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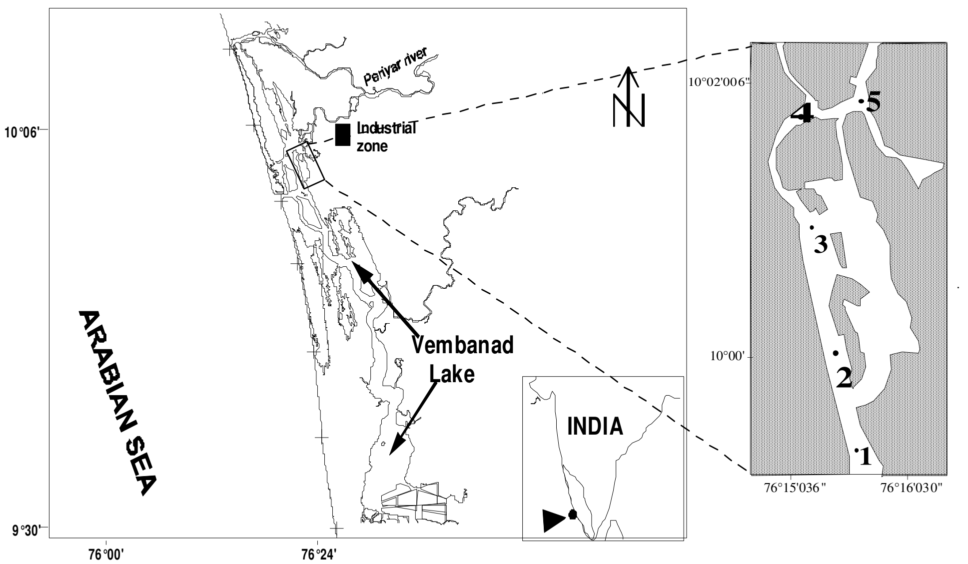

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POTENTIAL OF TENDU LEAF REFUSE FOR PHENOL REMOVAL IN AQUEOUS SYSTEMS

G. K. NAGDA* – A. M. DIWAN – V. S. GHOLE

Department of Environmental Sciences, University of Pune,
Pune – 410007, India
(phone: +912172310145)

e-mail: gnagda@rediffmail.com

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Abstract. The potential of tendu (Diospyros melanoxylon) leaf refuse from bidi industry waste to remove phenol from aqueous solution was studied. For this purpose, the tendu leaf refuse was carbonized by subjecting it to chemical treatments with sulfuric acid. Batch kinetics and isotherm studies were carried out under varying experimental conditions of contact time, phenol concentration, adsorbent dose and pH. Adsorption equilibrium of tendu leaf refuse and chemically carbonized tendu leaf refuse was reached within 2 hr for phenol concentration 10-25 mg/l and 1 hr for phenol concentration 20-200 mg/l, respectively. The adsorption of phenol decreases by the increase of the pH value of the solution. The kinetic data followed more closely the pseudo-second-order chemisorption model. The adsorption data were modelled by using both Langmuir and Freundlich classical adsorption isotherms. The maximum adsorption capacity of chemically carbonized tendu leaf refuse as per Langmuir model was 4 times higher than that of raw tendu leaf refuse. The results illustrate how tendu leaf refuse, a solid waste disposal menace from bidi industry, can be used as an effective biosorbent for phenol in aqueous solution.
Keywords: *Diospyros melanoxylon, biosorption, pseudo-second-order kinetics*

Introduction

Pollution by phenols is an important environmental issue. Phenol, being a basic structural unit for a variety of synthetic organic compounds, wastewater originating from many chemical plants and pesticide and dye manufacturing industries contain this chemical. Wastewater from other industries such as paper and pulp, resin manufacturing, gas and coke manufacturing, tanning, textile, plastic, rubber, pharmaceutical, and petroleum also contain different types of phenols. Decay of vegetation also contributes phenols to water bodies. [1]. Phenols are considered as priority pollutants since they are harmful to organisms at low concentrations and many of them have been classified as hazardous pollutants because of their potential harm to human health [2]. The majority of phenols are toxic substances and some are known or suspected carcinogens [3]. It is important to remove phenols and aromatic compounds from contaminated industrial aqueous streams before discharged into any water body.

Conventional processes for removing phenolic compounds include extraction, adsorption on activated carbon; steam distillation, bacterial and chemical techniques, irradiation, etc. fail to generate final effluent with the required discharge quality at affordable costs [4]. Literature contains number of methods such as oxidation with ozone/hydrogen peroxide [5], ion exchange [6], electrochemical oxidation [7], reverse osmosis [8], photo-catalytic degradation [9], and adsorption [10] which have been used for the removal of phenols. The treatment of with active carbon, silicates and polymer resins are considered to be an effective method for the removal of phenol from wastewater because of its large surface area, micro-porous nature, high adsorption

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capacity, high purity and easy availability [11]. Phenol removal by adsorption process remains the best treatment of choice as it can generally remove all types of phenols in a simple and easy operation. The adsorbent that is used in practice remains activated carbon [12]. However because of high cost of activated carbon, its use is sometimes restricted on economical considerations. As such, attempts have been made by different workers to develop alternative adsorbents, preferably of low cost.

Literature survey shows that a large number of alternative adsorbents have been studied to replace activated carbon. Both, the industrial wastes as well as some natural resource materials such as bentonite [13], fly ash [14], spent bleaching earth [15], apricot stone shells [16], rubber seed coat [17], waste tire rubber [18], etc. have been utilized for this purpose. Recently interest has turned to using various biomasses and agricultural wastes as biosorbent, which have the advantage of low cost and increased tolerance of environmental conditions. Biosorption can be defined as a process in which solids of natural origin are employed for sequestration or separation of pollutants from an aqueous environment [19]. Many natural materials were used as biosorbent for phenol removal, like human hair [20] and chicken feather [21], chemically modified pistachio shells [22], Peanut Shell [23] and tamarind nutshell [24} etc., presented with cheap viable options.

Tendu (*Diospyros melanoxylon*) leaf refuse, which itself is an environmental pollutant, generated during the manufacture of Indian crude cigarette bears this investigation. A bidi is smaller and less expensive than a cigarette and is considered a poor man's cigarette in India. Bidi is an indigenous crude cigarette in which tobacco is rolled in a small cut portion of tendu leaf and tied with a cotton thread. Tendu leaf is used as bidi wrapper on account of the ease with which it can be rolled and its wide availability. It also has agreeable flavor, resistance to decay and capacity to retain fires. Bidi rolling is a home industry covering thousands of homes in the industrial towns of Jabalpur and Solapur in India. This home industry of bidi making produces cuttings of tendu leaf as refuse which pollutes the dumping sites creating solid waste disposal problem of alarming scale. About 7200 tonnes of tendu leaf waste is generated per annum in the industrial town of Solapur, India [25]. Thus the aim of this work was to investigate the ability of tendu leaf refuse from bidi industry to remove phenol from aqueous solution and to determine its potential for use in the phenol removal from wastewater.

Materials and methods

Preparation of biosorbent

The tendu leaf refuse was obtained from the dumping sites near bidi industries in the town of Solapur, India. They were cut into small pieces of about 4-5mm, thoroughly washed with distilled water to remove all dirt, dried at 80ºC till constant weight. The dried tendu leaf refuse (TLR) was powdered and sieved with an 80-mesh siever and stored in desiccator until used. Chemically modified carbon from tendu leaf refuse (TLR-CM) was prepared, by treating five part of TLR with three parts of concentrated sulfuric acid and kept in air tight oven at 150^o – 160^oC for 48 hours. The carbonized mass was washed free of acid with distilled water to get the pH 6.5. It was further filtered and dried at 110^oC till constant weight. The material was pulverized and sieved through 150-mesh size and used as TLR-CM. (*Table 1)* gives the physical properties of thus prepared chemically carbonized tendu leaf refuse.

Table 1. Physical parameters of chemically carbonized tendu leaf refuse.

Parameters	Characteristic value
pH	6.45
Moisture (%)	9.15
Ash content (%)	4.04
Bulk density (g/ml)	0.48
Surface area (m2/g)	210
Pore volume (ml/g)	0.13
Iodine number	128
Cation exchange capacity (meq/g)	1.02

Chemicals

The test solutions were prepared by diluting of stock solution of phenol to the desired concentrations. A stock solution was obtained by dissolving 1.0g of phenol, (obtained from Merck, India), in de-ionized water and diluted to 1000 ml. Desired solutions of phenol were prepared using appropriate subsequent dilutions of the stock solution. The range in concentrations of phenol prepared from standard solution varied between 10mg/l to 200mg/l. Before mixing the adsorbent, the pH of each test solution was adjusted to the required value with diluted and concentrated sulfuric acid and sodium hydroxide solution, respectively. All pH measurements were carried out with a pH meter.

Absorption studies

Adsorption studies were performed in batch method; using 100ml aqueous phenol solutions of varying concentration in 250ml Erlenmeyer flasks and known amount of TLR and TLR-CM were added to each flask. The flasks were maintained at 30ºC under constant stirring on a magnetic stirrer at 100 rpm. Samples were removed at different time intervals, centrifuged and analyzed for phenol. The uptake was calculated from the difference between the initial and final phenol concentration. Data for adsorption isotherm were obtained by allowing the adsorbents to remain in contact with the phenol solution for 24 hours. The phenol concentrations used were in the range of 20 to 200 ppm. All experiments were carried out in triplicates with respect to each condition and mean values were used for further calculations. The maximum deviation was 2.5%.

Analysis of phenol

The concentration of phenol in the aqueous medium was determined with direct photometric method [26]. The absorbance of the coloured complex of phenol with 4-aminoantipyrine was measured spectrophotometrically at 500nm.

Results and discussion

The adsorption of phenol in aqueous solution on tendu leaf refuse (TLR) and chemically carbonized tendu leaf refused (TLR-CM) were examined by optimizing various physicochemical parameters such as; pH, contact time, and the amount of adsorbent and adsorbate.

Effect of pH on phenol removal

In any adsorbate-adsorbent system, pH of the system affects the nature of surface charge of the adsorbent, effects ionization and the extent and rate of adsorption. Several 100ml portions of 100mg/l of phenol solution were adjusted to assigned pH with sodium hydroxide or sulfuric acid and were mixed with different adsorbent doses with stirring for 1h. Measurement of initial and final phenol concentration gave the percent adsorption of phenol. The adsorption of phenol by TLR and TLR-CM was studied at various pH values as displayed in (*Fig.1.)* Adsorbed amount decreased with increasing pH value, which can be attributed to the phenol ionization to form phenoate ions and at the same time the presence of hydroxyl ions on the adsorbent prevents the uptake of phenoate ions [27]. Similar behaviour has been reported during the adsorption of phenol onto bentonite [13]. Decrease in adsorbed phenol was sharp at pH 10 in case of TLR-CM.

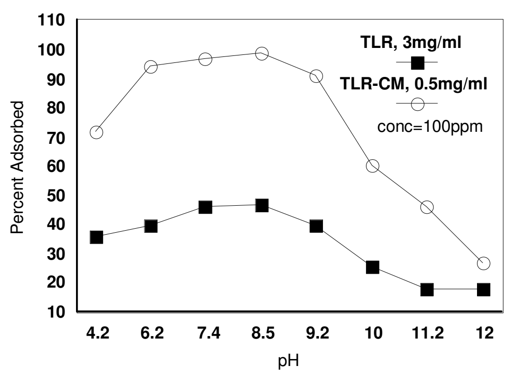

Figure 1. *Effect of pH on phenol removal.*

Adsorption kinetics

Phenol uptake by TLR and TLR-CM for different contact time were studied in batch experiments and shown in (*Fig. 2)* at different initial phenol concentration ranging from 10 to 200 ppm. The adsorbent mass used was 5mg/ml for TLR and 1mg/ml for TLR-CM at $30^{o}C$ ant at pH value of 7.2. The increase in initial phenol concentration results in an increase in the phenol uptake due to increase in mass transfer driving force resulting in higher phenol adsorption [12]. The results also showed that equilibrium time required for the adsorption of phenol on TLR and TLR-CM are 2hr and 1hr respectively, which is considered a relatively fast adsorption process.

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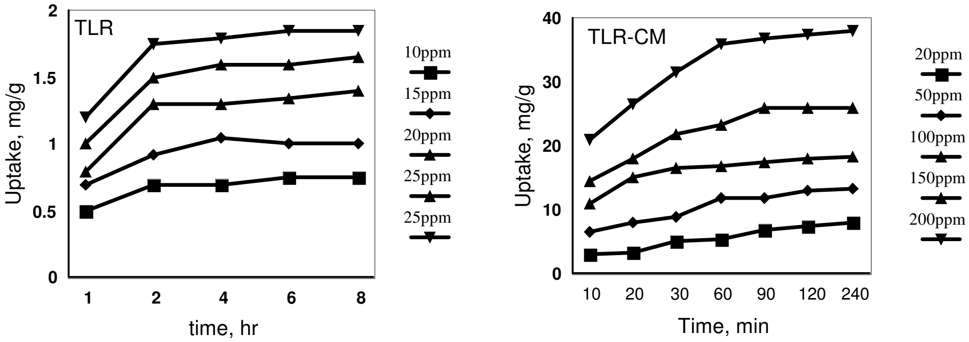

Figure 2. *Kinetics of Phenol uptake at various initial concentrations of phenol by TLR and TLR-CM.*

The rapid adsorption of phenol onto the biosorbent, suggest that intra-particle diffusion is the limiting factor in this latter portion of the process. The kinetic data fits best in the Ho's pseudo-second-order chemisorption model [28] as shown in (*Fig. 3*).

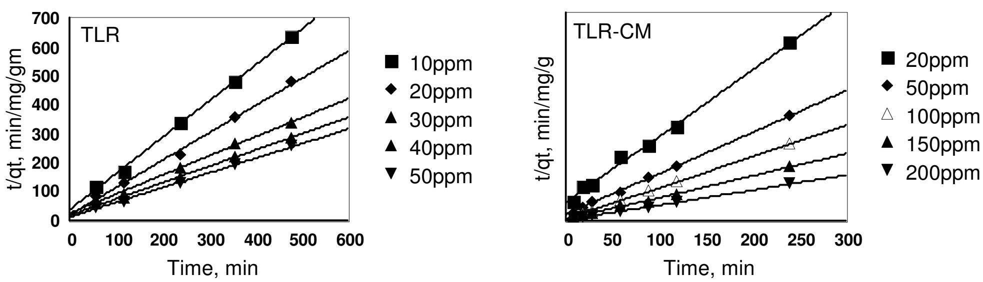

Figure 3. *Pseudo-second-order kinetics of phenol uptake by TLR and TLR-CM.*

The pseudo-second order equation is based on the sorption capacity on the solid phase. It predicts the behaviour over the whole range of studies supporting a pseudo-second order equation and is in agreement with chemisorption being the rate-controlling step. The pseudo-second-order rate expression of Ho [28] has been applied widely to the sorption of metal ions, dyes, herbicides, oils and organic substances from aqueous systems.

Effect of adsorbent concentration

The effect of adsorbent concentration on phenol removal was studied where various amounts of TLR and TLR-CM were contacted with a fixed initial phenol concentration. The residual phenol was measured in the solution at equilibrium and results are shown in *(Fig. 4.)* The percentage of adsorption increases with the increase in adsorbent concentration as the number of adsorbent particles increases and more phenol is attached to their surface.

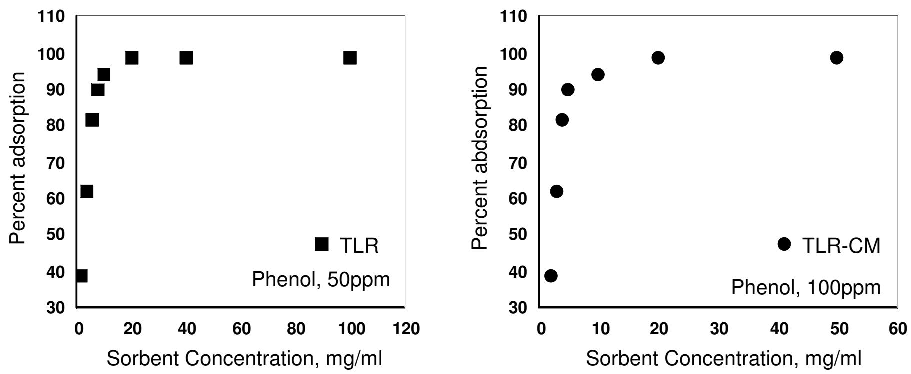

***Figure 4.** Effect of adsorbent concentration on phenol uptake by TLR and TLR-CM.*

Adsorption isotherms

It essentially expresses the relation between the concentrations of the solute in solution at dynamic equilibrium with the concentration of the solute adsorbed onto the adsorbent at constant temperature. Several models have been published in the literature to describe experimental data of which the most common are the Langmuir isotherm and the Freundlich isotherm [29]. In order to facilitate the estimation of the adsorption capacities at various conditions, the Langmuir adsorption isotherm, a typical model for monolayer adsorption was applied. The linearized Langmuir model can be written as

$$\frac{1}{Q_e} = \frac{1}{K_L} + \frac{1}{bK_L} \times \frac{1}{C_e} \quad \text{(Eq.1)}$$

Where Ce is the concentration of phenol at equilibrium (mg/l), q_e is the amount of phenol adsorbed at equilibrium (mg/g), K_L (mg/l) and b (mg/g) are the Langmuir constants, representing the maximum adsorption capacity for the solid phase loading and the energy constant related to the heat of adsorption. The constants b and K_L can be evaluated from the intercept and slope of the linear plot of the experimental data of 1/Qe versus 1/Ce, respectively.

The Freundlich isotherm has been widely adopted to characterize the adsorption capacity of organic pollutants using different adsorbents by fitting the adsorption data. The Freundlich isotherm in its linearized form can be written as:

$$\log Q_e = \log K_F - \frac{1}{n} \times \log C_e \quad \text{(Eq.2)}$$

Where, K_F is a Freundlich constant related to the adsorption capacity (mg/g), and 1/n is the intensity of adsorption. The values of K_F and $1/n$ can be determined from the intercept and slope, respectively of linear plot of log qe versus log Ce.

The linearized Langmuir and Freundlich adsorption isotherms of TLR and TLR-CM for phenol are shown in (*Fig. 5)* and (*Fig. 6)* respectively. The Langmuir and Freundlich constants are displayed in (*Table 2)*.

