maximum conidial production was analyzed during January, which reached upto 74930 conidia/cm^2/litre while the least conidial production (20870 condia/cm^2/litre) was accounted during August.

Pinus roxburghii Sarg.

The submerged needles of *Pinus roxburghii* were colonized by 15 species of water borne conidial fungi (Table 3). Total average conidial production in *Pinus roxburghii* was 92487 conida/cm^2/litre. The maximum contribution was made by *Flagellospora penicillioides*, which reached upto an average of 18060 conida/cm^2/litre whereas least number of conidia were contributed by *Heliscus lugdunensis* (411 conida/cm^2/litre).

As evident from Table 3 January month was found the most favourable for conidial production to have upto 198670 conidia/cm^2/litre while least conidial production was found in the month June i.e. 46720 conidia/cm^2/litre.

Table 1: Monthly variation in conidial production of water borne conidial fungi in per litre/ unit area of *Acer oblongum* Wall. ex DC leaf litter in captivity

S.No.	Fungi	Conidia produced												Average
		June	July	Aug	Sept	Oct	Nov	Dec	Jan	Feb	March	Apl	May	
1.	*Alatospora acuminata*	-	-	-	8210	6420	4550	-	-	-	8850	-	-	2336
2.	*A. flagellata*	-	-	-	-	-	-	-	-	14440	-	-	-	1203
3.	*A. pulchella*	-	-	-	4940	4210	-	7770	-	-	5270	-	-	1849
4.	*A. longissima*	4530	-	-	-	7530	-	-	-	-	-	-	2910	1248
5.	*Articulospora tetracladia*	-	-	-	-	-	-	35350	-	-	-	-	-	2946
6.	*Camposporium pellucidum*	-	-	-	-	-	-	-	-	-	7760	-	-	647
7.	*Clavariopsis aquatica*	-	-	-	3140	1150	-	2640	23320	13340	-	-	-	3633
8.	*Dimorphospora foliicola*	-	-	-	-	-	20290	-	15940	-	-	-	-	3019
9.	*Flagellospora penicillioides*	8160	-	2750	-	-	-	-	-	-	16460	-	-	2281
10.	*Heliscus lugdunensis*	-	-	-	-	2780	-	2880	-	-	-	-	-	472
11.	*Lemonniera cornuta*	-	-	-	-	-	-	-	31470	12160	-	-	-	3636
12.	*L. terrestris*	-	-	-	-	-	-	1980	-	-	-	-	-	165
13.	*Lunulospora curvula*	-	13100	2250	-	2820	8930	9240	14600	12260	-	10970	7980	6846
14.	*L. cymbiformis*	-	11710	2900	33990	1640	1140	2500	-	-	-	2270	-	4679
15.	*Tetrachaetum elegans*	-	12250	900	-	1170	2500	10090	-	-	-	-	-	2243
16.	*T. marchalianum*	-	-	-	-	-	-	1580	19410	-	-	-	-	1749
17.	*T. chaetocladium*	-	-	-	11380	3640	9070	11330	-	-	-	-	-	2952
18.	*Triscelophorus acuminatus*	4070	-	-	15750	-	-	-	-	-	-	-	-	1652
19.	*T. monosporus*	8890	22910	7340	34650	3830	7490	-	-	-	-	-	10320	7953
	Total	**25650**	**59970**	**16140**	**112060**	**35190**	**53970**	**85360**	**104740**	**52200**	**38340**	**13240**	**21210**	**51506**

Table 2: Monthly variation in conidial production of water borne conidial fungi in per litre/ unit area of *Lyonia ovalifolia* Wall) Drude leaf litter in captivity

S.No.	Fungi	Conidia produced												Average
		June	July	Aug	Sept	Oct	Nov	Dec	Jan	Feb	March	Apl	May	
1.	*Alatospora acuminata*	8810	5070	-	-	-	5220	-	5600	13450	4680	-	11870	4558
2.	*A. pulchella*	-	8460	-	-	-	-	-	-	-	-	-	-	705
3.	*A. longissima*	-	-	1410	-	3790	-	-	-	-	-	-	-	433
4.	*Articulospora tetracladia*	-	-	-	-	-	-	12970	1600	-	-	-	-	1214
5.	*Clavariopsis aquatica*	-	-	-	-	3790	6390	660	-	-	-	-	-	903
6.	*Dimorphospora foliicola*	-	-	-	-	-	6820	-	40820	-	-	-	-	3970
7.	*Flagellospora penicillioides*	-	5010	-	3380	-	-	-	-	-	-	-	3620	1001
8.	*Heliscina campanulata*	-	-	-	-	-	-	-	-	18580	-	-	-	1548
9.	*Lemonniera cornuta*	-	-	-	-	3280	9410	13540	-	-	-	-	-	2186
10.	*L. pseudofloscula*	-	-	-	-	1310	-	9470	-	-	-	-	-	898
11.	*L. terrestris*	-	-	-	-	2250	-	-	-	-	-	-	-	188
12.	*Lunulospora. curvula*	10450	10690	9300	8720	4900	2150	-	-	-	-	2690	11540	5037
13.	*L. cymbiformis*	3950	8550	-	-	5350	-	-	-	-	-	-	-	1488
14.	*Pestalotiopsis submersus*	-	-	-	-	-	-	-	-	8270	-	-	-	689
15.	*Speiropsis scopiformis*	-	4650	-	-	-	-	-	-	-	-	-	-	388
16.	*Tetrachaetum elegans*	-	-	2940	9130	7970	13190	12030	10910	9040	7810	-	-	6085
17.	*T. marchalianum*	-	-	-	-	4420	2250	-	-	-	-	-	-	556
18.	*Tricladium chaetocladium*	-	-	-	-	-	6930	12710	16000	8120	9830	-	-	4466
19.	*Triscelophorus acuminatus*	-	-	5640	1880	2270	-	-	-	-	-	2340	-	1011
20.	*T. monosporus*	8700	7210	1580	6850	3760	-	-	-	-	10560	7050	2490	4017
21.	*T. konajensis*	-	-	-	-	-	-	-	-	-	-	9650	-	804
	Total	**31910**	**49640**	**20870**	**29960**	**43090**	**52360**	**61380**	**74930**	**57460**	**32880**	**21730**	**29520**	**42144**

Table 3: Monthly variation in conidial production of water borne conidial fungi in per litre/ unit area of *Pinus roxburghii* Sarg. leaf litter in captivity

S.No.	Fungi	Conidia produced												Average
		June	July	Aug	Sept	Oct	Nov	Dec	Jan	Feb	March	Apl	May	
1.	*Alatospora acuminata*	14510	-	11220	-	26290	-	-	-	-	-	-	-	4335
2.	*A. pulchella*	-	-	7010	-	-	--	-	-	-	-	-	-	584
3.	*Clavariopsis aquatica*	-	-	-	-	-	-	23640	17810	-	-	-	-	3454
4.	*Flagellospora penicillioides*	13410	10400	19530	-	-	-	-	-	-	93260	39740	40380	18060
5.	*Heliscus lugdunensis*	-	-	-	-	-	-	4930	-	-	-	-	-	411
6.	*Lemonniera cornuta*	-	-	-	-	10940	-	-	17810	15920	-	-	-	3723
8.	*Lunulospora curvula*	-	26160	-	-	9730	30890	4450	41270	32230	6580	10050	19730	15091
9.	*L. cymbiformis*	-	-	-	-	-	11470	-	38000	-	-	-	-	4123
10.	*Pestalotiopsis submersus*	-	-	-	-	-	-	-	-	34770	-	-	-	2898
11.	*Setosynnema isthmosporum*	-	5780	-	-	-	-	-	-	-	-	-	-	482
12.	*Tetrachaetum elegans*	-	-	-	10200	13120	17290	46500	29220	-	-	-	7530	10322
13.	*Tetracladium marchalianum*	-	4370	-	3430	-	-	-	-	-	-	-	-	650
14.	*Tricladium chaetocladium*	-	4080	10410	23030	13760	10630	51830	54560	30910	8510	-	-	17310
15.	*Triscelophorus acuminatus*	2730	3820	7620	21780	-	--	-	-	-	-	-	-	2996
16.	*T. monosporus*	16070	19920	6300	-	18350	10410	7980	-	-	-	-	17570	8050
	Total	**46720**	**74530**	**62090**	**58440**	**92190**	**80690**	**139330**	**198670**	**113830**	**108350**	**49790**	**85210**	**92487**

Table 4: Comparative variation in occurrence of fungi and their rate of conidial production on different substrates in captivity

S. No.	**Fungi**	**Average conidial production on different substrate**		
		Acer oblongum	*Lyonia ovalifolia*	*Pinus roxburghii*
	Alatospora acuminata	2336	4558	4335
	A. flagellata	1203	-	-
1.	*A. pulchella*	1849	705	584
2.	*A. longissima*	1248	433	-
3.	*Articulospora tetracladia*	2946	1214	-
4.	*Camposporium pellucidum*	647	-	-
5.	*Clavariopsis aquatica*	3633	903	3454
6.	*Dimorphospora foliicola*	3019	3970	-
7.	*Flagellospora penicillioides*	2281	1001	18060
8.	*Heliscella campanulata*	-	1548	-
9.	*Heliscus lugdunensis*	472	-	411
10	*Lemonniera cornuta*	3636	2186	3723
11	*L. pseudofloscula*	-	898	-
12	*L. terrestris*	165	188	-
13	*Lunulospora curvula*	6846	5037	15091
14	*L. cymbiformis*	4679	1488	4123

15	*Pestalotiopsis submersus*	-	689	2898
16	*Setosynnema isthmosporum*	-	-	482
17	*Speiropsis scopiformis*		388	-
18	*Tetrachaetum elegans*	2243	6085	10322
19	*T. marchalianum*	1749	556	650
20	*Tricladium chaetocladium*	2952	4466	17310
21	*Triscelophorus acuminatus*	1652	1011	2996
22	*T. monosporus*	7953	4017	8050
23	*T. konajensis*	-	804	-
	Total Conidial Production	51506	42144	92487
	Total no of species	19	21	16