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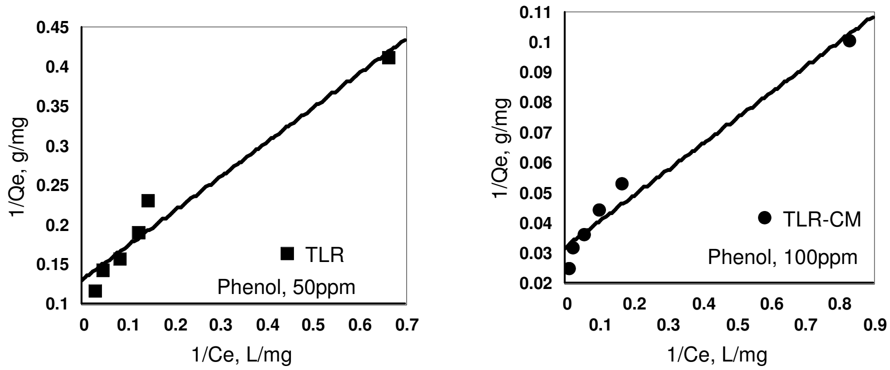

Figure 5. *The linearized Langmuir Adsorption Isotherm for Phenol with TLR and TLR-CM.*

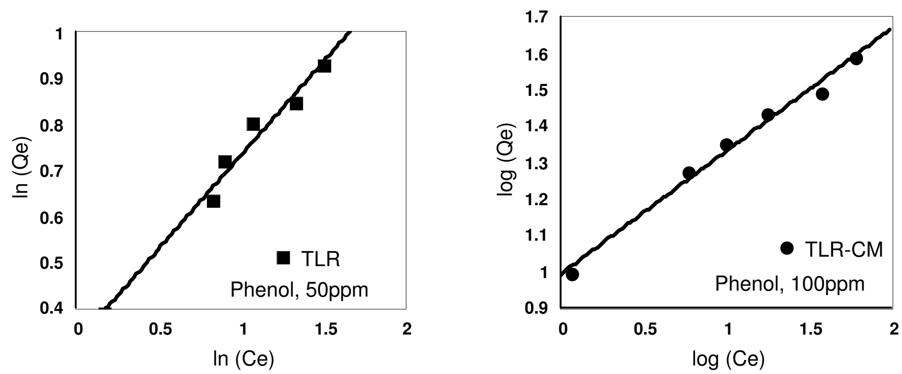

Figure 6. *The linearized Freundlich Adsorption Isotherm for Phenol with TLR and TLR-CM.*

Table 2. *Adsorption isotherm parameters for the adsorption of phenol.*

Adsorbent	Langmuir			Freundlich		
	K_L, (mg/g)	b, (mg/g)	R^2	K_F	n	R^2
TLR	0.299	7.6923	0.957	2.099	2.4631	0.977
TLR-CM	0.374	31.348	0.965	9.817	2.9586	0.986

The higher regression values showed that the equilibrium data for phenol fitted well to both the Langmuir and Freundlich isotherms in the studied concentration ranges. Based on the correlation coefficients (R^2), the equilibrium data was slightly better fitted in the Freundlich adsorption isotherm than the Langmuir equation (*Table 2*). The higher value of K_F, the Freunlich constant, showed easy uptake of phenol from aqueous solution [30]. Also the higher value of *n* reflects the intensity of adsorption signifies that the surface of biosorbents is heterogeneous in nature and high enough for effective separation [31].

In this study, the ability of tendu leaf refuse from local bidi industry, which itself is a solid waste disposal menace and its chemically carbonized product to adsorb phenol was investigated. The equilibrium data were fitted well by the Freundlich and Langmuir isotherms where the data were slightly better fitted by the Freundlich isotherm in terms of regression values (R^2). The adsorption capacity increased four times after its carbonization with sulfuric acid. The pseudo-second-order chemical reaction kinetics of phenol adsorption on tendu leaf refuse provides the best correlation of the experimental data. On the bases of this study, it may be concluded that tendu leaf refuse–a pollution menace was found to be effective, efficient and promising adsorbent for phenol from aqueous solutions, providing attractive alternative for activated carbon.

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EFFECT OF PLASTIC TUNNEL SIZE ON PRODUCTION OF CUCUMBER IN DELTA OF EGYPT

F. EL-AIDY – A. EL-ZAWELY – N. HASSAN – M. EL-SAWY

Dept. of Hort., Fac. of Agriculture, Tanta University
33516 Kafr El-Sheikh, Egypt

e-mail: faelaidy@yahoo.com.au

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Abstract. The experiments were carried out in the years of 2000, 2001 and 2003 at Kafr El-Sheikh. The main objective of this research was to study the influence of growing seasons and types of unheated plastic house on yields of two cucumber F_1 hybrids. Downy mildew and powdery mildew were lower in the winter season than during the early summer season. Likewise, they were lower inside the double span house than inside the single span house (at 60 days after transplanting).The highest total yield as number and weight was produced from Petostar hybrid grown inside the double span house during the early summer season.
Keywords: p*lastic tunnels, cucumber, seasons, cultivars*

Introduction

In the recent years, growing vegetables under protected cultivation in Egypt is expanding rapidly. The common types of protected cultivation in Egypt are the plastic low tunnels and the single span plastic houses. The number of single-arch greenhouses reached about twenty thousand, when about 12000 (60%) are used for cucumber production.

For obtaining higher energy efficiency and economic use of construction materials, several changes in the type of houses have been recommended. Most of the modifications in greenhouse construction were related to the construction of the multiple span houses instead of the single span. Multiple span houses with higher roofs are believed to be more suitable for the continental climate than the single houses.

Therefore, the present study aimed to evaluate two new cucumber F_1 hybrid cvs. (Primo and Petostar) under single span and double span plastic houses during the winter and early summer seasons, under Kafr El-Sheikh conditions.

Welles *et al.* (1999) [35] studied the influence of cultural factors on fruit quality of glasshouse cucumber. They found that cultivar choice had the largest impact. Air temperature, humidity, plant density and leaf area index had negative effects, but light intensity, fertilizers and certain growing media had positive effects on quality. Combining these factors improves fruit quality substantially, but isn't enough for giving quality guaranties to buyers. However, recent research data on a new growing system, the high wire system (HWS), plants did not top but allowed to grow up to 3.5 m high, showing that high yields of excellent fruit quality (keeping quality) can be obtained almost all year-round.

Papadopoulos *et al.* (2000) [31] studied the effects of day and night air temperature (DT and NT) on growth of long English cucumber in the spring season. They indicated that plant development rates (leaves number) were linearly increased with increasing daily average air temperature (MT), but weren't affected by day-night air temperature difference (DIF). Also, plant development rates increased with increasing air temperature regardless of DT or NT.

High temperature in summer under plastic house could cause plant injury to cucumber and tomato in Egypt [15].

The main effect for humidity is on leaf expansion which is favoured by high humidity (through improving water balance), but may be counteracted by a negative effect, in some crops caused by calcium deficiency in the leaves through a reduction of transpiration [2], [26]. Also, [2] found that the positive effect of air humidity on stomatal conductance results in only a minor effect on crop photosynthesis.

Regional climate differences have a major effect on ventilation choice. Natural ventilation is most common (61%), followed by fans (17%) and pads (11%).Gas-fired heaters are most common (61%) followed by hot water perimeter pipers (28%), [3].

The main problems are the high costs of materials required for protected cultivation, unsuitable ventilation of the existing tunnels and the unacceptable (to local consumers) intensive use of pesticides [18].

The results of long term work by El-Aidy (1979) [14] and Abou-Khaled and El-Aidy (1986) [1] introduced eleven types of plastic tunnels (types of I, II, III and IV) and greenhouses (types, V, VI, VII, VIII, IX and Nobaria) varying from low tunnels type to large volume type which cover 1200 m^2, in two generations of development. The second generation offers a simple solution to achieve sufficient ventilation in attempt to solve most of the first generation problems. Also, they reported that in spite of all presented types of the plastic tunnels or greenhouses correspond to a medium advanced stage.

Farag (2001) [21] studied the effect of natural ventilation (as side and top ventilation) on growth and yield of cucumber plant cv. Primo F_1 hybrid in unheated single plastic houses. He showed that using the side ventilation during the summer season reduced maximum and minimum relative humidity, and air temperatures as well as average soil temperature compared with top ventilation and control. Likewise, it surpassed vegetative growth and fruit yields, compared with top ventilation and control which had the lowest values.

Castilla and Lopez-Galvez (1994) [6] evaluated two new unheated prototype greenhouse (a north-south oriented multispan of symmetrical 17 roof slope and an east-west oriented singlespan of asymmetrical roof angles, 18 and 8, as possible alternatives to the conventional flat-roofed greenhouse. They found that around the winter solstice, the higher light transmission in the asymmetrical singlespan houses significantly increased cucumber yields, relative to those grown in a conventional plastic house. Also, the multispan structure generated lower radiation transmissivity in spring.

Chen *et al.* (1989) [8] indicated that increasing natural ventilation (in plastic greenhouse) by 3 h in the morning and at night every day from the end of April to the first 10 days of May, reduced the dew duration on cucumber leaves. The use of natural ventilation for the whole night within the second 10 days and the last 10 days of May reduced downy mildew by 86% and 46%, respectively.

Ten cucumber cultivars were compared for their yield, quality and resistance to diseases when cultivated in unheated greenhouse by Leber and Heck (1991) [29]. They showed that the highest yields were obtained with cv. Birgit (40.3 fruits/m^2 or 26.1 kg/m^2). Also, the same variety was more resistant to powdery mildew (*Erysiphe cichoracearum*).

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The influence of the planting date during the winter and summer seasons was studied by El-Aidy (1989 and 1990) [16], [17]. He showed that the best vegetative growth was obtained from the plants cultivated in October, while the worst one was obtained from November planting dates. The yield was decreased as the transplanting date delayed. The decreasing yield at late transplanting dates might be due to the highly increase in air temperature a result of the plastic covers. Also, the effect of production season on the average yield of some cucumber varieties and their interaction was studied. The differences were significant. All of the varieties used in the present study produced more yield during the early summer season. The differences were between 23% and 81%

Materials and methods

The study was conducted during four successive growing seasons starting from 2000 to 2002 at Sakha (Kafr El-Sheikh Governorate) Protected Cultivation Site, Ministry of Agriculture.

Treatments used: The experiment included three factors as follows:

A. Growing seasons: Two seasons were tested, the first was the winter season and the second was the early summer season. Dates of sowing in plastic seedling trays were 27 September in the winter season and 10 January in the early summer season in both years. Likewise, the dates of transplanting of cucumber under plastic houses were 10 October in the winter season and 2 February in the early summer season in both years.

B Type (size) of unheated plastic houses: Single span plastic house with top ventilation having round-arch (3.2 m height, 8.5 m width and 55 m length) which was covered an area of 467.5 m^2 as showing in *(Fig. 1).* It was divided into five ridges. Each ridge was 150 cm width.

Double span plastic house with side ventilation having round-arch (5.1 m height, 17 m width and 55 m length which was covered an area of 935 m^2) as showing in *(Fig. 2).* It was divided into ten ridges.

Both plastic house types were covered with UV polyethylene film with thickness of 200 microns.

C.Cucumber treatments:

Two hybrids of cucumber cvs Petostar and Primo were used.

Nursery material and cultural practices

In winter and early summer season, seeds of cucumber cvs Petostar and Primo F_1 hybrids were sown in seedling trays The seedlings were transplanted into an unheated plastic houses when they reached the second true leaf stage (13 days age) on 10 Oct. and 2 Feb. in both years.

Transplanting took place on both sides of each ridge at space of 45 cm (planting density was 3 plants/m^2).

Cucumber plants were trained vertically on single stem. All cultural practices (cultivation, nutrition, pests and diseases control and others) were carried out whenever they were necessary.

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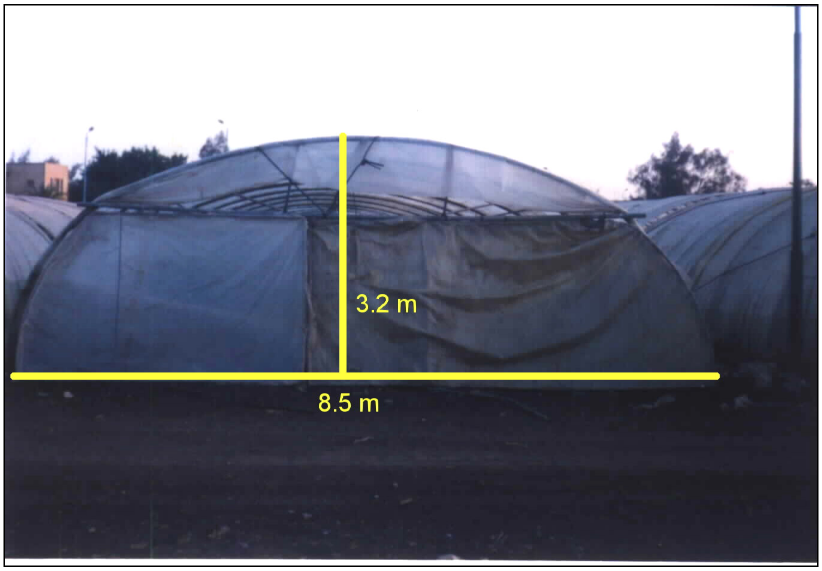
Harvesting started on Nov. 10th (after 31 days from transplanting date) and continued for 67 days until 15 Jan. in the winter season and on Mar. 18th (after 44 days from transplanting date) and continued for 82 days until June 7th in the early summer season in both years of 2001 and 2002. Number of pickings in winter and early summer seasons were 32 and 42, respectively.

Outside view



Inside view

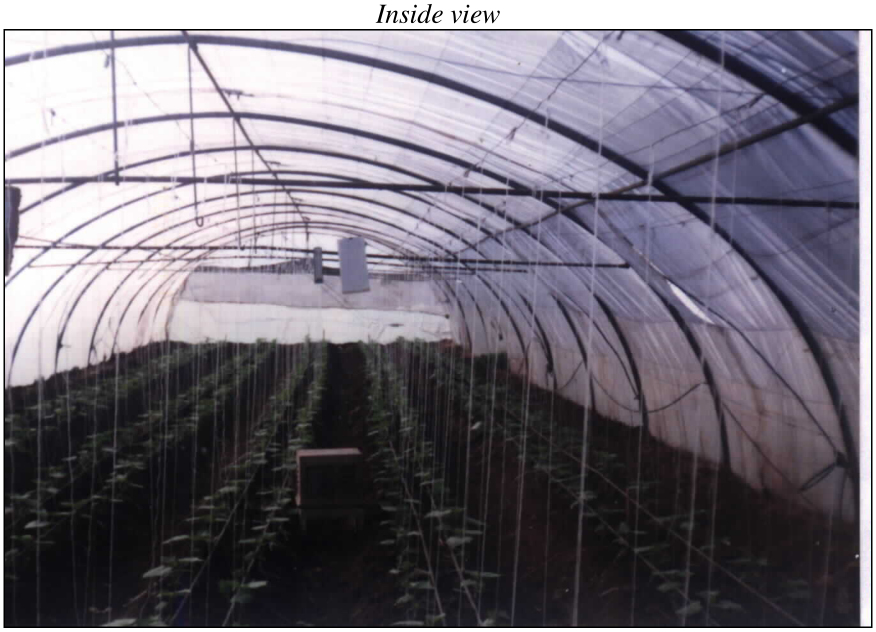


Figure 1. *Singlespan plastic house having one round-arch (3.2 m height, 8.5 m width and 55 m length) covered an area of 467.5 m^2.*

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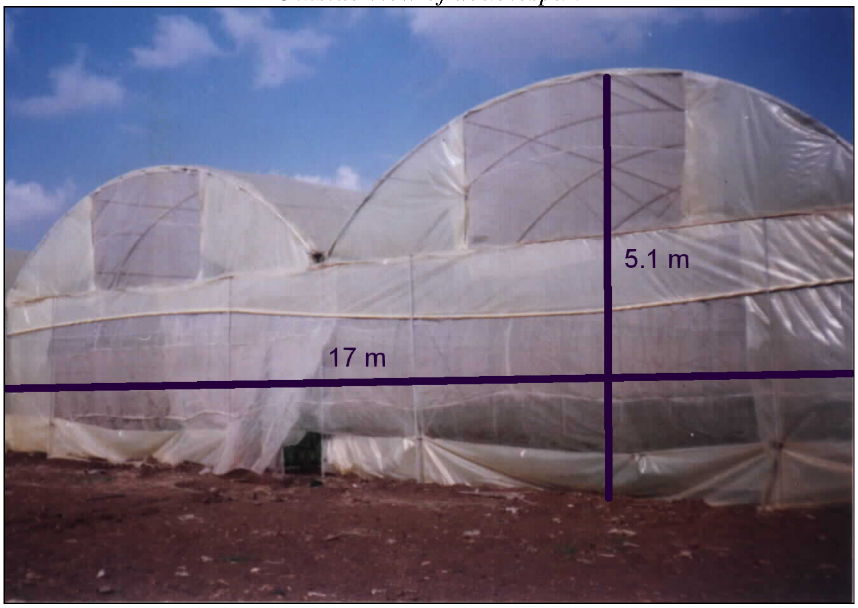
Outside view of doublespan



Inside view of doublespan

Figure 2. *Doublespan plastic house having two round-arches (5.1 m height, 17 m width and 55 m length) covered an area of 935 m*^{2}.

Experimental design and statistical analysis

The experiment included 8 treatments which were the combinations of two growing seasons, two types of plastic houses and two hybrids of cucumber. The previous treatments were arranged in a split-split-plot design with four replications. The main plots were assigned for growing seasons, the subplots for types of plastic houses and the sub-sub plots for the two cultivars. The experimental plot (sub-sub plot) consisted of 144 plants with an area of 48 m^2.

Data were tested by analysis of variance according to Little and Hills (1975) [30]. Duncan's multiple range test was used for comparison among treatments means [12].

Data recorded

Flowering

Accumulative number of opened female flowers per plant was recorded daily on five plants which were randomly chosen from each experimental plot (sub-sub-plot) starting from the first opened female flower until the end of flowering period.

Fruit yield and its components

Data included early and total fruits yields and earliness. Early fruits yield was determined (as weight and number of fruits per plant and per square meter) of the first 10 pickings. Total fruits yield was determined (as weight and number of fruits per plant and per m^2) for all pickings. Earliness was expressed as number of days from transplanting to the first picking of fruits [34].

Downy mildew and powdery mildew infection

Downy mildew and powdery mildew were estimated weekly as infection percentage inside the plastic houses according to the following formula:

$$\text{Infection\%} = \frac{\text{Number of infected plants}}{\text{Number of total plants}} \text{x100}$$

Economic evaluation

Economic evaluation of cucumber yield, production cost, income (crop value) and net profit (net return) were studied.