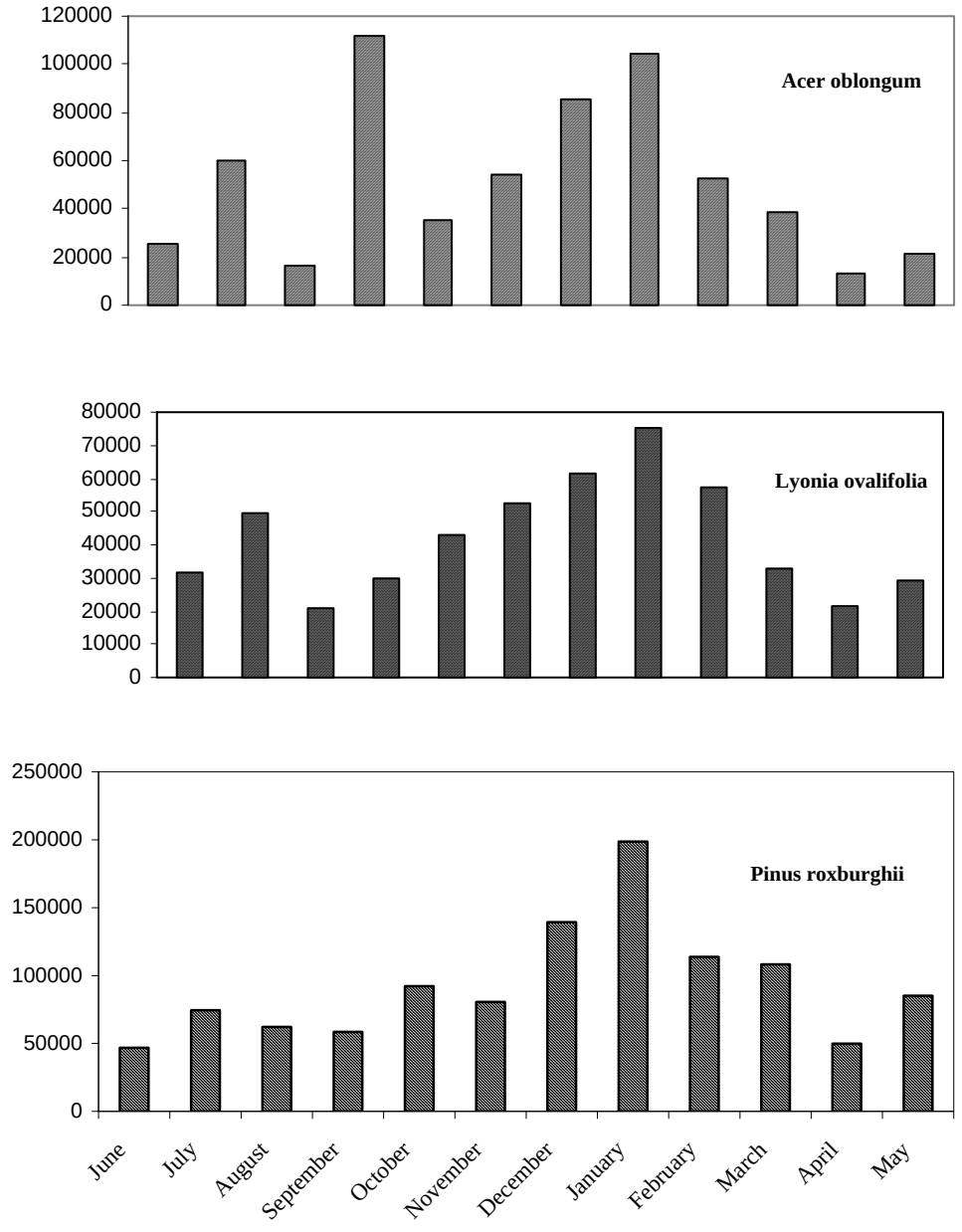

Fig.1: Total conidial production of water borne conidial fungi/litre/cm^2 in different host species

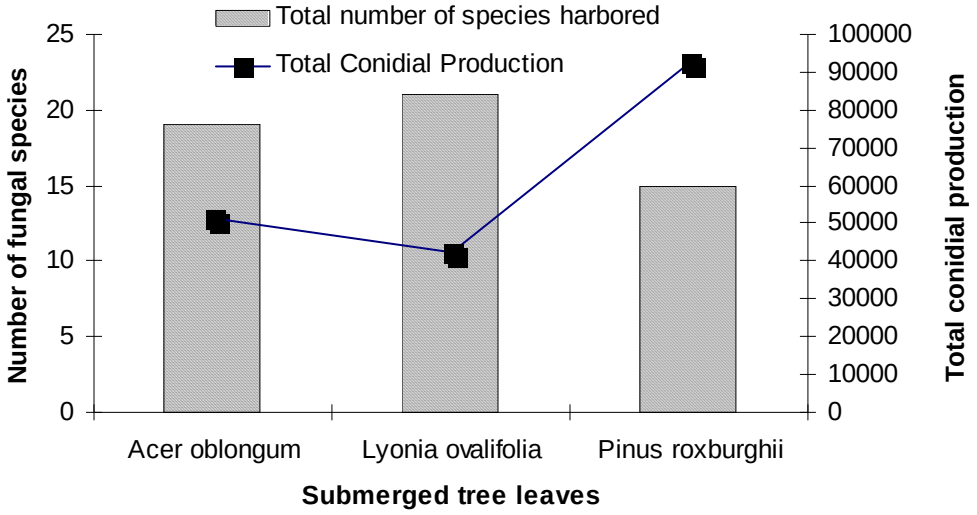

Fig. 2: Total average conidial production/cm^2/liter and total species colonization in different host species

DISCUSSION

On reconnaissance of table 1-3, an inclining trend of conidial production was found from autumn months to winter months i.e. September to February, when temperature remains low. It was interesting to note that during autumn months like September to November a maximum number of species were observed. The similar trend was followed by winter (December to February) and spring (February to April) months (Fig. 1). Relying upon these results it can be said that the maximum number of conidial production take place during winter and spring months while the maximum number of species variation take place during autumn and rainy months (Fig. 1). The result of present investigation confirms the findings of some of the previous workers (Iqbal and Webster 1973, Alasoadura 1968, Barlocher and Rosset 1981, Mer and Sati 1989, Thomas et al 1979).

As evident from fig. 2 *Lyonia ovalifolia*, which was colonized by highest number of water borne conidial fungi (i.e. 21 species) but the total average conidial production for this host remained in low profile (42144 conidia/cm^2/litre respectively). On the other hand, in *Pinus roxburghii* the less species colonization was reported, however the total average conidial production reached in its highest profile (92487 conidia/cm^2/litre). The total average conidial production on unit area of different studied three plant leaf litter in per liter of water is summarized in table 4. On the perusal of table 4 and fig. 2 it could be concluded that the gymnospermous leaf litter have maximum conidial production /cm^2/litre and support the view of Sati and Tiwari (1995).

Thus relying up on these observations it could be visualized that the number of species colonization and rate of conidial production varies species to species of leaf litter or might be depend on the nature of available substrate. On the basis of above said observation it could be concluded that the higher rate of conidial production might depend upon the nature and nutritive value of substrate. The present observation also support the findings of Willoughby and Archer (1973).

As evident from table 4, a total of 25 species of water borne conidial fungi were encountered. A maximum number of species were harbored on the submerged leaves of *Lyonia ovalifolia* (21 species) followed by *Acer oblongum* and *Pinus roxburghii* were colonized by 19 and 15 species of water borne conidial fungi respectively. However each species had different species composition (Table 4). This suggests a preferential occurrence of water borne conidial fungi on the nature of plant substrates. Therefore, it could also be envisaged that the occurrence of water borne conidial fungi much depend on the available substrate provided by the plant leaf litter.

On perusing table 4, 12 species viz., *Alatospora acuminata*, *A. pulchella*, *Clavariopsis aqautica*, *Flagellospora penicillioides*, *Lemonniera cornuta*, *Lunulospora curvula*, *L. cymbiformis*, *Tetrachaetum elegans*, *Tetracladium marchalianum*, *Tricladium chaetocladium*, *Triscelophorus acunminatus* and *T.*

monosporus were found to occur in all the studied plant leaf litter. It shows their habit tolerant nature as appear to be common in occurrence and do not show selective substrate requirement. On the other hand, 4 species of water borne conidial fungi i.e. *Alatospora flagellata*, *Camposporium pellucidum*, *Setosynnema isthmosporum* and *Triscelophorus konajensis* were restricted to only specific leaf litter of plant species showing specific habitat loving nature. Present observation also confirms the findings of Willoughby and Archer (1973).