Results and discussion

Early fruits yield

Effect of season

Data in *Table 1* indicate that the early summer season caused a highly significant increase in early fruits yield (as weight and number of fruits) when compared with the winter season in both 2000/01 and 2001/02 seasons. The increase in weight and number of fruits of early yield during the early summer season might be due to the positive influence of warmer weather (period from Mar. to Jun.) on vegetative growth and flowering which led to increase in early fruits yield (as number and weight). On the contrary, the reduction of early fruits yield during the winter season might be due to the negative effect of colder weather (period from Nov. to 15 Jan.) on decline vegetative growth and flowering which led to decrease in early fruit yield. The suitable soil and air temperatures and light for improvement of early cucumber yield were emphasized by many investigators. [9], [7], [11] and [31].

Table 1. *Effect of season on flowering and early and total yields of cucumber plant during 2000/01 and 2001/02 seasons.*

Traits Treatments Seasons	No. of female flowers/plant	Early fruits yield/m^2		Total fruits yield			
				/plant		/m^2	
		Wt. of fruits (kg)	No. of fruits	Wt. of fruits (kg)	No. of fruits	Wt. of fruits (kg)	No. of fruits
2000/01 seasons							
Winter season	25.8 b	1.512 b	19.8 b	1.670 b	21.7 b	5.010 b	65.1 b
Early summer season	46.1 a	2.994 a	36.6 a	3.325 a	40.5 a	9.975 a	121.5 a
F test	**	**	**	**	**	**	**
2001/02 seasons							
Winter season	22.5 b	1.323 b	16.8 b	1.397 b	18.5 b	4.191 b	55.5 b
Early summer season	42.5 a	2.592 a	34.5 b	2.958 a	38.1 a	8.874 a	114.3 a
F test	**	**	**	**	**	**	**

*, ** and NS indicate significant differences at P < 0.05, P < 0.01 and not significant, respectively, according to F test.
Values having the same alphabetical letter within each column are not significantly different at the 5% level, according to Duncan's test.

Effect of plastic houses type (size)

Data in *Table 2* show that using the double span plastic house caused a significant increase in weight of early fruits yield when compared with the single span in both 2000/01 and 2001/02 seasons. However, number of fruits of early yield was not significantly affected by the type of plastic houses in both 2000/01 and 2001/02. The increase in weight of early fruits yield inside the double span house might be due to that the doubles pan house offering better microclimate (increase in minimum and average air temperatures min. relative humidity and light intensity, and natural ventilation which led to increase in vegetative growth and in turn photosynthesis, subsequently increase average fruit weight.

Table 2. *Effect of plastic houses type on flowering and early and total yields of cucumber plant during 2000/01 and 2001/02 seasons.*

Traits Treatments Houses type	No. of female flowers/plant	Early fruits yield/m^2		Total fruits yield			
				/plant		/m^2	
		Wt. of fruits (kg)	No. of fruits	Wt. of fruits (kg)	No. of fruits	Wt. of fruits (kg)	No. of fruits
2000/01 seasons							
Single span	35.5	2.088 b	28.2	2.324 b	31.0	6.972 b	93.0
Double span	36.4	2.418 a	28.2	2.671 a	31.2	8.013 a	93.6
F test	NS	**	NS	**	NS	**	NS
2001/02 season							
Single span	31.8	1.794 b	25.2	2.070 b	27.8	6.210 b	83.4
Double span	33.3	2.121 a	26.1	2.285 a	28.7	6.855 a	86.1
F test	NS	*	NS	*	NS	*	NS

*, ** and NS indicate significant differences at P < 0.05, P < 0.01 and not significant, respectively, according to F test.
Values having the same alphabetical letter within each column are not significantly different at the 5% level, according to Duncan's test.

Table 3. *Effect of hybrid on flowering and early and total yields of cucumber plant during 2000/01 and 2001/02 seasons.*

Traits Treatments	No. of female flowers/plant	Early fruits yield/m^2		Total fruits yield /plant		Total fruits yield /m^2	
Hybrids		Wt. of fruits (kg)	No. of fruits	Wt. of fruits (kg)	No. of fruits	Wt. of fruits (kg)	No. of fruits
2000/01 season							
Brimo	32.1 b	2.022 b	24.6 b	2.248 b	27.2 b	6.744 b	81.6 b
Petostar	39.9 a	2.484 a	31.8 a	2.747 a	35.0 a	8.241 a	105.0 a
F test	**	**	**	**	**	**	**
2001/02 season							
Brimo	30.0 b	1.716 b	23.1 b	1.978 b	25.5 b	5.934 b	76.5 b
Petostar	35.0 a	2.199 a	28.2 a	2.377 a	31.1 a	7.131 a	93.3 a
F test	**	**	**	**	*	**	*

*, ** and NS indicate significant differences at P < 0.05, P < 0.01 and not significant, respectively, according to F test.
Values having the same alphabetical letter within each column are not significantly different at the 5% level, according to Duncan's test.

Table 4. *Effect of season-plastic houses type-hybrid interaction on flowering and early and total yields of cucumber plant during 2000/01 and 2001/02 seasons.*

Seasons	Houses type	Hybrids	No. of female flowers/ plant	Early fruits yield/m^2 Wt. of fruits (kg)	Early fruits yield/m^2 No. of fruits	Total fruits yield /plant Wt. of fruits (kg)	Total fruits yield /plant No. of fruits	Total fruits yield /m^2 Wt. of fruits (kg)	Total fruits yield /m^2 No. of fruits
2000/01 seasons									
Winter season	Single	Brimo	21.1	1.122	16.2	1.248	17.1 f	3.744	51.3 f
		Petostar	25.9	1.395	19.8	1.549	21.7 e	4.647	65.1 e
	Double	Brimo	24.5	1.524	19.2	1.684	21.0 e	5.052	63.0 e
		Petostar	31.7	2.004	24.6	2.198	27.2 d	6.594	81.6 d
Early summer season	Single	Brimo	44.2	2.700	35.4	3.007	39.4 b	9.021	118.2 b
		Petostar	50.9	3.135	41.4	3.492	45.9 a	10.476	137.7 a
	Double	Brimo	38.4	2.745	28.5	3.053	31.3 c	9.159	93.9 c
		Petostar	51.0	3.396	41.1	3.749	45.4 a	11.247	136.2 a
F test			NS	NS	NS	NS	*	NS	*
2001/02 seasons									
Winter season	Single	Brimo	20.1	1.062	15.0	1.161	16.6	3.483	49.8
		Petostar	23.6	1.281	18.3	1.419	20.1	4.257	60.3
	Double	Brimo	21.5	1.170	14.7	1.293	16.1	3.879	48.3
		Petostar	25.0	1.776	19.2	1.716	21.1	5.148	63.3
Early summer season	Single	Brimo	39.3	2.160	31.5	2.722	35.0	8.166	105.0
		Petostar	44.1	2.676	36.3	2.979	39.7	8.937	119.1
	Double	Brimo	39.1	2.475	31.2	2.737	34.3	8.211	102.9
		Petostar	47.5	3.057	39.0	3.395	43.4	10.185	130.2
F test			NS	NS	NS	NS	NS	NS	NS

*, ** and NS indicate significant differences at P < 0.05, P < 0.01 and not significant, respectively, according to F test.
Values having the same alphabetical letter within each column are not significantly different at the 5% level, according to Duncan's test

Effect of hybrid

Data in *Table 3* illustrate that the differences were highly significant in both 2000/01 and 2001/02. Petostar hybrid had higher number and weight of early fruits yield than those of Primo hybrid. The superiority of Petostar hybrid in early fruits yield (as number and weight) might be due to increase in vegetative growth and number of female flowers.

Effect of season – plastic houses type – hybrid interaction

Data in *Table 4* show that the interaction among season, plastic houses type and hybrid had no significant effect on early fruits yield (as weight and number of fruits).

Total fruits yield

Effect of season

Data in *Table 1* illustrate that the early summer season caused a highly significant increase in total fruits yield (as weight and number of fruits) when compared with the winter season in 2000/01 and 2001/02. Similar results were observed inside unheated plastic houses [16], [17] and [5].

The increment of total fruits yield during the early summer season more than during the winter season might be due to that the microclimate and soil temperature were better in the early summer season than in the winter one which led to more vigorous growth and increase in number of female flowers. The effect of air and soil temperatures and light were demonstrated by some researchers [27], [11], [32], [28] and [23].

Effect of plastic houses type (size)

Data in *Table 2* demonstrate that application of the double span house had a significant increase in weight of total fruits yield when compared with the single span house. However, number of total fruits yield was not significantly affected by type of unheated plastic houses. The increase in weight of total fruits yield inside the double span house might be due to offering better microclimate (increase in minimum and average air temperatures and light intensity than the single span house). In the same line, Hara et al. (1987) [25] mentioned that large and small greenhouses had higher fruit yield than that in large and small tunnels.

Effect of hybrid

Data in *Table 3* illustrate that the differences were highly significant in both 2000/01 and 2001/02. Petostar hybrid had higher total fruits yield (as weight and number of fruits) than those of Primo hybrid. The superiority of Petostar hybrid in total fruits yield (as number and weight) might be due to increase in vegetative growth and number of female flowers. In this concern, El-Aidy and Moustafa (1977) [19] found that there is a high positive correlation (at the age of ten weeks) between stem length of seven cucumber C.V.S. and total yield.

Effect of season – plastic houses type – hybrid interaction

Data presented in *Table 4* show that the interaction among season, plastic houses type and hybrid had a significant effect on number of total fruits yield in 2000/01 only. Such effect was not significant on weight of total fruits yield in both 2000/01 and 2001/02 seasons. The highest number of total fruits yield was produced from the cucumber plants cv. Petostar hybrid, which was grown inside the double span house during the early summer season.

Effect of plastic house type and season on infection with fungal diseases

Downy mildew infection

Data in *Table 5* show that downy mildew infection (%) on cucumber plants was lower inside the unheated plastic house of the double span than inside the single span house at both sampling dates (60 days after transplanting) during the winter and the early summer seasons of 2000/01 and 2001/02. This result might be due to that the natural ventilation inside the double span house was better than that inside the single span.

On the other hand, downy mildew infection was lower during the winter season than during the early summer season. The favourable weather conditions for downy mildew in fields and greenhouse of cucumber were rainy weather after mid summer [33] or high relative humidity [22], [24] and warm nights [22]. Moreover, daily ventilation in plastic greenhouse by 3 hr. in the morning and at night, from the end of April to the first 10 days of May, reduced the dew duration on cucumber leaves. The use of natural ventilation for the whole night within the second 10 days and the last 10 days of May reduced downy mildew by 86 and 46%, respectively. Bilgrami and Dube (1976) [4] reported that a relative humidity of 90-100% and the temperature range of 20-25 is most conductive. Furthermore, Dixon (1981) [10] noted that the optimal temperature required is 15^{o}C with 6 h moisture on the leaf.

Powdery mildew infection

Data in *Table 5* indicate that powdery mildew infection (%) on cucumber plants was higher inside the single span house than inside the double span house during the winter and the early summer seasons of 2000/01 and 2001/02. In addition, powdery mildew infection was lower during the winter season than during the early summer season. In this concern, Bilgrami and Dube (1976) [4] reviewed that higher incidence of powdery mildew was during May-June than in winter months as this is due to the effect of temperature rather than relative humidity. Moreover, the same authors noted that powdery mildew is more prevalent in dry area (moisture stress is most important) and dry months of the year, because of the high water content of the conidia which enables them to withstand high moisture stress. Furthermore, Dixon (1981) [10] reported that the pathogen is favoured by dry atmospheric and soil conditions, moderate temperatures, reduced light intensity, fertile soil and succulent plant growth. Optimal conidial germination is at 28^{o}C with a range of 22-31; conidia can germinate in the absence of free water and in RHs as low as 23 percent.

Economic evaluation

Data of economic evaluation in terms of total cost/m^2, crop value and net return as a result of the combined interaction of growing seasons, types of plastic houses and hybrids are shown in Table 6 in both 2000/01 and 2001/02 seasons. The results illustrate that there were slight differences in the total cost/m^2 of interaction treatments which ranged from 3.46 to 3.66 L.E.

As regards to crop value, the highest record was obtained from Petostar F_1 hybrid grown inside the double span house during the early summer season, followed by Petostar hybrid grown inside the single span house during the same season when compared with the treatment of Primo hybrid grown inside the single span house during the winter season which had the lowest crop value. With respect to net return (net income)/m^2, the highest value was obtained from Petostar hybrid grown inside the double span house during the early summer season compared with the other interactions, especially Primo hybrid grown inside the single span house during the winter season which had the lowest net return. Likewise, the highest net return during all year round (as two cropping seasons per year, i.e., total net return of the winter and the early summer seasons was obtained from Petostar hybrid grown inside the double span house, followed by Petostar hybrid grown inside the single span house.

Table 5. *Effect of season-plastic houses type interaction on downy mildew and powdery mildew infection and life period of cucumber plants during the 2000/01 and 2001/02 seasons.*

Traits		Infection with fungal diseases (%)						Period of cucumber plant life			
Treatments		Downy mildew			Powdery mildew			No. of days from			
Seasons	Houses type	30	60	Mean	30	60 days*	Mean	Seeds sowing till transplanting	Transplanting till 1st picking (earliness)	1st picking till end of harvest	Seeds sowing until end of harvest
2000/01 seasons											
Winter Season	Single	0.9	9.7	5.30	3.1	4.5	3.80	13	31	67	111
	Double	0.4	6.2	3.30	0.0	0.5	0.25	13	31	67	111
Mean		0.65	7.95	4.30	1.55	2.5	2.03				
Early summer Season	Single	0.3	22.0	11.20	2.2	20.8	11.50	23	44	82	149
	Double	0.0	4.0	2.00	0.3	18.2	9.25	23	44	82	149
Mean		0.15	13.0	6.60	1.25	19.50	10.38				
2001/02 seasons											
Winter season	Single	1.2	10.2	5.7	0.0	23.3	11.65	13	31	67	111
	Double	0.4	6.1	3.25	0.0	20.4	10.20	13	31	67	111
Mean		0.80	8.15	4.48	0.0	21.85	10.93				
Early summer season	Single	2.2	21.5	11.85	0.0	46.2	23.10	23	44	82	149
	Double	0.8	3.4	2.10	0.0	23.6	11.80	23	44	82	149
Mean		1.50	12.45	6.98	0.0	34.90	17.45				

*Number of days from transplanting
Optimal conidial germination is at 28°C with a range of 22-31; conidia can germinate in the absence of free water and in RHs as low as 23 percent.

Table 6. *Economic evaluation of different growing season and types of plastic houses for the two studied hybrids (L.E./m²) in 2000/01 and 2001/02 seasons.*

Treatments			Total cost L.E / m²	2000/01 seasons		2001/02 seasons	
Growing seasons	Types of plastic houses	Hybrids		Crop value L.E./ m2	Net return L.E. / m²	Crop value L.E./ m²	Net return L.E./ m²
Winter season	Single span (467.5 m²)	Primo Petostar	3.50 3.50	4.68 5.81	1.18 2.31	4.35 5.32	0.85 1.82
	Double span (935 m²)	Primo Petostar	3.66 3.66	6.32 8.24	2.66 4.58	4.85 6.44	1.19 2.78
Early summer season	Single span	Primo Petostar	3.46 3.46	9.02 10.48	5.56 7.02	8.17 8.94	4.71 5.48
	Double span	Primo Petostar	3.64 3.64	9.16 11.25	5.52 7.61	8.21 10.19	4.57 6.55

Average price of one kg cucumber during the winter and early summer seasons was 1.25 and 1.0 L.E.
* 1 US$ = 5.7 L.E.

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PITS CONSERVE SPECIES DIVERSITY IN AN OVERGRAZED GRASSLAND

S. TSUYUZAKI[1] – H. TAKAHASHI[2]

[1]*Graduate School of Environmental Earth Science, Hokkaido University Sapporo 060 0810 Japan;*
[2]*Kansai Research Center, Forestry and Forest Products Research Institute Kyoto 612-0855 Japan*

e-mail: tsuyu@ees.hokudai.ac.jp

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Abstract. Decrease in species diversity by overgrazing is one of the crucial indicators for ecosystem deterioration. Sika deer (*Cervus nippon* Tamminck) has grazed various plants on Nakajima Island, Hokkaido, Japan, for a few decades, due to low food availability. We examined if diverse microtopography supported high species diversity by conserving specific plants on the grassland of the island where overgrazing by deer occurred. Based on TWINSPAN analysis, three plant community types were classified: grasslands represented by short seed plants, ferns, and unpalatable forbs. Grasslands dominated by short and/or unpalatable seed plants established on the flat ground, while fern species except *Equisetum arvense* did not establish there. Soil hardiness was higher on short-plant grasslands than on unpalatable-forb grasslands, suggesting that palatability on plants was related to plant community differentiation on the flat ground. Of 10 fern species recorded, 9 species established mostly in deep pits. Pit depth was more important than pit area to maintain high fern diversity. Those results indicated that diverse microtopography, i.e., pit development, supports fern diversity, because of the multiplier effects of predator avoidance and preferable sites for fern establishment. Diverse and/or specific microtopography must be a prerequisite to conserve rare species and high diversity in such disturbed areas.
Keywords: *fern diversity, grassland, microtopography, overgrazing by deer, palatability, pit depth*

Introduction

Grazing by mammals, including deer, greatly affects plant species composition in relation to species attributes, such as palatability and morphological traits, and *vice versa* [8, 23]. Particularly, deer are over-populated on the world-natural heritages assigned by UNESCO in Japan, and modify the ecosystems [30]. Overgrazing by deer has been conspicuous for a few decades in a grassland on Nakajima (or Nakanoshima) Island, Hokkaido, northern Japan, because grassland often shows higher productivity than forest under story [15]. Palatable plants decline greatly in the grassland on Nakajima Island after the explosion of deer density, and then deer tend to eat whatever food is available when food availability is low, e.g., in winter [24]. Palatability is related to taxa and/or life-forms, for example, livestock-grazed sites have a higher proportion of prostrate species, early flowering, cryptophytes (plant whose growth buds survive seasons with adverse conditions below ground), etc. than ungrazed sites in grasslands, Central Spain [17, 20]. Totally, moose has negative effects on vascular plants but has few effects on mosses [5].