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Population Dynamics of *Quercus floribunda* Lindl. Seedlings Under Denser and Lighter Canopied Microhabitats

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ABSTRACT: Population dynamics of *Quercus floribunda* seedlings in a fenced and protected forest plot were studied over a 14 months period to determine the influence of temperature and drought in seedling mortality in different seasons. The experiment involved two forest microhabitats (denser and lighter canopied microhabitats) located at an altitude of 2100 m in Central Himalaya. Total seedling mortality of 74.6% was observed in lighter microhabitat and 51.6% in denser canopied microhabitat. Seedling mortality in different months correlated negatively with predawn and midday water potential. Significant correlation also existed between seedling mortality, soil temperature and air temperature at both microhabitats. [Nature and Science. 2009;7(1):84-90]. ISSN: (1545-0740).

Keywords: Population dynamics; predawn water potential; midday water potential; microhabitats; seedling density.

INTRODUCTION

In terms of species richness, Mexico, North America and China are the major centres of the genus *Quercus* and Himalaya is relatively unimportant. However, the four oak species that occur in Central Himalaya dominate more forest areas than many species do in any other part of the world. They occupy most of the areas (approximately 20,000 km^2) from 1000 to 3000 m altitude in Central and Western India (Kumaun, Garhwal and Himanchal Pradesh) (Singh et al. 2000).

Q. floribunda (Moru oak) is the largest of the Western Himalayan oaks, occurring between 2100-2700m elevation although found on all aspects, it avoids very dry situations and favours moist, cool localities and northerly aspects. It also stands more shade than the other oaks of this region (Troup, 1921). The Nainital Hills where the study was under taken is one of the major centres of this oak where it occurs on cool moist slopes.

In 1991, *Q. floribunda* produced a mast seed crop which resulted in recruitment of seedlings in large numbers of similar-aged plants during the warm and wet rainy season (July-August) over a range of microhabitats.

Although changes in species composition have been related to environmental variation in grassland communities (Watt, 1981), the role of environmental variation in regulatory forest community dynamics has received less attention. We monitored these seedlings from the time of recruitment till they were one year old to determine the extent to which temperature (soil and air), soil moisture (at 30 cm depth) and water potential of seedlings (as an indicator of stress) are responsible for large scale mortality of newly recruited seedlings of *Q. floribunda* in different seasons in the monsoon climate.

The present study attempts to determine a suitable microhabitat for this important oak species of Central Himalaya where green felling is banned completely.

MATERIAL AND METHODS

Study site

The study site was protected and enclosed by a barbed wire fence (fencing done by Department of Botany Kumaun University, Nainital) as a result human and cattle pressure was negligible. The study site is located in Nainital catchment on eastern aspect at 2150 m elevation, between 29°22'47"-29°23'04" N latitude and 79°26'5"-79°28'36" E longitude in Central Himalaya. The study site was disturbed about 20 years ago, as a consequence of road construction which involved hill cutting in the upper part of the slope. The basic climatic pattern is governed by the monsoon rhythms. Severe frosts are usual throughout the winter season and snow falls frequent, snow persists for months. The annual rainfall was 2086 mm during the study year however, during the winter season just 48 mm of rainfall occurred. Mean minimum and maximum temperatures were -2.0 and 20ºC. However, on certain days in January temperature as low as -6.0 ºC were common. Within the study site two microhabitats of 1 ha were selected, a denser canopied

microhabitat with 78% cover and a lighter canopied microhabitat with 48% canopy cover estimated by a densiometer.

The soil was always moister in denser canopied microhabitats than in lighter canopied microhabitats. The lighter canopied microhabitat was covered with gravel and had little soil and the denser canopied microhabitat had good and uniform soil cover (Table 1).

Population dynamics and growth behaviour

Ten seed traps of 50x50 cm size, constructed of a wooden frame attached to a fibre glass screen base, were placed were placed directly on the forest floor with sides extending upwards ≈5 cm at different position(i.e. under canopy, under overlapping canopies and under the canopy gaps) in both microhabitats. The seeds falling within the seed traps (15 July-30 August 1997) were counted fortnightly to calculate the seed fall density (Donna et al. 1989). Next to each seed trap in each plot were placed 1x1 m permanent quadrat to observe seedling recruitment and mortality at monthly interval. The emerging seedlings were located and marked within these permanent quadrats by white paint.

To determine dry weight changes seedling of size and diameter similar to that of seedling within permanent quadrats were marked in both microhabitats. Only three seedlings were harvested at monthly interval from April to October to estimate the biomass allocation to different components (e.g. leaf, stem and root) of seedling. Net primary productivity was calculated by positive increase method (Singh and Yadava, 1974).

Shoot Water potential

For assessing the water stress in seedlings, water potential was measured at two times of the day, first during early morning(predawn) when the seedling water potential is most favourable, and second during mid-day when water stress is most severe (Turner, 1987). The data was collected over 12 months (autumn to rainy).

Data were analyzed by linear regression (Snedecor, and Cochran, 1968). Analyses of variance (ANOVA) were undertaken for seedling mortality and shoot water potential following Snedecor and Cochran (1968) and to test the significance of differences in seedling mortality between microhabitats and seasons.

RESULTS

Seed fall density and seedling recruitment

Seedling recruitment was recorded between August-September. Seed fall was significantly higher ($P<0.01$) in the denser canopied microhabitats (38.0 seeds m^{-2}) than in lighter canopied microhabitats (28.8 seeds m^{-2}), 56.1% of those germinated in denser canopied microhabitat and 52.1% in lighter canopied microhabitats. The greater seed fall in denser microhabitat was possibly due to greater crown cover.

Population dynamics

Initially the mean seedling number was greater in denser canopied microhabitats (21.3 seedlings m^{-2}) than lighter canopied microhabitats (15.0 seedlings m^{-2}).

Seedling numbers started to decline from October-November (autumn) and continued to decline until summer. Total seedling mortality was greater in lighter canopied microhabitats (74.6%) than denser microhabitats (51.6%). The seedling mortality was the maximum during winter (Table 2) and much less in spring and summer. By summer the live seedling density had declined to $10.3m^{-2}$ in denser microhabitat and $3.8m^{-2}$ in lighter canopied microhabitat. Therefore, by end of year, the density had decreased by 51.6% in denser microhabitat and 74.7% in lighter canopied microhabitat.

Water potential

Pre-dawn and mid-day water potentials of seedlings under denser and lighter canopies did not vary significantly between seasons. The predawn seedling water potential in denser microhabitats ranged between -0.3 and -1.9 MPa and -0.4 to -2.3 MPa under lighter canopied microhabitat, respectively. Water potential was most negative during the winter months (December- January) and most favourable during monsoon months (Table 3).