Diverse microtopography supports high species diversity and/or rare species in disturbed ecosystems by changing micro-environments [21, 28, 29]. Microsites may provide safe sites for plant establishment by escaping from grazing pressures. We suspect that plants can escape from grazing by two ways: unpalatable habits and the utilization of microtopography. Furthermore, when plants can escape from grazing by using microtopography, the characteristics of microtopography, such as shape and size, influence grazing pressures [1]. We evaluate: (1) Palatability is related to plant community differentiation, in particular, unpalatable plants still remained on overgrazed areas. (2) Specific species establish in distinct microhabitats by avoiding grazing. (3) What microhabitat characteristics regulate species diversity? To demonstrate those, we examined how the effects of deer overgrazing modified plant community structure with reference to plant taxa, i.e., seed plants vs ferns, and microtopography, i.e., flat vs pit.

Study area and methods

Nakajima Island (497 ha, summit = 455 m a.l.s.) is enclosed by a caldera lake, Lake Toya. Volcanic upheaval formed this island 40,000-50,000 BP. On this island, three major vegetation types are recognized: deciduous forest represented by *Acer mono* and *Tilia japonica*, needle-leaved plantation, and grassland [15]. The snow period is from December to April and the maximum snow depth is ca 50 cm on February in usual years.

On the island, a male adult sika deer (*Cervus nippon* Tamminck) was introduced in 1957, an 1-year-old female was introduced in 1958, and a pregnant was introduced in 1965. All deer on this island in the present is considered to be originated from the three individuals. The population became over-populated, i.e., 31.5/km^2, in 1980, and then palatable plants, such as *Angelica ursina*, *Polygonum sachalinense*, *Cacalia hastata* var. *orientalis* and *Miscanthus sinensis*, were fed and their abundance declined [11], while unpalatable plants, e.g., *Pachysandra terminalis*, *Cynanchum caudatum*, and *Senecio cannabifolius*, became dominant in areas where palatable plants decreased in cover. Deer density was 57.5/km^2 in maximum recorded in 1983, and then crashed in the spring of 1984 due to starvation, and then sika deer began feeding on fallen leaves and unpalatable plants [24].

The vegetation surveys were conducted in the late June of 1996 on the grassland where depressions had been established. When the survey was conducted, the whole ground surface on the grassland was completely modified by deer grazing and thus we could not establish ungrazed plots. Of the depressions, pits that had perpendicular side-wall were developed conspicuously *(Figure 1).* These pits seemed to be developed by ancient volcanic activities, i.e., depression holes and fumaroles. A few conical depressions were also observed. To investigate the topographical effects on plant community patterns, we measured long/short axes and depth of depressions. Surface area is calculated as oval shape. Since the surface area of depressions averaged 0.78 m^2, 27 1 m × 1m quadrates were additionally set up on the flat ground, i.e., elevation difference was zero. Hereafter, we call depressions and quadrates ‘plot’ for the convenient sake. Cover on each taxa was recorded in each plot. Nomenclature follows [18] for seed plants, [16] for ferns, and [7] for mosses. Litter thickness was measured by a ruler, and the soil hardiness was measured by a hardiness tester (Type A-0858, Yamaoka System, Tokyo) on each plot. On the measurement of litter thickness and soil hardiness, five points were measured and the averaged value was used for each plot. On four pits selected, soil temperature, soil moisture and photon flux density (PFD) were measured at 0, 10, 20 and 40 cm deep in the summer of 2005. The ranges of pit-section diameter and depth were, respectively, from 22 cm to 130 cm and from 40 cm to 110 cm. Air temperature and PFD at 20 cm above the ground surface were also measured. In each depth, the measurements were conducted three times. Soil temperature was measured by inserting the sensor of a portable thermometer (Digimult Model D611, Takara Thermsitor Instruments Co., Ltd, Yokohama) into soil. Soil moisture was measured by time-domain reflectance (TDR), using a portable water content meter (Hydrosense, Campbell Scientific, Inc., Logan, Utah). The unit of water content is volumetric (%). Photon flux density (PFD) was measured by two sensors (Quantum LA-190SA, LI-COR Inc., Lincoln, Nebraska). One sensor was used for control and another is for the reference. For the reference, PFD was measured adjacent to the side-wall of pit. Then, PFD was expressed as relative PFD by (PDF at the target)/(PFD at control) × 100. The relationship between relative PFD and distance from the ground surface was examined by a logarithmic regression [31].

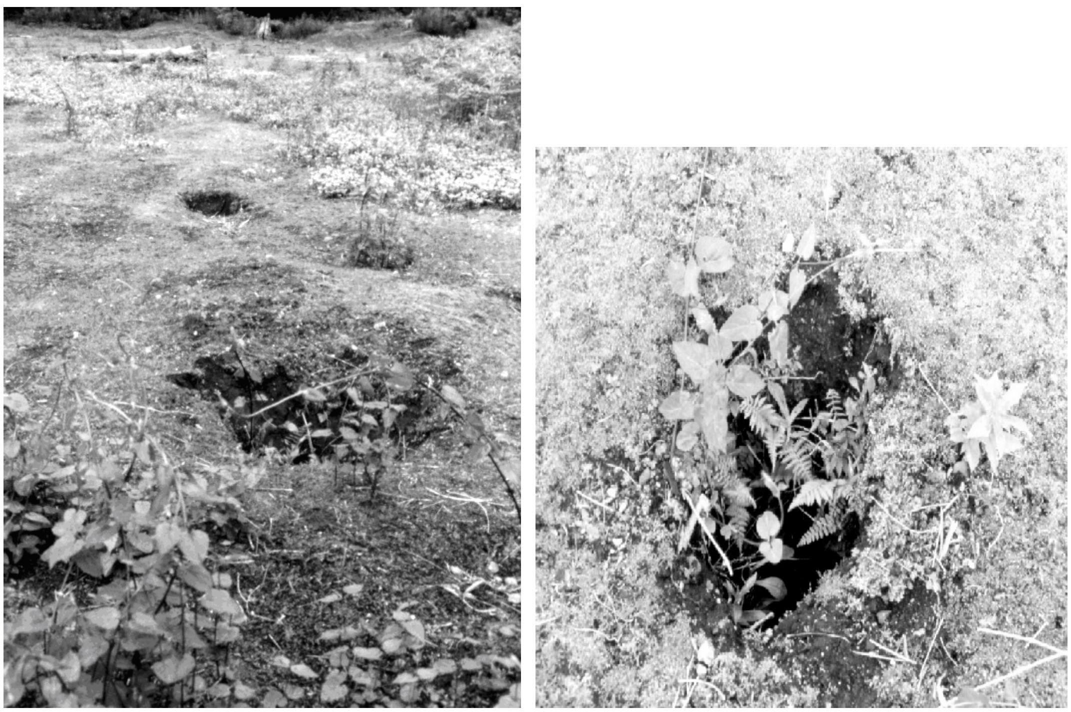

Figure 1. *Vegetation patterns in the surveyed area.*
A: Three surveyed pits are shown by enclosed circles. B: Close-up of a pit. The arrow indicates the establishment of fern.

Three taxa were used in the present study: moss, fern, and seed plants. Plant community types were extracted by TWINSPAN cluster analysis [9]. Detrended correspondence analysis (DCA) was performed to inspect the establishment patterns of species and plots [10]. On TWINSPAN and DCA, species that were recorded in two or more plots were used after square-root transformation. Species richness may not be appropriate for the comparison of community diversity, because plot size varied. Therefore, not only species richness but also Shannon-Wiener's species diversity (*H*') was calculated on each plot based on cover values [31]. *H*' is more robust against sample size effects than species richness [13]. The multiple comparisons were conducted by Scheffe's tests when ANOVA had the significant difference at $P < 0.05$.

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To confirm the significant characteristics of pits on fern diversity, zero-inflated Poisson regression was applied by using number of fern species as dependent variable and depth and area as independent variables [14]. On the regression, 1 m × 1 m quadrates established on the flat were not used, because the area did not represent the area of concave part. We examined three models, fern richness explained by pit depth, by area, and by depth and area as follows.

$$P(Y = 0|x, z) = 1 - p(x) + p(x)\exp(-\lambda(z)), \quad \text{(Eq. 1)}$$

$$P(Y = r|x, z) = p(x)\frac{\exp(-\lambda(z))\lambda(z)^r}{r!}, \text{ where } r > 0 \text{ (integer)}. \quad \text{(Eq. 2)}$$

Here $p(x)$ is the probability of observing at least one fern species in a plot, $\lambda(z)$ is the truncated Poisson distribution of the number of observed fern species. Based on Akaike's Information Criteria, we selected the best model [22].

Results

Plant community patterns

In total, 47 seed plants, 10 ferns and 4 mosses were recorded. The most frequent seed plant taxa were *Sagina japonica* and *Cynachum caudatum*, both of which frequencies were > 80% *(Table 1)*. Based on TWINSPAN cluster analysis, three plant community types (hereafter, groups A, B and C) are recognized *(Figure 2)*. Groups A and B are separated from group C by the dominance of a moss, *Polytrichum juniperium*, and by low cover and frequency of *Senecio cannabifolius* and *Pachysandra terminalis* (Table 1). *S. cannabifolius* is a tall forb, and *P. terminalis* is a stoloniferous shrub-like forb. Both of them are unpalatable for deer. Group A was separated from group B by the dominance of *Sagina japonica*. Group B did not have any representative species for community type, but if pressed *S. japonica* and *Cynanchum caudatum* established most frequently with low cover. Furthermore, group B and was characterized by the establishment of ferns, represented by *Athyrium vidalii*, *Matteuccia struthiopteris*, and *Dryopteris austriaca*. The other ferns were *Equisetum arvense*, *Aachnioides standihii*, *Dennstaedtia wilfordii*, *Lunathyrium pycnosorum*, *Adiantum pedatum*, *Cyrtomium fortunei* var. *clivicola*, and *Matteuccia orientalis*. Four moss species were recorded from 90% of the plots examined. The common mosses were *Ceratodon purpureus*, *Polytrichum juniperium*, *Myuroclada maximoviczii*, and *Marchantia polymorpha*. Although a few other minor mosses were also established infrequently with low cover, the identification of those minor moss species was difficult in the filed. Therefore, the minor moss species were recorded together with any common moss species.

Total plant cover per plot averaged 35% for all the plots *(Table 1),* but differed greatly between the three community types. Group C showed the highest total cover, i.e., 65%, but group B showed only 5%. Ferns established less in groups A and C, while they established frequently in group B with < 1% in averaged cover. Averaged moss cover was < 10% in any plant community groups, although mosses established in 90% of plots.

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Table 1. *The leading species of seed plants and ferns based on TWINSPAN cluster group. Mean coverage is shown with percentage frequency in parentheses. +: less than 1%. -: no individuals observed. Mean plot depth, litter thickness and soil hardiness are shown with standard error. The same letters indicate non-significantly different at* $P < 0.01$ *(Scheffe's test). Pseudospecies grouped by TWINSPAN are boldface.*

Cluster group code	A	B	C	Total
Number of plots	27	36	31	94
Depth (cm)	11 ± 12 [a]	80 ± 7 [b]	11 ± 3 [a]	37 ± 4
Litter thickness (cm)	2.5 ± 0.4 [a]	5.0 ± 0.7 [a]	3.1 ± 0.4 [a]	2.8 ± 0.3
Soil hardiness (kg/cm^2)	3.4 ± 0.3 [a]	2.3 ± 0.2 [b]	1.8 ± 0.3 [b]	2.5.± 0.3
Seed plant				
Sagina japonica	**23.5 (100)**	0.1 (72)	3.3 (87)	7.9 (85)
Cynanchum caudatum	4.3 (78)	0.2 (72)	5.3 (94)	3.2 (81)
*Veronica arvensis**	0.1 (67)	+ (67)	0.2 (90)	0.1 (74)
Oxalis corniculata	0.7 (81)	0.1 (61)	0.9 (71)	0.5 (70)
*Taraxacum officinale**	1.3 (85)	+ (39)	1.7 (77)	1.0 (65)
Poa annua	1.8 (93)	0.1 (56)	0.8 (56)	0.8 (66)
*Erigeron annuus**	0.1 (56)	0.1 (61)	0.1 (29)	0.1 (49)
Pachysandra terminalis	-	0.7 (11)	**29.0 (74)**	9.8 (29)
Senecio cannabifolius	-	0.1 (11)	**17.8 (58)**	5.9 (21)
Fern				
Athyrium vidaliii	+ (4)	0.1 (39)	-	+ (16)
Matteuccia struthiopteris	-	0.2 (33)	-	+ (13)
Dryopteris austriaca	-	0.1 (33)	-	+ (13)
Total cover				
Seed plant	34.1 (100) [a]	2.3 (100) [b]	61.2 (100) [c]	30.9 (100)
Fern	+ (7) [a]	0.6 (61) [b]	+ (3) [a]	0.2 (27)
Moss	7.3 (96) [a]	1.9 (92) [a]	3.9 (84) [a]	4.1 (90)
All	41.4 (100) [a]	4.8 (100) [b]	65.1 (100) [c]	35.2 (100)

* naturalized plants.

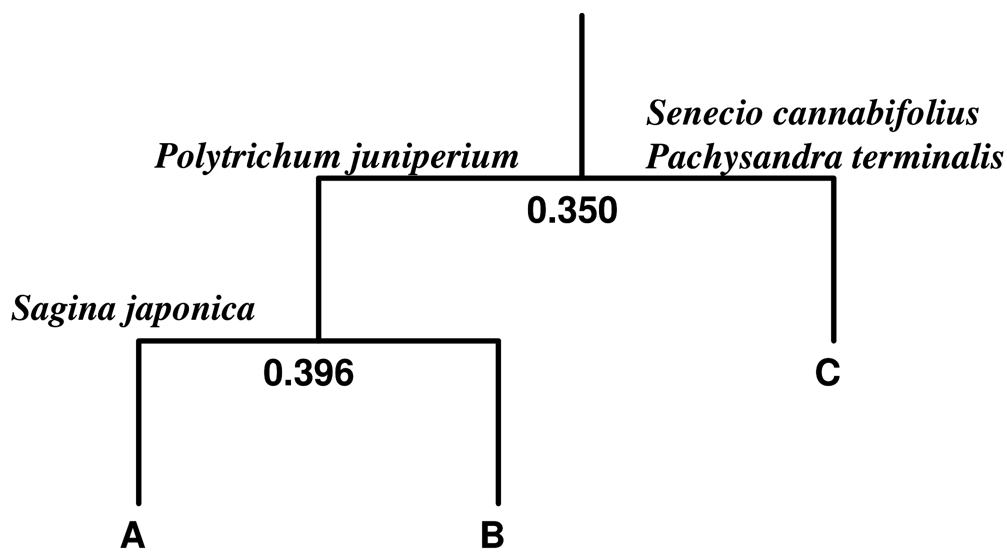

Figure 2. *Plant communities determined by TWINSPAN. Eigenvalues and indicator species for each division are shown.*

Biodiversity

Species richness (total number of species per plot) did not differ significantly among the three groups, and species diversity was also not different *(Table 2)*. Seed plant richness and diversity were not different among the groups, as well as total species richness and diversity, because major species composition was from seed plants. In contrast, fern species richness and diversity were significantly higher in group B than in groups A and C.

Because one or less fern species established in depressions less than 50 cm deep, fern diversity was zero there *(Table 2)*. Therefore, fern species richness and diversity were clearly higher in pits with > 50 m deep *(Figure 3)*. Zero-inflated Poisson regression detected that the model only using pit depth was best to explain fern species richness, indicating that pit depth is more important than pit area to conserve fern diversity. The equations, $p(x)$ and $\lambda(z)$ are:

$$\log(\lambda(z)) = a + b\cdot(\text{depth}) = 4.665 - 0.101\cdot(\text{depth}) \quad \text{(Eq. 3)}$$

$$\text{logit}(p(x)) = \log(p(x)/(1 - p(x))) = c + d\cdot(\text{depth}) = 0.341 + 0.004\cdot(\text{depth}) \quad \text{(Eq. 4)}$$

Here, a, b, c and d are respective coefficients. The coefficients a and b were not significant and c and d were significant at $P < 0.05$. Those implied that the depth is positively related to presence/absence of ferns, although fern species richness is not explained by Poisson distribution.

Table 2. *Comparison of species richness and diversity between cluster groups determined by TWINSPAN in relation to total species, seed plant, and fern. Each value shows mean with standard error. The same letters indicate non-significantly different at P < 0.01 (Scheffe's test).*

Cluster group code	A	B	C	Total
All				
Species richness	8.6 ± 0.3 [a]	10.3 ± 0.7 [a]	9.4 ± 0.4 [a]	9.5 ± 1.0
Species diversity	1.14 ± 0.09 [a]	1.41 ± 0.12 [a]	1.08 ± 0.08 [a]	1.22 ± 0.13
Seed plant				
Species richness	7.1 ± 0.2 [a]	6.8 ± 0.5 [a]	8.2 ± 0.3 [a]	7.3 ± 0.8
Species diversity	0.98 ± 0.09 [a]	1.23 ± 0.09 [a]	0.91 ± 0.07 [a]	1.05 ± 0.11
Fern				
Species richness	0.1 ± 0.1 [a]	1.7 ± 0.3 [b]	0.0 ± 0.0 [a]	0.7 ± 0.1
Species diversity	0.00 ± 0.00 [a]	0.39 ± 0.09 [b]	0.00 ± 0.00 [a]	0.15 ± 0.02

Environmental characteristics and plant communities

Soil moisture was highest 40 cm below the ground surface of pits, i.e., 33% ± 2 (s.e.) at 0 cm deep, 34% ± 4 at 10 cm, 32% ± 3 at 20 cm, and 42% ± 2 at 40 cm. Air temperature ranged between 18.7°C and 22.2°C, and averaged 20.2°C ± 0.6. Soil temperature at 0 cm in depth was slightly higher than air temperature, i.e., 20.9°C ± 0.4, probably because of direct solar radiation. Then, soil temperature decreased to 19.7°C ± 0.5 at 10 cm in depth, and 19.2°C ± 0.5 at 20 cm. At 40 cm in depth, soil temperature was lowest, i.e., 17.6 ± 0.6. RPFD was 69% ± 7 at 20 cm above the ground surface, and was 7% ± 5 on the deepest locations, i.e., 40 cm in depth. As expected, RPFD

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decreased logarithmically with increasing pit depth: RPFD = 43·log(pit depth) – 164 (r^2 = 0.974, significant at $P < 0.01$). In summary, pits of which depth was more than 40 cm support high soil moisture and low temperature, and avoided direct solar radiation.

Group B established significantly deeper plots than groups A and C *(Table 1)*, while the depths of plots were not different between groups A and C *(Table 1).* Groups A and B established on shallower sites, i.e., elevation difference in plot less than 50 cm, and the depths of plots in group C ranged from 20 cm to 205 cm *(Figure 3).* The establishment sites for ferns were restricted in these pits, in particular, of which depth was more than 50 cm *(Figure 4).* A fern recorded from groups A and C was *Equisetum arvense*, a micorphyllous fern that established only on shallow pits even in group B, while macrophyllous ferns were restricted to establish in deep depressions, i.e., more than ca 50 cm in depth. Those implied that the establishment sits for ferns were restricted to pits, while groups A and C were not differentiated by topography.

Litter thickness did not differ between the three groups. Soil hardiness on group A differed from that on groups B and C. In particular, soil was the hardest in group A and was the softest in group C, suggesting that trampling and/or its related factors were related to the differentiation between groups A and C.

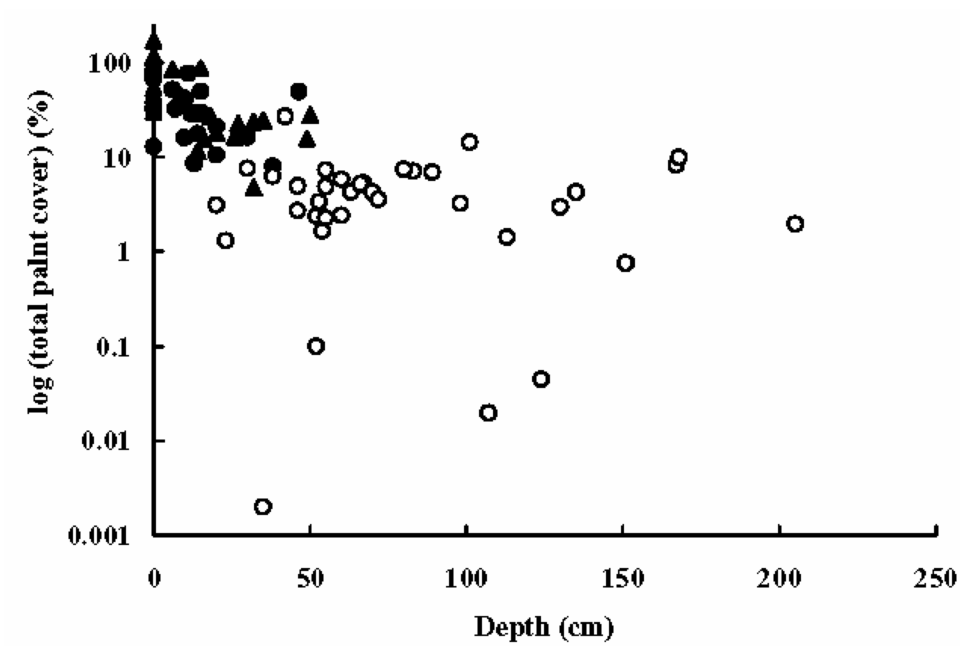

Figure 3. *Relationship between plot depth and total plant cover. Closed circles, open circles and closed triangles indicate TWINSPAN cluster groups, A, B and C, respectively.*

Detrended correspondence analysis

Detrended correspondence analysis (DCA) showed that ferns tended to aggregate the scores on the first two axes *(Figure 5),* i.e., the ferns showed the lowest scores on axes I and II. Four seed plants, *Hydrangea petiolaris*, *Aruncus dioicus* var. *tenuifolius*, *Galium spurium* var. *echinospermon* and *Gnaphalium uliginosum* were also clustered with ferns. Those implied that those seed plants as well as ferns restricted to establish in deep pits. In total, the scores of the three plant community groups were ordered as: C > A > B along axis I, and A > C > B along axis II. The species scores on the three unpalatable plants, i.e., *Cynnanchum caudatum*, *Senecio cannabifolius*, and *Pachysandra terminalis*, showed higher scores on axis I, indicating that group C was supported by unpalatable plants. Plots in group A showed higher scores on axis II. On the species scores of axis II, the highest scores on seed plants were obtained by *Zoysia japonica*, *Mazus japonicus*, and *Erigeron canadensis*. A moss, *Ceratodon purpureus*, also showed high score on axis II. *Z. japonica*, *M. japonicus* and *C. purpureus* are innately short. *E. canadensis* became short in the grazed area, suggesting that grazing and trampling modified plant community structures via the alteration of plant morphological traits. Those also suggested that plants in group B avoided grazing effects.

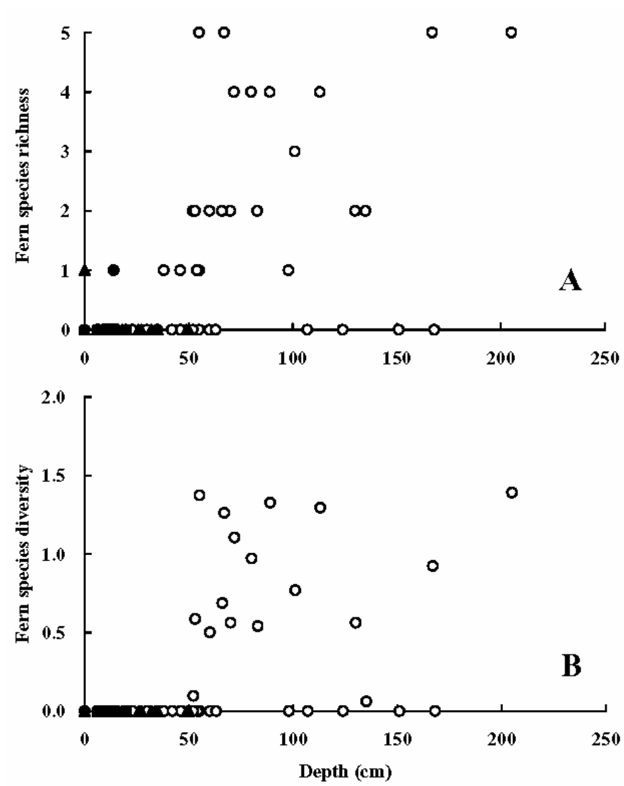

Figure 4. *Relationships between plot depth and fern species richness (A) and between plot depth and fern species diversity (B). Symbols, same as in Fig. 3.*

Discussion

Species composition in pits was characterized by the presence of ferns. In particular, pits with more than 50-cm deep support most ferns. The size of treefall pits does not affect species richness in a tropical rainforest, Puerto Rico, but affects the establishment of specific species [28]. In the present study, pit depth influences fern diversity more than pit area. Spatial heterogeneity, including microtopography and its related environmental factors such as light and soil moisture, is one of the determinants on plant community structures [1, 25]. Pit conserved fern species diversity on Nakajima Island, by the avoidance from deer feeding. Pothole develops more variable plant

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community than barren areas on Mount St. Helens, USA, although the plant community patterns are not well-documented by surface area of pothole and the properties of the volcanic deposits [6]. While, the pits on Nakajima Island support fern diversity, because the pit depth is related to light intensity and soil moisture and is important for the specific (macrophyllous) fern establishment. Those mutual advantages for ferns could support fern diversity in the pits.

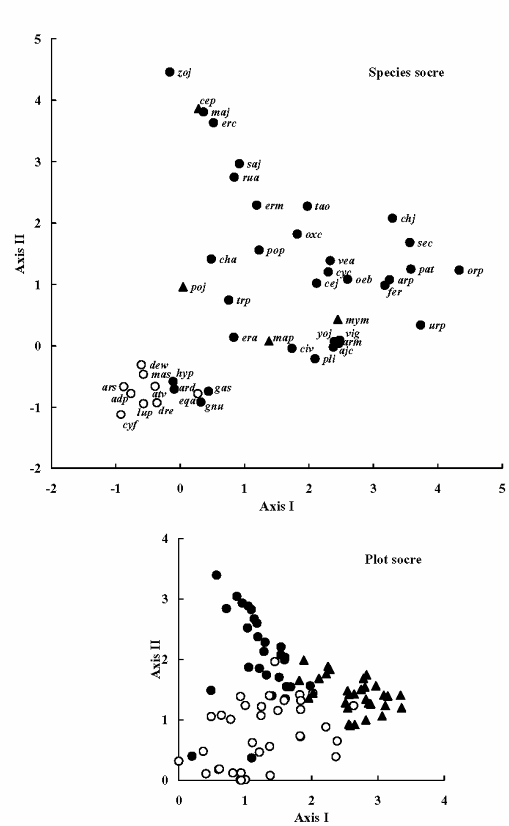

Figure 5. *Species (A) and plot (B) scores on detrended correspondence analysis. Species with frequencies more than 1 were used.*

A: Seed plants (closed circles); saj = Sagina japonica, vea = Veronica arvensis, oxc = Oxalis corniculata, cyc = Cynachum caudatum, erm = Eragrostis multicaulis, tao = Taraxacum officinale, era = Erigeron annuus, yuj = Youngia japonica, pat = Pachysandra terminalis, sec = Senecio cannabifolius, pop = Poa pratense, oeb = Oenothera biennis, hyp = Hydrangea petiolaris, rua = Rumex acetosella, arp = Arisaema peninsulae, vig = Viola grypoceras, gas = Galium spurium var. echinospermon, zoj = Zoysia japonica, pli = Plectranthus inflexus, trp = Trifolium pratense, civ = Cirsium vulgare, erc = Erigeron canadensis, cej = Cercidiphyllum japonicum, arm = Artemisia montana, orp = Oreorchis patens, maj = Mazus japonicus, chj = Cloranthus japonicus, gnu = Gnaphalium uliginosum, ajc = Ajuga ciliata var. villosior, urp = Urtica platyphylla, fer = Festuca rubra, cha = Chenopodium album, ard = Aruncus dioicus var. tenuifolius. Ferns (open circles); atv = Athyrium vidalii, mas = Matteuccia struthiopteris, dra = Dryopteris austriaca, eqa = Equisetum arvense, Ars = Arachniodes standichii, dew = Dennstaedtia wilfordii, lup = Lunathyrium pycnosorum, adp = Adianthum pedatum. Mosses (closed triangles); mym = Myuroclada maximovizicii, map = Marchantia polymorpha, poj = Polytrichum juniperinum, cep = Ceratodon purpureus. B: Closed circles, open circles and closed triangles show scores on TWINSPAN cluster groups A, B and C, respectively.

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Plant communities were differentiated by the presence/absence of palatable plants on the flat ground. A few plant species persist and dominate on heavily-grazed areas where palatable plant species decline [8]. On the flats, i.e., in groups A and C, *Pachysandra terminalis* and *Senecio cannabifolius*, both of which were unpalatable plants for deer [12, 24], were dominant in group C, while these two species were not common in group A. *Cynanchum caudatum* that was also unpalatable for deer was common in groups A and C. Soil hardiness was lower in group C than in group A, suggesting that differences in environmental factors were also related to the intensities of grazing and trampling. Those results indicated that the intensities of trampling and/or feeding were lower in group C where unpalatable plants were dominant. Therefore, plant communities differentiated with the gradient of grazing intensities. The grazing of red deer influence productive grasslands more than unproductive ones on the Isle of Rum in Scotland [27], and tall plants decrease richness and height even by weak deer browsing on a riparian deciduous forest in central Japan [17]. Livestock grazing increases prostrate life form and clonal reproduction in Dehesa grasslands, due to trampling (Peco et al. 2005), but increases short-lived plants in south-western Pyrennees [3]. The mortality of tussock grass declines by grazing [19]. Short plants were common on the flats, as well as mosses. Mosses were widespread in the grassland on Nakajima Island, because mosses are not greatly damaged by cervids (Crete et al. 2001). Plant taxa and/or life forms are related to the palatability of deer, i.e., the short morphological form, including mosses, and therefore feeding by deer affected differences in species composition between groups A and C.

Food habits of sika deer varies greatly between regions in the Japanese Archipelago [2], i.e., deer eat whatever they can eat when food resources are limited. For example, reindeer eat goose droppings when food resources become low [26]. Even though white-tailed deer is one of the most common herbivores, it behaves as a carnivore when food resources are deficient [4]. We emphasize that diverse microtopography supports higher landscape-level species richness and diversity by the establishment of various taxa, even though overgrazing eliminates various plants.

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ZONATION OF CONSERVATION PRIORITY SITES FOR EFFECTIVE MANAGEMENT OF TROPICAL FORESTS IN INDIA: A VALUE-BASED CONSERVATION APPROACH

S. SOOSAIRAJ[1*] – S. JOHN BRITTO[2] – B. BALAGURU[3] – N. NAGAMURUGAN[4] – D. NATARAJAN[2]

[1]*Department of Botany, St.Joseph's College (Autonomous), Tiruchirappalli, 620 002, Tamil Nadu, India*

[2]*Rapinat Herbarium, St.Joseph's College (Autonomous), Tiruchirappalli, 620 002*

[3]*Research Associate, National Innovation Foundation, Ahmedabad, India*

[4]*Department of Biotechnology, Kurinji College of Arts and Science, Tiruchirappalli, 620 002*

e-mail: pspsoosai@yahoo.co.in

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Abstract. Zonation of conservation sites has been modelled by overlaying different layers such as vegetation type, species richness, endangered, endemic, economic status maps and socio-cultural value map using remote sensing and GIS in the Pacchaimalai hills part of Eastern Ghats in India that is spread to an area of 527.61 sq. km. These hills are situated at the mid regions of Tamil Nadu with latitudes 11°09'00" to 11°27'00" N and longitudes 78°28'00" to 78°49'00" E. They harbour eight vegetation types of which tropical dry deciduous forests are widespread with rich diversity. As in other hills, the forest cover is under severe anthropogenic pressure in spite of being protected as a reserved forest. This study proposes about 0.93% (4.95 sq. km) of the total area of the Pacchaimalai hills for immediate conservation. The effectiveness of the proposal would be based on the attitude and awareness of local people towards vegetation who inhabit the areas adjacent to proposed sites and the effective monitoring by foresters and participatory approach of both the people and forest authorities to accomplish the goal of the conservation of biodiversity and sustainable use of natural resources.
Keywords: *conservation, remote sensing, geographical information system, species richness, Margalef index*

Introduction

Formal protection of natural resources in reserves has tended to be *ad hoc* favouring the biodiversity of areas that are least valuable for commercial use, in public tenure but earnest to reserve, most charismatic, and with least need for protection [19, 11] which is quite sentimental to protection strategy. Therefore methods for identifying priority areas are only one aspect of overall biological conservation planning and management [10, 4]. In Indian context ecosystems are rich in natural resources, but are under constant stress and exploitation [7]. Due to this heavy pressure, India has lost 7,422 km^2 forests during the intervening period between 1987 and 1999 assessment [18]. This fact reminds us that the rate of biodiversity loss is more in tropical countries like India especially in the regions of Eastern Ghats, a rugged hilly terrain running almost parallel to the eastern coast of India, characterized by the broken hills with its small patches of vegetation with manmade plantations and habitations. Stresses on this adjoining biota are increasing due to easy accessibility in the pertinent. Even after decades of superficial establishment of protected areas in these hills there is a negligible difference in deforestation rates inside and outside protected areas. This study focuses on a segment of Eastern Ghats *i.e.*

Pacchaimalai hills, which includes vegetation types such as Dry evergreen, Dry mixed deciduous, Savannah and grasslands. The hills are most significant socio-culturally because most of the forest patches are regarded as sacred groves (vestiges of habitation reserved for deity as in abode). These hills have been studied earlier mainly for the floristic analysis [12], besides this there is no significant developmental studies such as biodiversity and conservation. Though it is ideal to protect all places that contribute to biodiversity conservation, it is high cost and time consuming. Developing countries like India could not afford to spend on such a heavy task. Current protected areas contain relatively few biodiversity surrogates or those that are not in most urgent protection and there is very uneven representation of biodiversity in existing protected areas. Thus scoring and ranking procedures were developed as an attempt to make systematic priority setting effectively explicit [25, 26]. Thus the present study focuses on identification of conservation priority sites based on the scoring and ranking procedures. Our objectives include the estimation of the exhaustive knowledge of species diversity and distribution and the zonation of conservation values sites in the existing reserve areas and ranking the conservation priority sites.

Review of literature

There are many methods used for priority settings such as scoring and ranking procedures [11]. Two types of information are necessary for setting conservation priorities; the conservation value of an area and its vulnerability [13]. These pieces of information should include multiple criteria such as vegetation type [33], species richness [22], socio-economic and socio-cultural value sites [21], endemism [6], concentration of red listed plants [8, 1] and stresses on the biota [28]. Few vegetation types, though they are less important, should maintain the ecological balances, for the representativeness [23] and such vegetation types should also be included on the conservation priority sites. Each criterion is assigned with weight and then the scores are combined for each candidate area. The areas are then ranked accordingly and highest priority is given to the areas with the highest scores. Nevertheless there had been many critical reviews of these procedures and the criteria used [11].

Materials and methods

Study area

The Pacchaimalai hills are situated at the central region of Tamil Nadu, India, with latitudes 11° 09’ 00’’ to 11° 27’ 00’’ N and longitudes of 78° 28’ 00’’ to 78° 49’ 00’’ E *(Map 1).* They occupy an area of about 527.61 sq. km and altitudes range of 160 to 1072 m msl. The vegetated area is distributed into 35 reserved forests. The Pacchaimalai hills enjoy a sub-tropical climate with temperatures varying from 25°C to 31° C and annual rainfall ranging from 800 to 900 mm. The total tribal population is around 12,000 who live in 70 hamlets scattered all over the plateau area. The area is underlain by the crystalline rocks of the Archaean age comprising gneisses, charnockites and granites with little soil cover *i.e.,* red loamy and black. The alluvium is found in narrow patches along the river courses and is restricted in thickets. The crystalline terrain exhibits multi spectral and poly metamorphic complexity. Dominant plant species of these areas are *Nothopegia colebrookiana, Aglaia elaegnoidea,*

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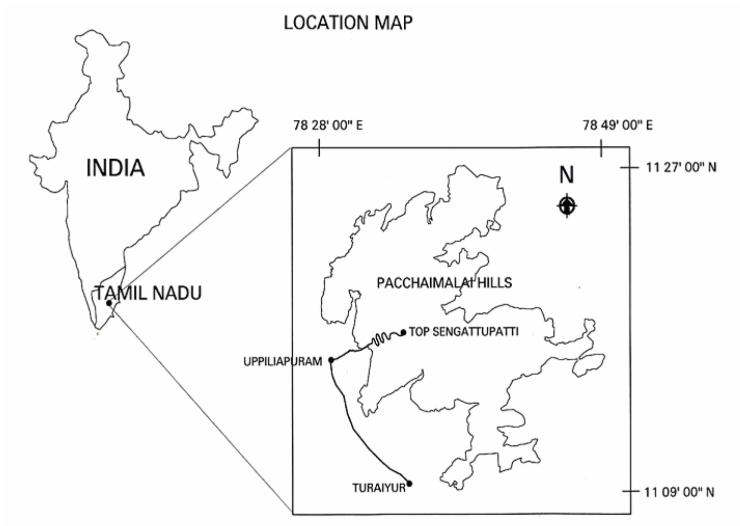
Cipadessa baccifera, Diospyros ferrea, Glycosmis mauritiana. Tarenna asiatica. Carissa carandas, Chloroxylon swietenia, Albizia amara, Commiphora caudata, Commiphora berryi, Pterolobium hexapetalum and *Celtis philippensis.* A few important species are *Santalum album, Cycas circinalis* and *Canarium strictum.* The Pacchaimalai harbour spotted deer, Hyenas, Sloth bear and Monkey like animals.