Seedling dry mass

Peak seedling mass, 3.92 g seedling^{-1} vs. 5.52 g seedling^{-1}, recorded a net increment of 1.04 and 1.58 g from April to October in denser and lighter-canopied microhabitats, respectively. The difference between seedlings of the two microhabitats (3.92 g seedling^{-1} vs. 5.52 g seedling^{-1}) was highly significant ($P<0.01$). At both microhabitats proportional contribution of leaves to total seedling mass increased marginally from April to July, rapidly during August-September and declined thereafter (Figure 1). The stem contribution was almost equal to seedling weight throughout the study period. The contribution of root declined from April to August and thereafter increased marginally. A declining root:shoot ratio was recorded throughout the study period with a marginal increase towards October (Figure 2).

Table 1. Comparison of microhabitats for soil characters. Soil texture and soil chemical data are on dry weight basis.

	Denser microhabitat	Lighter canopied microhabitat
Soil texture		
Gravel and other Coarse material	80.4±0.013	88.9±0.034
Sand	8.9±0.03	6.2±0.006
Silt	6.4±0.012	3.0±0.005
Clay	4.0±0.004	1.7±0.003
Soil pH	8.14±0.023	8.03±0.004
Soil organic carbon	1.41±0.012	1.02±0.005
Soil nitrogen	0.10±0.023	0.15±0.006
Air temperature		
Maximum (ºC)	19.5±0.03	20.0±0.132
Minimum (ºC)	-2.1±0.002	-2.0±0.002
Light intensity (lux)	57.8 x 100±0.387	152.4 x 100±0.768

Table 2. Seed fall density and population dynamics of 1-yr old seedlings of *Quercus floribunda in denser and lighter canopied microhabitat*

Parameters	**Denser microhabitat**	**Lighter canopied microhabitat**
Period of seed fall density	June – Sep.	June –Sep.
Seed fall (seed m^{-2})	38.0±0.45	28.8±0.35
Period of peak seed germination	Late Aug. – Sep.	Late Aug. – Sep.
Percent seed germination	56.1	52.1
Total number of newly recruited seedlings (no. m^{-2})	21.3+0.32	15.0+0.035
Period of seedling mortality		
Total percent annual seedling mortality	Oct. – June	Nov. – June
Seasonal seedling mortality (%)	35.2	55.6
Summer		
Rainy	8.7	13.3
Autumn	0.0	0.0
Winter	5.5	11.2
Spring	47.3	56.9
	15.4	3.3

Table 3. Seasonal variation in predawn and midday water potential of *Quercus floribunda* seedlings in denser and lighter canopied microhabitats

	Predawn		Mid-day	
Season	Denser canopied	Lighter canopied	Denser canopied	Lighter canopied
Autumn	-0.7	-0.68	-1.3	--1.26
Winter	-1.47	-1.5	-1.65	-1.76
Spring	-0.83	-1.08	-1.33	-1.36
Summer	-0.67	-0.85	-1.35	-2.35
Rainy	-0.38	-0.58	-1.03	-2.09

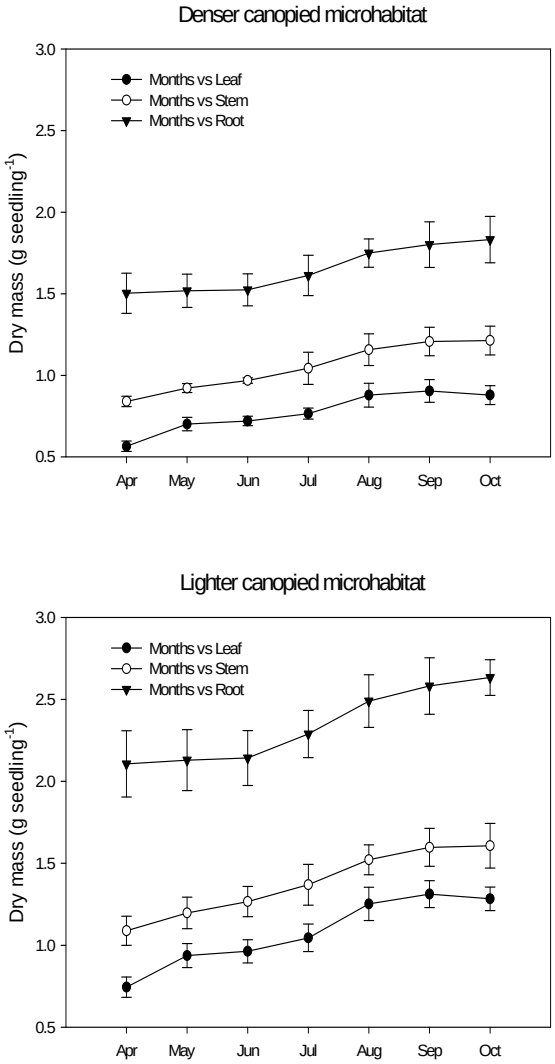

Figure 1 Monthly variation in seedling dry mass (g seedling^{-1}) in denser and lighter canopied microhabitats.

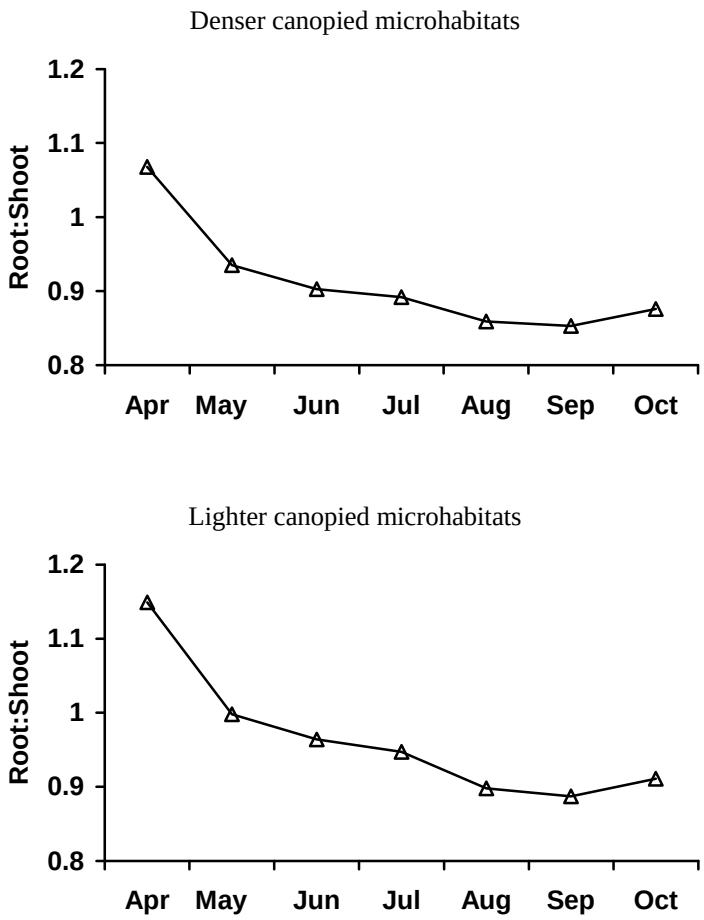

Figure 2 Monthly variation in root:shoot ratio in denser and lighter canopied microhabitats.

DISCUSSION

The study began to monitor the seedlings of *Q. floribunda* when they were 1 to 2 months old. Other studies (Pandey, 1979; Upreti et al., 1985) on vegetational analysis in the same catchment, elevation and aspect have reported that in the non-mast years seedling density ranges between 1.2 seedlings m^{-2} and 1.3 seedlings m^{-2} (Negi et al., 1996). In this study during a year of good seed population at the time of recruitment seedling density was comparable with Negi *et al.* (1996) who reported about four-times greater seedling density in mast years compared to normal years.

The greater number of seedlings in denser canopied microhabitats could be attributed to larger seed production at this site because of a denser canopy and high shade tolerance capacity in *Q. floribunda* seedling (Rao and Singh, 1989). Limited mortality due to frost in this denser canopied microhabitat too cannot be ruled out for higher seedling density compared to the micro habitat with lighter canopy density at the end of the year.

However, winter drought in the first year was the major contributor to the seedling mortality (47.3% and 56.9% in denser and lighter-canopied microhabitats, respectively). The low winter air temperatures and only 19 mm of rainfall may have led to maximum seedling mortality in both these microhabitats. For *Eucalyptus pauciflora*, Osmond *et al.* (1987) found temperatures less than -5 ºC to be most lethal.

Soil temperatures of -2.3 and 2.0ºC during January were experienced by the seedlings of *Q. floribunda.* For the most part, plants experience the highest and the lowest temperatures near the soil surface. Thus, mortality of juveniles during establishment usually determines the ecological impact of temperature tolerance limits. The smallest seedlings suffer the most extreme thermal stress (Nobel, 1984).

It is likely that water stress, like temperature stress, has a major influence on plant distribution during seedling establishment. The most rapid change in water availability take place at the soil surface,

and several studies show that seedling mortality during water stress is higher than adult mortality (Wellington, 1984). Tree seedlings experience more severe water potentials than larger individuals on the same site (Crombie, 1997). In a study on *Q. floribunda* saplings on the same site during an unusually severe drought in 1999 when only 26.5 mm of rain was recorded over a period of about 8 months after the monsoon of 1998. Pre-dawn water potentials were found to range between -3.1 MPa to -5.5 MPa resulting in most of saplings having dead withered leaves. This oak population keeps its stomata open and fixes carbon even at a heavy cost in transpirational water loss (Singh et al., 2000). During winters in the present study the values of predawn water potential i.e. -1.9 and -2.3 MPa in denser and in lighter canopied microhabitats suggest severe water stress. Large scale mortality in *Q. floribunda* seedlings may have occurred due to this winter drought, when soil moisture content at 30 cm depth was -26.4% and mean root length of seedling was only 8.7 cm.

A significantly greater annual gain in seedling dry mass in lighter canopied microhabitat than in denser canopied microhabitat indicates that the conditions for healthy growth were better at former microhabitat, where seedlings were fairly widely spaced and competition was low. In this respect the opportunities for the development of healthy seedlings at denser canopied microhabitat were poor, where the seedlings were more in number and may hold chance of self thinning in the future course of growth.

The net gain in seedling dry mass strongly coincided with rainy season, for about 89% of the total gain in dry weight occurred during rainy season. However, seasonal pattern of dry matter build up in aboveground and belowground parts was different. The decreasing root:shoot ratio from March to August suggests that there occurs translocation of minerals and food reserves to the aboveground parts during favourable growing period to maximize photosynthetic gain (Joshi and Rawat, 1996). A substantial increase in root:shoot ratio from September onwards indicates the accumulation of food reserve in belowground parts to ensure the supply in the next growing season. This strategy helps seedlings to survive in conditions of stress and have been fairly documented (Kozlowski 1971).

In conclusion, it can be emphasized that the two microhabitats having different soil characteristics and light conditions have their own advantages with regard to growth and survival of *Q. floribunda* seedlings. While denser canopied microhabitat, possesses potential of supporting a large number of seedlings with more water potential, but hold risks for self shading and thinning in longer run for the demand of nutrients, moisture, sunlight etc. On the other hand lighter canopied microhabitat has a potential to establish few healthy individuals with greater gain in dry weight, likely to grow into healthy individuals. Therefore, in the regeneration programmes of *Q. floribunda* consideration of large seed crop, removal of biotic interference and microhabitat conditions within the disturbed forest sites should be kept in mind.

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Transforming growth factor in diabetes and renal disease

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Abstract: Renal lipid metabolism may play important roles in renal inflammation, glomerulosclerosis and tubulointerstitial injury in diabetic nephropathy. These alterations in lipids are associated with (1) decreased expression of PPAR-γ mRNA and protein, (2) increased abundance of the sterol regulatory element binding protein-1 (SREBP-1), key regulator of fatty acid synthesis, (3) decreased abundance of farnesoid X receptor (FXR), a negative regulator of fatty acid synthesis and promoter of fatty acid oxidation, (4) downregulation of peroxisome proliferator-activated receptor delta (PPAR-γ), key regulator of fatty acid oxidation, (5) increased abundance of the sterol regulatory element binding protein-2 (SREBP-2), key regulator of cholesterol synthesis, and (6) downregulation of ATP binding cassette A1 (ABCA1), key regulator of cholesterol efflux. These lipid alterations are also associated with marked downregulation of the podocyte markers podocin and zonula occludens-1 (ZO-1) and proteinuria. Treatment of ZDF rats with the PPAR-γ agonist rosiglitazone results in normalization of the renal lipid metabolism pathways and prevention of lipid and adipophilin accumulation, restoration of podocin and ZO-1 expression, and prevention of proteinuria. Thus, our results indicate that renal lipid accumulation significantly contributes to renal cell injury and treatment with PPAR agonist significantly ameliorates podocyte injury, glomerulosclerosis, tubulointerstitial fibrosis, and proteinuria. PPAR-FXR-SREBP pathway may play a critical role in regulation of lipid homeostasis and fibrosis in the kidney. [Nature and Science. 2009;7(1):91-95]. (ISSN: 1545-0740).