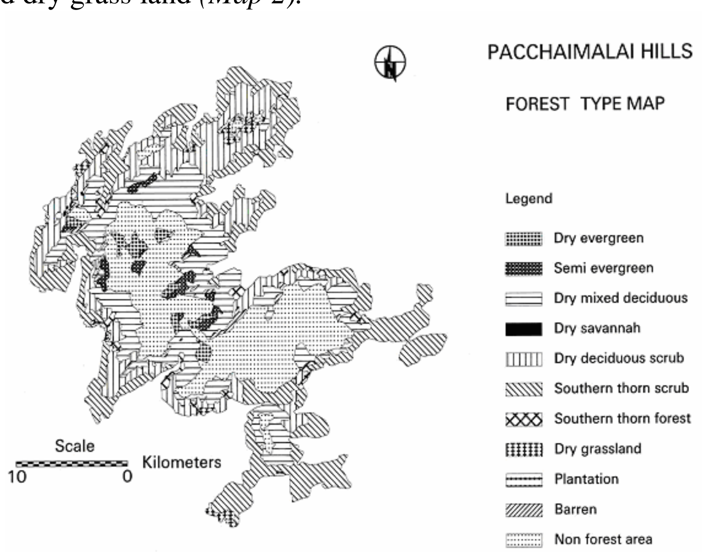


Map 1. *Location map of Pacchaimali. Eastern Ghats of Tamil Nadu, India*

Data generation

Mapping vegetation map

Vegetation type map (Forest type) of Pacchaimalai hills [27] cover 73.95% (390200 ha.) under reserved forests comprising about nine major forest types i.e., evergreen, semi evergreen, dry mixed deciduous, southern thorn scrub, dry savannah, southern thorn forest and dry grass land *(Map 2).*



Map 2. *Forest types of Pacchaimalai Hills*

The level of biological similarity of a particular site with other similar sites or with an ecosystem type in terms of the number and percentage of defining characteristics would determine its representativeness [23]. Since it is impossible to locate a site that represents an entire ecosystem, sites can be prioritized on the basis of levels of similarity. To evolve potential conservation strategy various elements are used as base for conservation priority, whereas the dry deciduous scrub and southern thorn scrub forests are excluded, as they are highly degraded forms of dry mixed deciduous forests.

Generation of species data base

This includes the preparation of floristic species richness, red list, endemism and economic value map. All the maps were prepared using the vegetation types with the ground data. For species richness map, sampling quadrates with the size 20 m x 20 m were laid in each vegetation type and frequency and number of all vascular plant species were collected in each quadrates and later utilized to estimate Margalef richness index [9] *(Eq. 1)*.

$$Dmg = S - 1 / \log N \qquad \text{(Eq. 1)}$$

Where S - is number of species recorded, N - total number of individual of all the species; In - $\log_e$. Each polygon of the respective vegetation type was replaced by Margalef diversity index and species richness values of all polygons were classified under three classes, *i.e.* low, medium, high.

Mapping of the Red listed and Endemic species *(Table 1)* is normally done based on the data collected from the above described quadrates and cross checked with the appropriate literature. Weights were attributed based on the summation of species under endemism and Red listed category [15] and again regrouped into three categories.

For preparation of economic value map, the economic values (i.e., medicinal (5), edible (2) and others (fuel -2, fodder-2 and timber-2) of each species that is encountered in each quadrate were summed and the summed values were attributed to the respective polygons as described above to reclassify similar valued polygons to form the final economic value map.

Table 1. *List of plant species and their status*

Name of species	Status	Value
Alstonia scholaris (L.) R.Br.	V	2
Andrographis lineata Wallich ex Nees	E	5
Aphanamixis polystachya (Wall.) Parker	V	4
Asystasia crispata Benth.	E	5
Balanophora fungosa Forster and Forster	E	5
Barleria acuminata Nees	E	5
Barleria longiflora L.f.	E	5
Bridelia crenulata Roxb.	E	5
Canarium strictum Roxb.	V	4
Canavalia cathartica Thouars	R	1
Cayratia pedata (Lour.) A. J. Juss. ex Gagnepain	En	5
Celastrus paniculatus Willd.	V	4
Ceropegia juncea Roxb.	E	5
Cinnamomum macrocarpum auct. non Hook.f.	R	1
Crotalaria medicaginea Lam.	R	1
Curcuma neilgherrensis Wight	E	5
Cycas circinalis L.	T	2

Name of species	Status	Value
Elaeagnus indica Servettaz	E	5
Embelia tseriam cottam (Roemer & Schult.) A. DC.	V	4
Gloriosa superba L.	En	5
Gnetum edule (Willd.) Blume	En	5
Gymnema elegans Wight & Arn. ex Wight	R	1
Habenaria heyneana Lindley	En	5
Hemidesmus indicus (L.) R.Br.	E	5
Mallotus stenanthus Muell. Arg.	E	5
Miliusa eriocarpa Dunn	E	5
Moringa concanensis Nimmo ex Dalz. & Gibson	V	4
Mucuna pruriens (L.) DC.	E	5
Naravelia zeylanica (L.) DC.	V	4
Ochna obtusata DC.	R	1
Peperomia dindigulensis Miq.	E	5
Pseudarthria viscida (L.) Wight & Arn.	L	3
Pterospermum xylocarpum (Gaertmer) Santapau & Wagh	T	2
Randia candolleana Wight & Arn.	E	5
Rubia cordifolia L.	En	5
Santalum album L.	T	2
Sapindus emarginata Vahl	L	3
Smilax zeylanica L.	T	2
Stephania japonica (Thunb.) Miers	V	4
Terminalia arjuna (DC.) Wight & Arn.	L	3
Tetrastigma sulcatum (M. Lawson) Gamble	E	5

V-Vulnerable, E- Endemic to Peninsular India, R-Rare, En- Endanger, T-Threatened, L-Lower risk

Deforestation risk zone

The anthropogenic pressures are found to be high near the hamlets and road network (metalled, unmetalled and foot path) and it might create secondary and tertiary routes to and within the forest area thereby increasing accessibility and vulnerability of the forest stand in the proximity. One km buffer for the road network and hamlet is generated to form the biotic pressure map. In this study threat posing as risk from human activities too was included as one of the thematic layers for identifying conservation priority zone.

Socio-cultural valuable sites

Sacred groves are patches of forests dedicated to deities, which are not usually disturbed. There is a strong need to promote such indigenous practice based packages for further rejuvenation and revitalization and as means of biodiversity conservation. Such socio-culturally protected areas would have been more imperative if one could value biodiversity in terms of economic value, utility, culture and religion. Due to modern civilization timber extraction was encountered in these sacred groves. However the wood extraction is strictly prohibited in the vicinity of the deity. This map has been prepared in terms of distribution of sacred groves in the Pacchaimalai hills. Distribution of sacred groves in the study area is mapped using GPS reading collected from the field, satellite imagery and SOI toposheets.

GIS overlay analysis

The components of various units from the themes of vegetation type, floristic richness, endemism and red lists with respective scores *(Figure 1)* are quite essential to develop conservation priority zones.

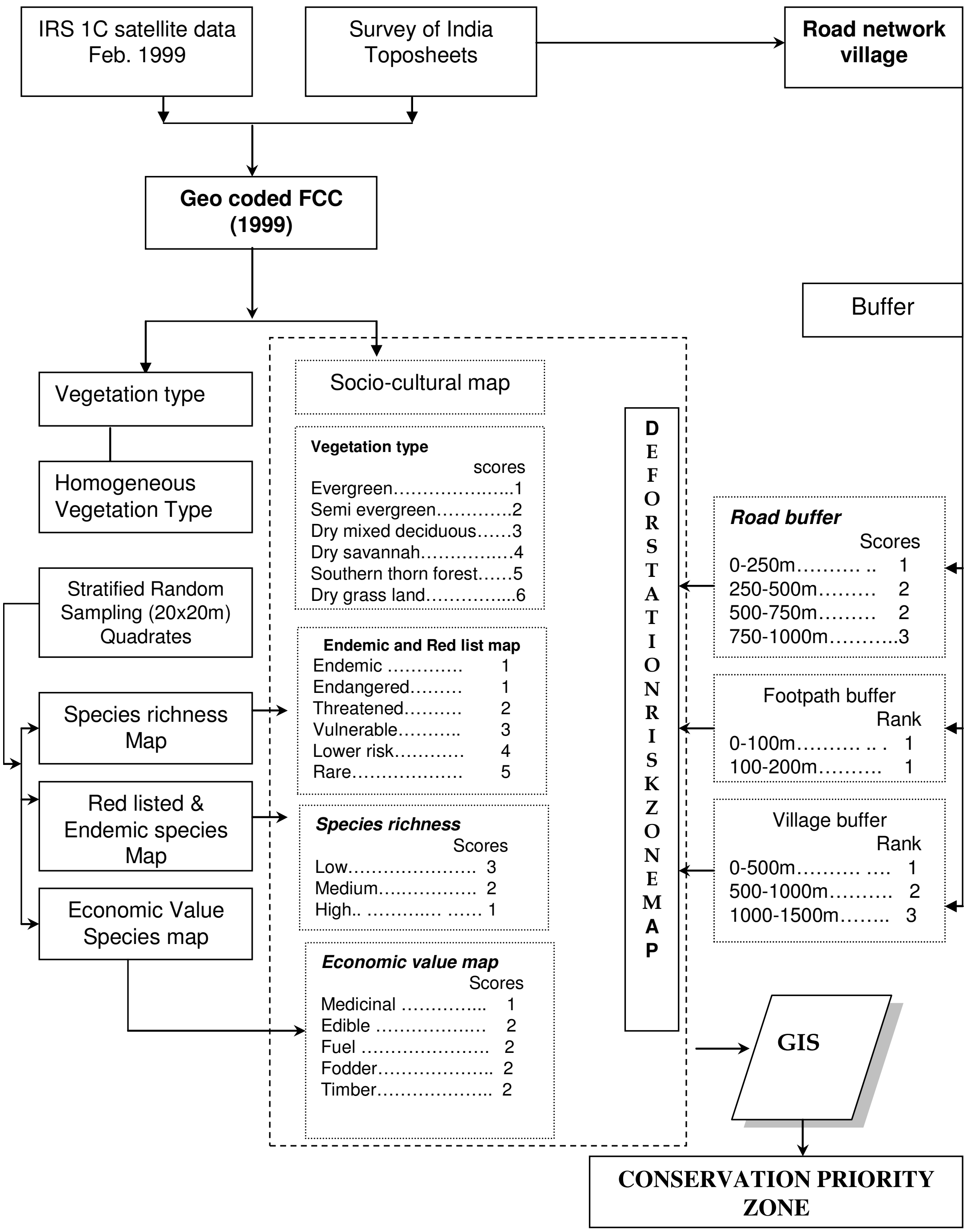
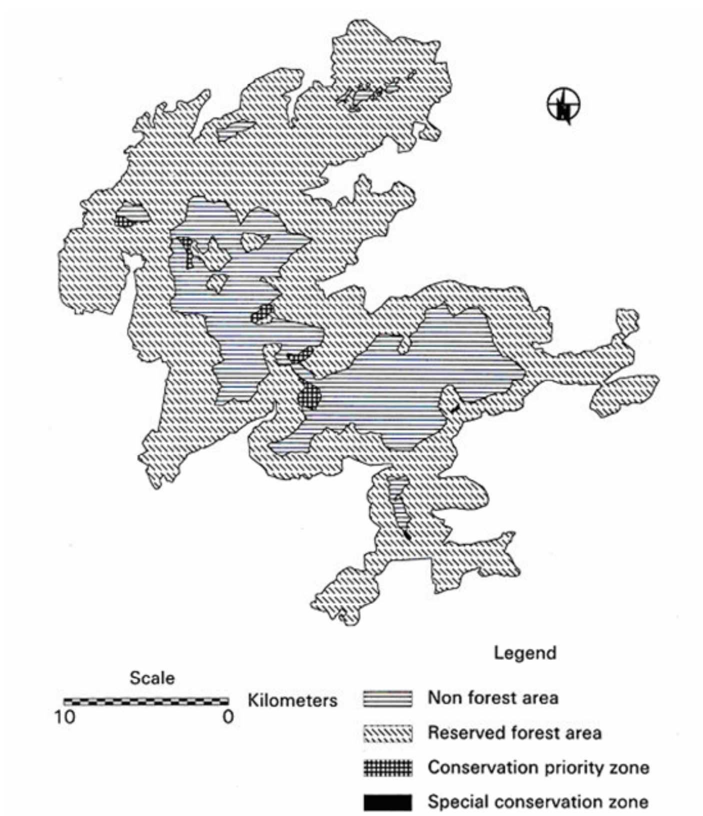


Figure 1. *Conceptual diagram illustrating the building of Conservation Priority Zone*

Considering conservational importance and status for each unit of thematic layer, the weighting was designated to identify such Priority areas. Overlay or superimposition creates a composite output GIS file by combining a number of input GIS files based on the minimum or maximum values of the input files are summed [14]. These scores are then combined for each candidate area. The areas are then ranked and highest priority/rank is given to the areas with the highest scores. For preparation of conservation priority maps, thematic maps are overlaid on one another wherein the vegetation type forms the lowermost tier and other layers are superimposed in sequence i.e. species richness, red list and finally endemism map. The thematic maps are rasterized and coded using the Criteria function in ERDAS 8.3.1 and the product was used as bases for modelling in the software using yet another tool i.e., model maker wherein, the different features of the thematic layers were intersected/ extracted and new class values were attributed to them and finally integrated (union) according to above mentioned sequence and finally conservation priority zone (*Map 3*) was prepared. The resulting polygons were classed according to the priority status. The authenticity of the areas/zones proposed for conservation priority is confirmed with ground justification. The endemic, red listed and biotic pressure zone maps were stored as temporary raster layers and used during GIS overlay analysis.



Map 3. *Conservation Priority zone map of Pacchaimalai Hills*

Results

The priority area that has been modelled comprises 4.95 sq. km, about 0.93% of the total hill area and is at five different localities. The site I has about 172.62 ha, and conspicuous for its rich diversity, and comprises a large number of endemic, endangered and vulnerable species. The slope is gentle to moderate. Huge woody liane like *Gnetum edule, Aganosma cymosum, Symphorema involucratum* and others inhabit the site. Tall trees growing up to 25 - 30 m are common, namely *Syzygium cumini. Alstonia scholaris, Canarium strictum, Nothopegia colebrookiana* and occasional presence of *Cinnamomum macrocarpum, Aglaia eleagnoidea* and *Atrocarpus heterophylus* are noteworthy. The villagers violating the ethos of sacred grove have noticed occasional grazing of cattle along the periphery, besides a few instances of encroachment. Proper fencing would surely help in conserving the rich natural resources.

Site II occupies an area of about 78.95 ha. and consists of trees growing up to 20 m. Dominant tree species are *Putranjiva roxburghii. Pterospermum xylocarpum, Chukrasia tabularis* and *Psydrax dicoccos.* Large expanse of degraded areas surrounding this site is a clear evidence of the disturbance and fire wood collection from this site. Lianes such as Gnetum edule, Symphorema involucratum, Elaeagnus indica are frequent. Slope is moderate to steep. Ground vegetation is sparse owing to dense tree cover.

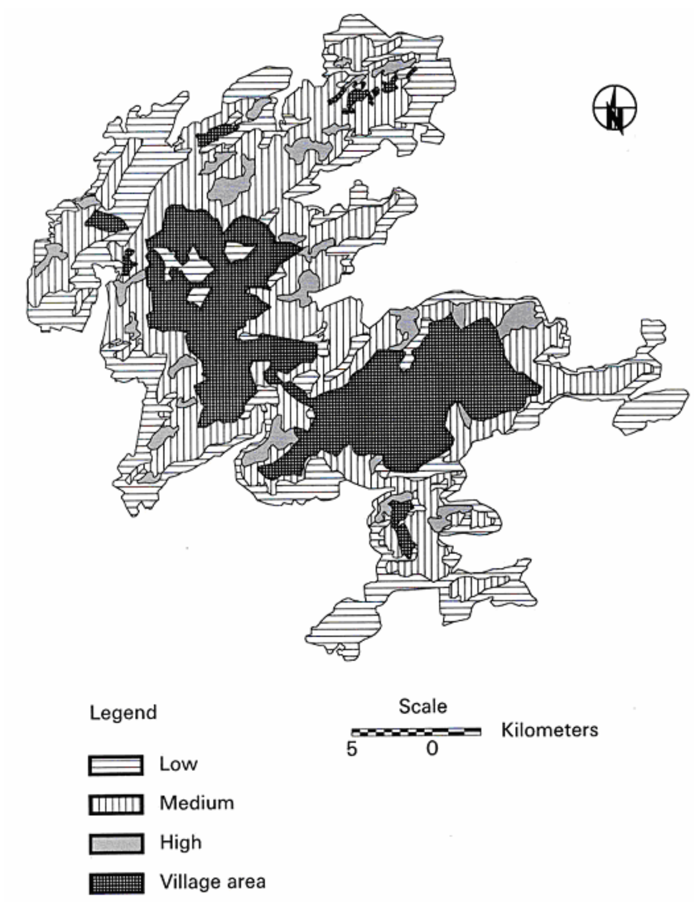
Site III with 86.85 ha has been suggested for conservation. The slope is moderate and the slope facing the north is conserved well due to the dwelling of Kaliamman deity, however the slope facing the south is drastically disturbed. Constituent tree species are *Nothopegia colebrookiana, Scolopia crenata, Mimusops elengi, Psydrax dicoccos* and *Diospyros ovalifolia.* Twiners like *Clematis gouriana* and *Rubia cordifolia* have been collected from this site. Frequent visits of cattle are encountered and occasional felling is noticed.

Area demarcated for conservation for the s*ite IV* is 77.18 ha. The highest elevation point 1071 m msl in the Pacchaimalai hills is located here and slope is moderate to steep. It is a sacred grove where in the stretches surrounding the area has been cleared, and the exotic tree *Plumera rubra* has been extensively planted. The forest is highly degraded due to casual cutting and felling thus leading to heavy run-off and subsequent erosion. *Diospyros ovalifolia, Ochna obtusata, Nothopegia colebrookiana* and *Gymnema sylvestre* have been recorded.

In the site V a big temple has been constructed in honour of the resident deity Perumal. The vegetation is dense and the common species are *Memecylon edule, Nothopegia colebrookiana, Diospyros ovalifolia, Schleichera oleosa* and *Alseodaphne semecarpifolia* that grow up to 10 - 15m tall. Frequent grazing by cattle does occur besides cutting and felling. *Curcuma neilgherrensis* is found only at this site in the hills.

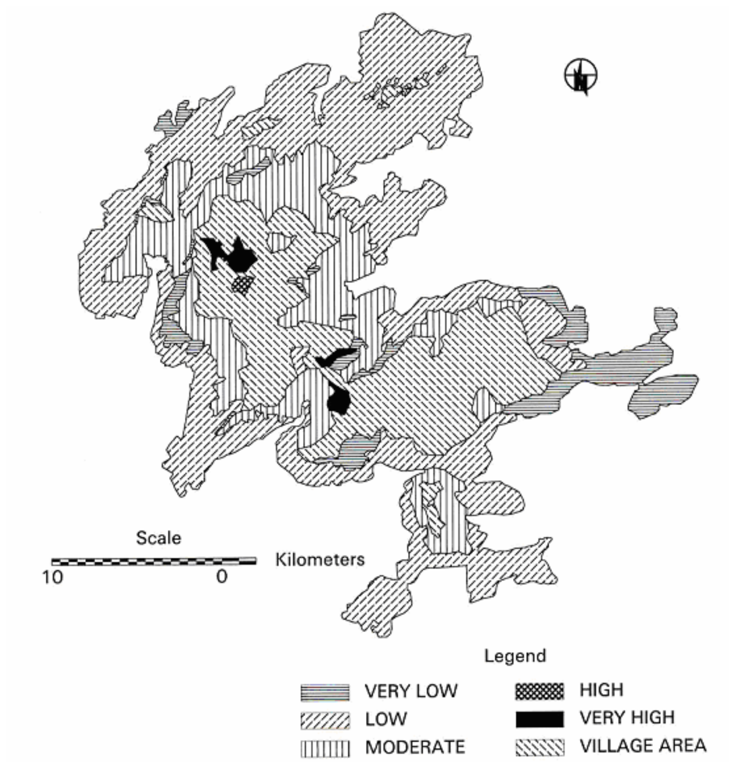
Discussion

The Pacchaimalai hills support many types of vegetation of this the dry evergreen forests have been considered significant due to their dense cover, besides their distinct composition. Evergreen forests are relatively common in the Western Ghats of Peninsular India; however, dry evergreen forests are meagre in distribution and are confined to higher elevations of the Eastern Ghats that are discontinuous and in small patches. Presence of such vegetation is an indication, that the lower elevation hills like the Eastern Ghats could harbour good vegetation and also they are the vestiges of the luxuriant vegetation cover of the past era in the Eastern Ghats, and hence needs to be protected. Species richness map *(Map 4)* refers to the number of species contained in an area and its measurement per unit area is perhaps the most objective of all the measures. Hence, it would be desirable to have a richness index independent of sample size. Vascular plant species richness has been used to characterize an ecosystem and that of biodiversity of an area [5, 30]. The species richness has been observed high not in completely covered or fully open vegetation rather, in partially degraded vegetation. Similar view has been also reported that the diversity of species tends to be greater in disturbed than undisturbed environment [24]. The dense cover area prevents the growth of herbaceous species while the open scrubland is unfit to harbour tree population.



Map 4. *Species richness map of Pacchaimalai Hills*

Temporal patterning of disturbance plays an important role in determining species abundance and diversity [2]. The most widely published prioritization of species for conservation action has been the threatened species categories defined by the IUCN and used in its Red data book [8]. The species that are endangered, vulnerable, threatened and endemic requires prime importance in conservation measures [20, 16]. There are 41 species under these criteria in the study area and tabulated herewith as endangered and endemic. The survival of these species, which have already reached critically low levels of population numbers, is dependent on certain critical factors of ecology, population dynamics or extrinsic factors such as exploitation and habitat loss or degradation. Hence, their distribution has been mapped *(Map 5)* in order to conserve them from extinction. *Gloriosa superba, Cayratia pedata, Gnetum edule, Habenaria heyneana* and *Rubia cordifolia* are the endangered species. Seventeen species are endemic to Peninsular India while eight species are vulnerable.



Map 5. *Endangered/endemic species distribution value map of Pacchaimalai Hills*

Association of trees with deities and demarcation of certain area as sacred are an ancient practice in India and other parts of the world. The associated tree and its surrounding habitat are revered and worshipped by people as sacred. The sacred groves that existed as conservation areas were now getting degraded and they needed to be studied and conservation strategies need to be prioritised for their preservation and perpetuation [21]. The sense of sanctity and the fear of the unknown, which helped their conservation, are gradually losing their hold. Hence, the task of conservation has assumed a greater urgency. In the Pacchaimalai hills, six groves are present namely Kannimar kovil, Kaliamman kovil, Masimalaian kovil, Perumal kovil (Kilkarai), Periya samy kovil (Periya shola) and Perumal kovil (Manmalai) and invariably all of these groves comprise dry evergreen vegetation in 848 ha and are repositories of a large number of rare and endemic plant species.

The forest loss and degradation are associated directly with proximity to roads and villages up to 6 Km [13, 29] that confirms the disturbing effect of roads by means of logging, mining, grazing, agriculture and urban development. Around 6500 ha of the reserved forest come into the risk area. This area would likely to increase if we include the footpath, which are numerous in the Pacchaimalai hills. The fuel wood consumption is calculated to be 2.4 Kg / household / day [32]. Based on the 1991 census, the fuel wood requirement would be of the order 7.2 tones per day, which is likely to increase due to present growth rate of the population and the influence of the people who live surrounding the hills.

It is essential to consider the following three principles while selecting area for conservation and that an area should be efficient in capturing all the species and ecosystems [31]. This has become a difficult task since the sustenance requirement of each species differs considerably. Besides, different variables are considered as prerequisites for conservation of a species, for example the size of the protected area [3]. In addition to size, conditions and connectivity to maintain even the most sensitive species with the prevalent ecological processes are an essential foundation of any conservation strategy [17].

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THREATS AND THEIR RELATIVE SEVERITY TO WILDLIFE PROTECTED AREAS OF KENYA

J. W. KIRINGE* – M. M. OKELLO

The School for Field Studies, Center for Wildlife Management Studies, P.O. Box 27743 – 00506, Nairobi, Kenya

e-mail: okelinge@africaonline.co.ke or jkiringe@yahoo.com

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Abstract. Little information is available on the threats against biodiversity in Kenya. This is critical in prioritizing conservation strategies and instituting mitigation procedures to contain and or eliminate these threats for the survival of biodiversity in protected areas. This study aimed at documenting relative severity of threats and how serious protected parks are threatened. Two hundred protected area officers were interviewed. The most relatively severe threat factors were bush meat trade; poaching for trophies; human – wildlife conflicts; human population encroachment; loss of migration corridors and dispersal areas. Thirty-two (64%) protected areas were susceptible to over half of the threat factors, while over 70% of them were threatened by an index over 0.5. All marine and nearly all forested/montane protected areas were highly susceptible to the identified threat factors. Further, protected areas popular with tourists were also highly susceptible and threatened. Protected areas around urban/industrial and agricultural areas were threatened mostly by a variety of threat factors. These findings imply that threats to Kenya's protected areas are serious. They are critical in helping the Kenya government to prioritize its strategies in protected areas management, rather than the current haphazard approach.
Keywords: *biodiversity, conservation, ecosystems, dispersal areas*

Introduction

Conservationists viewed the establishment of the first protected area in Kenya in the mid-1940 as a milestone towards preserving diminishing wildlife species and their habitats. Since then, a chain of such areas has been designated in various parts of the country encompassing ecologically diverse ecosystems specifically for biodiversity conservation (KWS, 1990; Sindiga, 1995; Mugabe *et al*. 1998; Mugabe, 1998; Kameri, 2002). Going by the economic returns from wildlife based tourism and tremendous loss of biodiversity globally; one appreciates Kenya's initiative to designate rich biota landscapes exclusively for nature preservation (Mugabe *et al*., 1998; Mugabe, 1998; Kameri, 2002). Further, since Kenya's national economy is predominantly hinged on biological resources, wildlife protected areas are an important asset from which a significant amount of foreign exchange has been derived in the past few decades (Okello *et al*., 2001). Even though tourism has recently declined for a variety of reasons, and the country currently faces a myriad of wildlife conservation challenges (Sindiga, 1995; Mugabe *et al*. 1998; Mugabe, 1998; Smith, 1999; Johnstone 2000; Okello & Kiringe 2004) conservation of biological resources still remains one of the key national obligations of the Kenya Government (Mugabe *et al*. 1998; Kameri, 2002).

The current biodiversity conservation problems and largely unviable of protected areas in Kenya are partly precipitated by the government's protectionist approach and local communities alienation before and after independence (Mugabe *et al*. 1998; Kameri, 2002). Kenya Wildlife Service (KWS), which has been the custodian of the countries biodiversity since 1990, and its predecessor institutions have not taken any meaningful initiative to critically review the conservation strategies of the country. A

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protectionist approach towards conservation and failure to address the plight of rural communities has strongly been engrained since the pre-independence days (Beresford & Phillips, 2000). There is also a tendency to mitigate superficially and in isolation threats to survival of protected areas without any clear goals.

In the last century, increased human population has created a high demand for land as well as exerting an incredible amount of pressure and threat to wildlife and other biodiversity types in Kenya (Mwale, 2000). For instance, in high potential areas of Western Kenya, Nyanza, Central and parts of the Rift Valley Provinces where agriculture is the predominant land-use, most biodiversity types have nearly been exterminated including substantial alteration and loss of wildlife habitats (Kameri, 2002). Human encroachment on critical biodiversity depository sites in search of agricultural land has since the 1970's and 1980's shifted to low potential rangelands which coincidentally are the prime wildlife ecosystems (Sindiga 1995; Mwale 2000). This has created a myriad of problems like competition for water resources, human-wildlife conflicts, habitat fragmentation and blocking of wildlife migratory routes and dispersal areas and negative perception towards conservation (Sindiga, 1995; Norton-Griffiths, 1997; Ottichillo, 2000). Similarly, mountain Ecosystems like Aberdare National Park, Mt. Kenya National Park, Mt. Elgon, the Mau Escarpment among others have in the past few decades seen substantial human influx for subsistence farming opportunities, collection of both animal and plant resources. The Forest Department and more recently Kenya Wildlife Service have been faced with a new challenge of regulating and containing this encroachment as a means of minimizing habitat degradation, loss and subsequent biodiversity destruction.

Institutions that have been in-charge of wildlife conservation and management of protected areas have taken little proactive approach to regularly evaluate status and threats of these areas. Various research works (Nyeki, 1993; Sindiga, 1995; Mwangi, 1995; Western, 1997; Smith, 1999; Ottichilo, 2000), have outlined some of the critical threats to protected areas that need to be seriously addressed. Attempts have been made to address and mitigate these threats but with mixed success. Consequently, the Kenya Wildlife Service and the government in particular should; re-examine wildlife conservation approaches, policies and objectives. They should urgently undertake a comprehensive or holistic assessment on threats undermining biodiversity conservation initiatives within and outside protected areas as well as their genesis. The findings will provide key insights on the formulation of a workable conservation action plan specifically targeting conservation problems of each protected area. The current approach of applying the same strategies across the existing protected areas network to mitigate threats to biodiversity and associated habitats or ecosystems will not have any meaningful gains even in the years to come.

The objectives of this study were to:

- Establish the relative severity of previously identified threat factors to protected areas in Kenya (see Okello & Kiringe 2004)
- Prioritize and rank protected areas based on the relative severity of threat factors operating against them.
- Make appropriate recommendations on prioritization of management actions and activities in mitigating threats to protected areas of Kenya.

Study country

This study focused on threats to biodiversity within and outside the current network of protected areas of Kenya, which is one of the three countries that make up East Africa *(Figure 1)*. Kenya is located within latitudes 4° 40' to the north and 4°20' to the south. It shares a common border with other countries of the larger Eastern Africa Region namely; Sudan, Somalia and Ethiopia. The landscape from Lake Victoria to the Indian Ocean in the East is extremely diverse. Further, the Great Rift Valley, which traverses from Lake Turkana in the North West to Tanzania and its associated features like Mt. Kenya, Mt. Elgon, the Mau Escarpment and Aberdare Ranges adds a complex landscapes comprising of various vegetation associations and biota types.

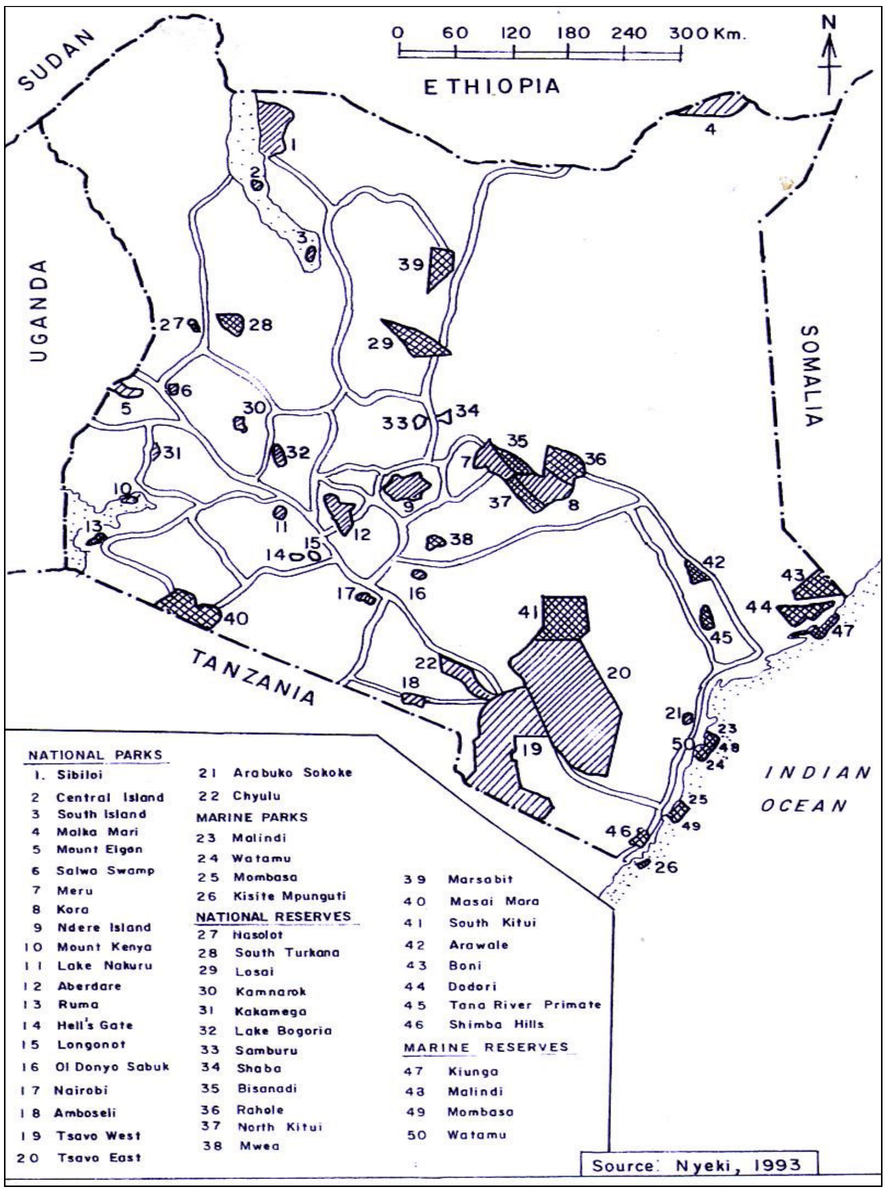

Figure 1. *A network of protected areas in Kenya. Most protected areas are small in size and quite representative of geographical areas in Kenya.*

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Kenya is endowed with an enormous diversity of ecosystems and wildlife species. In particular, it is renowned for its diverse assemblage of large mammals like elephant (*Loxodonta africana*), black rhino (*Diceros bicornis*), leopard (*Panthera pardus*), buffalo (*Syncerus cafer*) and lion (*Panthera leo*) numerous species of ungulates. This rich wildlife together with other attractions has for decades made the country an important tourist destination and hub for the lucrative tourist industry. The rich biodiversity is partly attributed to diversity in landscape, ecosystems, habitats and convergence of at least seven bio-geographic units (IUCN, 1990; Young, 1996; Medley & Hughes, 1996). Overall, the interactions between relief, geology, climate and soils have a profound influence on the types of habitats, ecosystems and biota life forms within the country (IUCN 1990; Young 1996; Medley & Hughes, 1996). Thus, most landscapes are characterized by peculiar climatic factors, wildlife life forms and vegetation associations which give rise to distinguishable geographical regions in the country (Grove, 1978; Leifer, 1977; Ojany & Ogendo 1973).

Methods

Information on threats to Kenya's protected areas was collected from field officers as a first preliminary step by Okello & Kiringe (2004). This work was followed up by a deeper probing of the opinions of the protected area officers on magnitude of each of the threat factors to their protected area using a brief questionnaire. Fifty protected areas (parks and reserves) in Kenya were included in this survey. The protected area officers who were interviewed or who provided information through the questionnaire were considered knowledgeable in view of their involvement in protected area management over time. These were long serving overall wardens, community wardens, law and policy enforcement ranger, and a research scientist.

The primary four protected area officers were asked, independent of each other, to rank from one (lowest threat level) to five (highest threat level) the key ten threats to protected areas identified from an initial preliminary survey (Okello & Kiringe, 2004). Each protected area officer was only allowed to provide ranks for the threat factors on the protected area under which they served. Scoring for each threat factor on ordinal scale by protected area officers was assumed to be adequate for the purpose of assessing status and threat index of each protected area.