Keywords: transforming growth factor (TGF) ; diabetes ; renal disease; kidney

1. Introduction

Diabetes mellitus is the leading cause of cardiovascular and renal disease. The pathogenesis of diabetic nephropathy is multi-factorial. Hypertension, hyperglycemia, profibrotic growth factors, including angiotensin II, transforming growth factor (TGF) and vascular endothelial growth factor (VEGF), proinflammatory cytokines, oxidative stress, and advanced glycation end products (AGEs) have been determined to play important roles in the pathogenesis of diabetic nephropathy (Wendt et al. 2003; Bohlender et al. 2005; Brownlee 2005; Chen et al. 2005; Cohen et al. 2005; Nicholas et al. 2005; Wellen and Hotamisligil 2005; Yamagishi et al. 2007; Goh and Cooper 2008). In addition, abnormal lipid metabolism and renal accumulation of lipids have also been proposed to play a similar important role in the pathogenesis of diabetic nephropathy (Moorhead et al. 1982; Sun et al. 2002; Jiang et al. 2005a; Jiang et al. 2005b).

Several studies in human subjects and in experimental animals with diabetes have shown a correlation between serum lipids, renal lipids, and proteinuria and progressive decline in renal function (Bonnet and Cooper 2000; Spencer et al. 2004). Renal lipid accumulation mediated by increased renal lipid synthesis is involved in the nephropathy seen in animal models of type I diabetes, diet induced obesity and insulin resistance, and aging (Sun et al. 2002; Jiang et al. 2005a; Jiang et al. 2005b).

Diabetic renal disease is associated with lipid deposits in the kidney and TNF-α, TGF-β1, TGF-β2, plasminogen activator inhibitor-1 (PAI-1), nephrin, podocin, ABCA1, α-actin, PPAR, VEGF, COX-2, and HIF expressions, etc. (Sun et al. 2002; Lim et al. 2005).

Transforming growth factor-βs (TGF-βs) in diabetes and renal disease

Transforming growth factor alpha (TGF-α) is upregulated in some human cancers. It is produced in macrophages, brain cells, and keratinocytes, and induces epithelial development. It is closely related to EGF, and can also bind to the EGF receptor with similar effects . TGFα stimulates neural cell proliferation in the adult injured brain (Fallon et al. 2000). TGFα was cited in the 2001 NIH Stem Cell report to the U.S. Congress as promising evidence for the ability of adult stem cells to restore function in neurodegenerative disorders.

TGF-β acts synergistically with TGF-α in inducing cellular transformation. Specific receptors for TGF-β activation trigger apoptosis when activated. Many cells synthesize TGF-β and almost all of them have specific receptors for this peptide. TGF-β1, TGF-β2, and TGF-β3 all function through the same receptor signaling systems.

The peptide structures of the three members of the TGF-β family are highly similar. They are all encoded as large protein precursors; TGF-β1 contains 390 amino acids and TGF-β2 and TGF-β3 each contain 412 amino acids. They each have an N-terminal signal peptide of 20-30 amino acids that they require for secretion from a cell, a pro-region (called latency associated peptide, and a 112-114 amino acid C-terminal region that becomes the mature TGF-β molecule following its release from the pro-region by proteolytic cleavage. The mature TGF-β protein dimerizes to produce a 25 KDa active molecule with many conserved structural motifs. TGF-β has nine cysteine residues that are conserved among its family; eight form disulfide bonds within the molecule to create a cysteine knot structure characteristic of the TGF-β superfamily while the ninth cysteine forms a bond with the ninth cysteine of another TGF-β molecule to produce the dimer. Many other conserved residues in TGF-β are thought to form secondary structure through hydrophobic interactions. The region between the fifth and sixth conserved cysteines houses the most divergent area of TGF-β molecules that is exposed at the surface of the molecule and is implicated in receptor binding and specificity of TGF-β.

TGF-β induces apoptosis in numerous cell types. TGF-β can induce apoptosis in two ways: The SMAD pathway or the DAXX pathway. The SMAD pathway is the classical signaling pathway that TGF-β family members signal through. In this pathway, TGF-β dimers binds to a type II receptor which recruits and phosphorylates a type I receptor. The type I receptor then recruits and phosphorylates a receptor regulated SMAD (R-SMAD). SMAD3, an R-SMAD, has been implicated in inducing apoptosis. The R-SMAD then binds to the common SMAD (coSMAD) SMAD4 and forms a heterodimeric complex. This complex then enters the cell nucleus where it acts as a transcription factor for various genes, including those to activate the mitogen-activated protein kinase 8 pathway, which triggers apoptosis.

TGF-β may also trigger apoptosis via the death associated protein 6 (DAXX adapter protein). DAXX has been shown to associate with and bind to the type II TGF-β receptor kinase. TGF-β plays a crucial role in the regulation of the cell cycle.

A study at the Saint Louis University School of Medicine of USA has found that cholesterol suppresses the responsiveness of cardiovascular cells to TGF-β and its protective qualities, thus allowing atherosclerosis to develop. It was also found that statins, drugs that lower cholesterol levels, enhance the responsiveness of cardiovascular cells to the protective actions of TGF-ß, thus helping prevent the development of atherosclerosis and heart disease. TGF increases with the renal disease.

Discussion

Since the description by Kimmelstiel and Wilson of the classical nodular glomerulosclerosis and presence of lipid deposits in the diabetic kidney, several investigators have shown presence of lipid deposition in the kidneys of diabetic humans and experimental animals. The results from our longitudinal studies in ZDF rats indicate that at the initial stage of diabetic nephropathy, there are multiple disturbances in the lipid metabolic pathways and significantly increased lipid deposition in kidney, including cholesterol, triglyceride, ceramide and glucosylceramide. Analysis of transcriptional factors and their target enzymes that play an important role in regulation of lipid metabolism demonstrated significant a) augmentation of *de novo* fatty acid synthesis and b) concomitant decreased fatty acid oxidation in the initial stage of diabetic kidney development. Moreover, there was c) increased cholesterol synthesis and uptake, and d) decreased cholesterol efflux in the young ZDF rat kidney. Thus, the combined effects of these disturbances in renal

lipid metabolism result in the net increased accumulation of lipids in the kidney. One of the novel and interesting findings in our present study is the demonstration of decreased FXR expression in ZDF rat kidney. FXR has been shown to inhibit SREBP-1c expression in the liver. In the liver FXR has also been shown to induce fatty acid oxidation via stimulation of PPAR and to have anti-fibrotic effect via decreasing TGF-M expression. Thus, the decrease in FXR activity in the kidney could mediate the upregulation of fatty acid synthesis, downregulation of fatty acid oxidation, and increased expression of TGF-M in the ZDF rat kidney.

In view of the toxic effects elicited by lipids on various target tissues and cells, we speculate that the ectopic accumulation of excess lipids in the kidney ultimately result in lipid-mediated cell injury or renal lipotoxicity. This could contribute to the pathogenesis of diabetic nephropathy. The lipotoxicity encompass various pathophysiological events including lipid-mediated cell injury. Lipotoxicity has been well documented in several non-adipose tissues including pancreatic cells, heart, liver and skeletal muscle, and has a profound impact in the pathogenesis and target organ damage in the metabolic syndrome.

We have observed that the increases in renal lipid content was already evident in 6 week old ZDF rats, prior to onset of hyperglycemia, glomerulosclerosis, and proteinuria indicating that these lipid alterations may play an important role in the progression of the diabetic renal injury. In support of this hypothesis, studies in renal mesangial and tubular cells grown in culture have shown that incubation of these cells with low density lipoprotein (LDL) or very low density lipoprotein (VLDL) cause upregulation of growth factors, including TGF-b, PDGF, and plasminogen activator inhibitor-1 (PAI-1), extracellular matrix proteins, proinflammatory cytokines including interleukins and tumor necrosis factor, adhesion molecules including monocyte chemotactic protein-1 (MCP-1), intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1), and lipid peroxidation and glycoxidation, processes which play a role in the pathogenesis and progression of diabetic kidney injury. Our studies in ZDF rats indicate that *in vivo*, accumulation of lipids in the kidney is associated with a) increased expression of TGF-b1, VEGF, PAI-1, b) increased expression of collagen and fibronectin, c) reduced expression of podocyte markers including podocin, ZO-1, and d) mesangial expansion. These functional and structural changes likely contribute to the development of glomerulosclerosis, tubulointerstitial fibrosis, and proteinuria. We provide evidence indicating that rosiglitazone decreases lipid accumulation in ZDF kidney by 1) prevention of fatty acid biosynthesis by suppression of nuclear SREBP-1 protein abundance; 2) induction of fatty acid oxidation via PPAR, ACO and CPT-1; 3) prevention of increased cholesterol biosynthesis by suppression of nuclear SREBP-2 protein abundance; 4) prevention of LDL uptake via inhibition of elevated ox-LDLR expression; and 5) augmentation of cholesterol efflux via increased expression of ABCA1. The prevention of renal lipid accumulation was coupled with i) simultaneous decreases in the expression of profibrotic growth factors and proinflammatory cytokines including TGF, VEGF, and IL-6, ii) prevention of extracellular matrix protein accumulation, and iii) prevention of podocyte injury and loss. These result in the significant amelioration of the development of glomerulosclerosis, tubulointerstitial fibrosis, and proteinuria.

PPAR agonists (thiazolidinedione or TZDs) have been shown to protect against the development of diabetic nephropathy in both human and animal models. Nevertheless, the molecular mechanism underlying the TZD-mediated renal protection has not been fully characterized. Although effective normalization of hyperglycemia and hyperlipidemia by TZD treatment may play an important role in the prevention of renal complications of diabetes, several lines of evidence also support a direct role for TZDs on the kidney. For example, renal glomerular mesangial cells express PPAR receptors and PPAR agonists have anti-fibrotic action in both *in vivo* and *in vitro* studies In addition, PPAR agonists have been shown to be renal protective in models of type I diabetes, independent of any alterations in systemic blood glucose or lipid levels. In the streptozotocin diabetic rat treatment with troglitazone was shown to prevent the increased expression of TGF, fibronectin and type IV collagen. Troglitazone prevented the increase in glomerular diacylglycerol (DAG) content, protein kinase C (PKC) activity and ERK2 phosphorylation while inducing an increase in DAG kinase activity. Troglitazone and pioglitazone also had similar effects in cultured mesangial cells, as they both prevented the high glucose induced increases in DAG, PKC and ERK2 phosphorylation. In another study in the streptozotocin diabetic rat troglitazone prevented the increased expression of PAI-1. Troglitazone has also been shown to be protective against glomerulosclerosis and proteinuria in the 5/6 nephrectomy model of nondiabetic renal disease, by preventing the increased expression of PAI-1 and TGF. Altogether, these studies therefore indicate that in addition to their systemic effects, PPAR agonists also have direct renal effects and modulate diabetic and non-diabetic renal disease by multiple cellular mechanisms, including modulation of renal lipid metabolism

as supported by our current study. Another intriguing finding of the current study is the demonstration of the significant lipid accumulation in the podocytes and the concomitant reduction of podocyte markers podocin and ZO-1 in the ZDF rat kidney, and the corrective effect of rosiglitazone. Podocyte injury is closely related to development and progression of diabetic nephropathy in humans. We demonstrated that podocin and ZO-1 expression was markedly reduced suggesting that decrease in podocin or ZO-1 may be the determinants of increased glomerular permeability and urinary protein loss.

Augmentation of podocyte proteins including podocin, nephrin, and ZO-1 by rosiglitazone demonstrates an important mechanism for the PPAR mediated decrease in proteinuria and renal protective effect in the setting of diabetes mellitus. In summary, we conclude that ZDF rats exhibit a primary alteration in renal lipid metabolism. The accumulation of triglyceride and cholesterol in the kidney glomerular and tubular cells is mediated via simultaneous increase in fatty acid synthesis and decrease in fatty acid oxidation, increase in cholesterol synthesis and uptake, and decrease in cholesterol efflux. The increase in lipid deposition is also associated with podocyte injury and increased expression of TGF-M, VEGF, PAI-1, IL-6, accumulation of extracellular matrix proteins, and proteinuria, suggesting the existence of renal lipotoxicity. Treatment of ZDF rats with the PPAR agonist rosiglitazone depletes the ectopic deposition of excess lipids in the kidney, and significantly ameliorates lipotoxicity-associated renal pathological abnormalities.

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