Data analysis

A tally of the threat factors mentioned for each protected area was computed, and the proportion of the sum of the threat factors in each protected area out of the total ten was considered a measure of the ***Protected Area Susceptibility Index (PASI)*** to the threat factors. The following were calculated as indicators of how serious a threat factor was against protected areas, and vulnerability of protected areas to these threats:

- ***Mean score of each threat factor*** = (sum of all the scores for that particular threat factor) / (the total number of respondents, 200)
- ***Relative Threat Factor Severity Index, RTFSI*** = (The mean score for a particular threat factor) / (the maximum possible score, 5)
- ***Protected Area Relative Threatened Index, PARTI*** = total score of the ten threat factors from the interviewed officers of a given protected area) / total responses (40)

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A ranking system based on RTFSI showed which of the threat factors was more serious across protected areas in Kenya, while a ranking based on both PASI and PARTI showed which protected areas were most vulnerable to the identified threat factors. The relationship of the each of the ten threat factors with the protected area relative threatened index (PARTI) was determined by performing a non – parametric Spearman Rank Correlations (Zar 1999) to determine key threat factors that influence the threat vulnerability of the protected areas. Analysis was done using STATIGRAPHICS (Version 4.0 for Microsoft Windows 1999) software. Comparisons of protected area vulnerability in terms of dominant ecosystem types they have, and the predominant adjacent land use was done by a non-parametric Kruskal – Wallis test followed by a Box – and – whisker Multiple Comparison Procedure (Zar, 1999).

Results

The protected areas are faced by threat factors operating at relatively higher relative threat factor severity (RTFSI) level of 0.57, and ranging from 0.51 to 0.63 *(Table 1)*. Specifically, the threat index of illegal killing of wildlife for local and regional bush meat was highest (0.84) across protected areas *(Table 1)*.

Table 1. *Threat factors that operate against biodiversity in Kenya's protected areas, their perceived threat index and Prevalence.*

Threat Factor identified by protected area officers	Mean threat factor score (Mean ± SE)	Relative Threat Factor Severity Index (RTFSI)
Illegal killing of wildlife for their bush meat for the local or regional markets	4.20 ± 0.12	0.84
Large mammal poaching for international commercial purposes	3.70 ± 0.20	0.74
Direct and indirect danger to biodiversity arising from the nature and intensity of human – wildlife conflicts	3.40 ± 0.17	0.68
Loss, conversion and degradation of wildlife migration and dispersal corridors important for the protected area	3.34 ± 0.19	0.67
Human encroachment in terms of their densities and distribution around protected areas	3.26 ± 0.21	0.65
Unsustainable use, demand and exploitation of natural resources (e.g. water, plant resources and minerals) by local communities surrounding protected area	2.94 ± 0.24	0.59
Recent agricultural expansion and other incompatible land use changes to biodiversity requirements	2.60 ± 0.22	0.52
Pollutants from external sources of a protected area that harm biodiversity directly or indirectly	1.84 ± 0.21	0.37
Negative and persistent tourism impacts to the welfare of biodiversity and their habitats	1.66 ± 0.20	0.33
Fencing of a protected area entirely or in part by certain form of fencing materials	1.44 ± 0.17	0.29
Mean value (± SE)	2.84 ± 0.09	0.57 ± 0.06

Killing of wildlife for their trophies for international commercial trade (poaching) had a threat index of 0.74, followed by negative consequences of human – wildlife conflicts that had a threat index of 0.68. The loss, conversion and degradation of migration corridors and dispersal area had a threat index of 0.67, while human density

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and encroachment had a threat index of 0.65. Unsustainable use, demand and exploitation of other natural resources had a threat index of 0.59 while recent agriculture expansion and other land uses had a threat index of 0.52. Other threats had a threat index less than 0.5 across protected areas *(Table 1)*.

A majority of Kenya's protected areas were highly susceptible to most of the ten threat factors identified. Thirty-two (64% of protected areas in Kenya) were susceptible to over half of the identified threat factors. Twenty-seven (54% of the protected areas) were susceptible to over 60% of threat factor types. Sixteen (32% of the protected areas) were susceptible to over 70% of the threat factor types, and three (6% of protected areas) were susceptible to over 80% of the threat factor types. The protected areas most susceptible to the majority of the threat factors were Maasai – Mara National Reserve, Ndeere Island National Park, Lake Nakuru National Park, Aberdares National Park, Mount Elgon National Park, Kiunga Marine Park, Mt. Kenya National Park, Mombasa Marine Park, Watamu Marine Park, Ruma National Park, Kisite - Mpunguti Marine Park, Malindi Marine Park, Mwea National Reserve, Kamnarok National Reserve, Rimoi National Reserve, and Nairobi National Park *(Table 2)*.

Table 2. *Kenya's protected areas and the major threat factors against biodiversity in and around them with Vulnerability Index (PAVI)*

Protected Area	**Protected Area Relative Threatened Index, PARTI (rank)**	**Predominant ecosystem type**	**Adjacent predominant land use**
Masai-Mara National Reserve	0.88 (1)	Savanna rangelands	Traditional pastoralism and Agriculture
Ndeere Island National Park	0.78 (2)	Inland wetland	Urban and Industrial
Lake Nakuru National Park	0.72 (4)	Inland wetland	Urban, Industrial and Agriculture
Aberdare National Park	0.74 (3)	Montane / Forested	Agriculture
Mt. Elgon National Park	0.66 (13)	Montane / Forested	Agriculture and Traditional pastoralism
Kiunga Marine	0.72 (4)	Marine	Urban and Industrial
Mt. Kenya National Park	0.68 (11)	Montane / Forested	Agriculture
Mombasa Marine	0.72 (4)	Marine	Urban and Industrial
Watamu Marine	0.72 (4)	Marine	Urban and Industrial
Ruma National Park	0.72 (4)	Savanna rangelands	Agriculture
Kisite-Mpunguti Marine	0.72 (4)	Marine	Urban and Industrial
Malindi Marine	0.72 (4)	Marine	Urban and Industrial
Mwea National Park	0.66 (13)	Savanna rangelands	Agriculture
Kamnarok National Reserve	0.62 (16)	Savanna rangelands	Agriculture
Rimoi National Reserve	0.62 (16)	Savanna rangelands	Agriculture
Nairobi N. Park	0.62 (16)	Savanna rangelands	Urban, Industrial and Traditional pastoralism
Tana River Primate National Reserve	0.62 (16)	Forested	Agriculture and Traditional pastoralism
Ngai Ndeithya National Reserve	0.68 (11)	Savanna rangelands	Agriculture
Saiwa Swamp National Park	0.64 (15)	Inland wetland	Agriculture
Kakamega Forest National Park	0.60 (21)	Forested	Agriculture
Oldonyo-Sabuk National Park	0.56 (30)	Savanna rangelands	Traditional pastoralism and Agriculture
Shimba Hills National Park	0.62 (16)	Forested	Agriculture

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Protected Area	Protected Area Relative Threatened Index, PARTI (rank)	Predominant ecosystem type	Adjacent predominant land use
Amboseli National Park	0.52 (22)	Savanna rangelands	Traditional pastoralism and Agriculture
Tsavo-West National Park	0.58 (22)	Savanna rangelands	Traditional pastoralism / ranching and Agriculture
Nasalot National Reserve	0.58 (22)	Savanna rangelands	Traditional pastoralism
Chyulu National Park	0.58 (22)	Savanna rangelands	Traditional pastoralism and Agriculture
South Turkana National Reserve	0.58 (22)	Savanna rangelands	Traditional pastoralism
Arabuko-Sokoke National Park	0.56 (30)	Forested	Agriculture
Tsavo-East National Park	0.58 (22)	Savanna rangelands	Traditional pastoralism / ranching and Agriculture
South Kitui National Reserve	0.58 (22)	Savanna rangelands	Agriculture
Hell's Gate National Reserve	0.52 (32)	Savanna rangelands	Agriculture and Traditional Pastoralism
Lake Bogoria National Reserve	0.58 (22)	Inland wetland	Traditional pastoralism
Sibiloi National Park	0.46 (38)	Savanna rangelands	Traditional pastoralism
Marsabit National Reserve	0.44 (39)	Savanna rangelands	Traditional pastoralism
Losai National Reserve	0.44 (39)	Savanna rangelands	Traditional pastoralism
Bisanandi National Reserve	0.50 (33)	Savanna rangelands	Agriculture and Traditional pastoralism
North Kitui National Reserve	0.50 (33)	Savanna rangelands	Agriculture
Kora National Park	0.50 (33)	Savanna rangelands	Traditional pastoralism
Rahole National Reserve	0.50 (33)	Savanna rangelands	Traditional pastoralism
Meru National Park	0.50 (33)	Savanna rangelands	Agriculture and Pastoralism
Samburu National Reserve	0.40 (42)	Savanna rangelands	Traditional pastoralism
Shaba National Reserve	0.40 (42)	Savanna rangelands	Traditional pastoralism
Buffalo Springs National Reserve	0.40 (42)	Savanna rangelands	Traditional pastoralism
Malkamari National Park	0.42 (41)	Savanna rangelands	Traditional pastoralism
South Island National Park	0.38(48)	Inland wetland	Traditional pastoralism
Central Island National Park	0.38 (48)	Inland wetland	Traditional pastoralism
Arawale National Reserve	0.40 (42)	Savanna rangelands	Traditional pastoralism
Boni National Reserve	0.40 (42)	Savanna rangelands	Traditional pastoralism
Dondori National Reserve	0.40 (42)	Savanna rangelands	Traditional pastoralism
Mt. Longonot National Park	0.38 (48)	Savanna rangelands	Traditional pastoralism and Agriculture

Thirty-seven (74%) of protected areas in Kenya had a relative threatened index of 0.5 and above. Twenty-one (42% of the protected areas) had a threat index of over 0.6, while ten (20% of the protected areas) has a threat index of over 0.70. The ranks of protected areas, in terms of susceptibility index, were different (paired Wilcoxon Signed Rank test, $T = 2.25$; $n = 50$; $p = 0.025$) from their ranks based on the threatened index. The fifteen most threatened protected areas in Kenya were: Masai – Mara National Reserve, Ndeere Island National Park, Aberdares National Park, Lake Nakuru National Park, Kiunga Marine, Mombasa Marine, Watamu Marine, Ruma National Park, Kisite –

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Mpunguti National Park, Malindi Marine, Mt. Kenya National Park, Ngai Ndeithya National Reserve, Mt. Elgon National Park, Mwea National Park and Saiwa Swamp National Park *(Table 2).*

All (100%) marine protected areas in the country were very susceptible and threatened by the threat factors (with both PASI and PARTI of over 0.7). Another group of highly susceptible and threatened protected areas were the natural / montane forests, with all of them (100%) having a susceptibility index of over 50%. Three of them (Aberdares, Mt. Elgon and Mt. Kenya, 43% of protected natural forests) were highly susceptible (with a PASI of over 0.7 and PARTI of over 0.65), while two more (Kakamega and Shimba Hills) natural forests (bringing to about 75% of protected natural forests) were also susceptible and threatened (with both PASI and PARTI of over 0.6). Only two protected forest ecosystems (Chyulu Hills National Park and Arabuko – Sokoke) had a susceptibility and threat index of less than 0.6. Of the twenty protected areas with a high ranking as tourist destinations (Okello *et al.* 2001), a majority of them were highly susceptible and threatened by the factors. Eighty percent (80%) of the top twenty protected areas for tourism were both susceptible and threatened (both PASI and PARTI of over 0.5), with half of them (50%) having susceptible and threat indices both of over 0.6.

The susceptibility to threat factors (PASI) differed (Kruskal – Wallis, KW = 11.92, $p = 0.0077$) among protected areas classified as Savanna Rangelands (0.49 ± 0.03), Inland Wetlands (0.60± 0.09), Forested and Montane Ecosystems (0.63 ± 0.03), and Marine Ecosystems (0.70 ± 0.01). Significant differences in susceptibility occurred only between Marine Ecosystem and Savanna Rangelands (from Box – and – whisker graphical distribution). Susceptibility to threat factors (PASI) among protected areas surrounded by urban / industrial areas (0.73 ± 0.02), agriculture (0.59 ± 0.03), and pastoralism (0.42 ± 0.03) land use practices differed (K-W= 24.07, $p < 0.001$). The protected areas surrounded by these land use practices were all significantly different from each other.

The severity of threat factors (PARTI) differed (K-W = 15.68, $p = 0.0013$) among protected areas classified as Savanna Rangelands (0.52 ± 0.02), Inland Wetlands (0.60± 0.07), Forested and Montane Ecosystems (0.64 ± 0.02), and Marine Ecosystems (0.72 ± 0.01). This difference was between Marine Ecosystem and Savanna Rangelands, but others had similar threatened index. Relative Threatened Index among protected areas surrounded by urban / industrial areas (0.72 ± 0.02), agriculture (0.61 ± 0.02), and pastoralism (0.47 ± 0.02) land use practices differed (KW = 28.0029, $p < 0.001$). The protected areas surrounded by these different land use practices were all significantly different from each other.

There was a positive and significant correlation between the protected area threatened index (PARTI) with the following threat factors: Human encroachment ($r = 0.90$, $p < 0.001$), agriculture expansion and other land use changes ($r = 0.75$, $p < 0.001$), unsustainable over - utilization of other protected area resources ($r = 0.73$, $p < 0.001$), discharge of pollutants from external sources into the protected area ($r = 0.59$, $p < 0.0001$), negative impacts of tourism ($r = 0.46$, $p = 0.012$), negative effects of fencing to wildlife movements and ranging ($r = 0.38$, $p = 0.0084$), and presence of human – wildlife conflicts ($r = 0.28$, $p < 0.0049$). Prediction of protected area relative threatened index had its important predictors as Human encroachment (explaining the majority of variability in the threat index of 75.73%), followed by unsustainable over-utilization of resources (9.01%) and human wildlife conflicts (4.94%).

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Discussion

Even though the ten threat factors are identified, findings identify human encroachment and threats directly related to actual killing of wildlife (bush meat, trophy poaching, and human – wildlife conflicts) as the main threats while those indirectly affecting them through habitat conversions and harassment are relatively less serious. This means that even though most protected areas are susceptible to all of the threats, dealing with human encroachment around protected areas and dealing decisively with poaching (for bush meat trade and commercial trophies) would secure most protected areas. Controlling human encroachment and associated activities is a difficulty endeavour (Osemeobo, 1993), yet critical in avoiding insularization of protected areas in Kenya (Western & Ssemakula 1981). Further, human – wildlife conflicts which is a function of human population increase and encroachment to protected areas, arises from conflicts between human and wildlife needs. Incidences of these conflicts are now considered the biggest threat to protected areas in Kenya (KWS, 1994). Any action such as controlling problem animals as well as reconsidering compensation to property as well as increasing compensation amounts would reduce negative attitudes to protected areas (Sindiga 1995, Seno & Shaw 2001).

The findings that a majority of Kenya's protected areas are threatened by a majority of threat types implies that conservation in the country is more at crisis than previously thought. The ever-increasing land demand in the country due to the increasing human population in rural areas, and especially in marginal arid and semi - arid lands, has put more pressure on biodiversity and protected areas. The list of protected areas most susceptible is important in focusing conservation action. Mismanagement of funds that could be used for conservation, changing land uses and human encroachment, and uncontrollable off – road driving are some specific issues the Mara needs to address in order to survive. Lake Nakuru is dealing with pollution from Nakuru town, shrinking lake due to negative land use changes catchments of the rivers that drain into it, as well as ecological changes within the park as a result of insularization by the town and electric fencing. These need to be addressed to save this ornithological world heritage site. Most forest and montane parks are critical water catchments areas and sources of most important rivers (Tana and Athi) but illegal logging, forest cultivation and poaching of plant and large mammal resources are threatening their status as biodiversity areas.

These few examples illustrate the importance of these findings in that they can be used to identify specific problems ailing each conservation area in a prioritized manner and deal with them on individual basis based on their severity indices (such number of snares, illegal poachers arrested, and cases of human - wildlife conflicts) or relative threat factor severity index (RTFSI). It is critical for any conservation agency to have structured and focused priorities for its protected areas. We therefore recommend that most of management actions should be based on actual measurement of threat indices or a reliable index such as RTFSI in addressing specific threat factors. Further prioritization of parks most affected should be done based on a threatened status using indices such as PARTI, rather than on susceptibility which is simply a catalogue of threats recorded without considering its magnitude or severity. From our findings, the tourism industry, often times strongly accused to have negative impacts on biodiversity in protected areas seemed to be a less important threat factor. This is possibly because only 32 % of Kenya's protected areas have a meaningful tourism potential, and only 24% of them have achieved or exceeded this potential (Okello *et al.*, 2001). Tourism is low in most of Kenya's protected areas, but is concentrated heavily in a few of them, which are accessible and are endowed with tourist facilities and large mammal diversity. However, the potential of negative tourism impacts as a threat factor was identified by its significant correlation with the threat index of protected areas and the fact that a majority of popular tourist destination protected areas seems to rank highly as threatened and susceptible protected areas in Kenya. As tourism activities have revealed in Maasai Mara and Amboseli, luck of active management of negative tourism impacts can pose immediate and severe threats to a protected area (Smith, 1999). It is therefore important to reduce the negative impacts of tourism by managing tourist behaviour and impacts of tourist facilities such as lodges and campsites. Diversifying tourism attractions to target cultural, physical features, historical and archaeological sites (and not only wildlife –based) attractions can help reduce pressure on protected areas (Okello *et al.* in press). The use gate fee adjustments periodically to target few but high paying tourists in order to limit tourist traffic would be a worthwhile idea. Small and over sold popular protected areas such as Amboseli, Nairobi, Nakuru, and Maasai Mara could benefit from these strategies. When tourism activities are high, with a high number of lodges and related activities, with diminishing migratory routes and dispersal areas for wildlife, the cumulative effects on biodiversity conservation become more pronounced and serious, especially in relatively small – sized protected areas (Johnstone, 2000). This is the dilemma facing Amboseli, Nairobi and Lake Nakuru National Parks where ecosystems are not large enough to cushion the impacts of multiple threats to their biodiversity and viability as conservation units. Therefore controlling tourism impacts is another critical step in maintaining ecosystem and biodiversity integrity of Kenya's protected areas.

Management actions can effectively deal with threats if it also focuses mainly (but not entirely) on protected areas that are highly threatened. Results suggest that one of the most threatened protected areas is natural forests and mountainous ecosystems. Natural forests and mountainous protected areas are critical for ecological services (such as air purification, water catchments, reservoir for biodiversity resources) but are now increasingly becoming endangered ecosystems. They have faced numerous excisions in the recent times for mundane purposes such as resetting the landless people, and for rural based agriculture (Rodgers, 1997). Further, they are getting degraded through a variety of land uses such as livestock grazing, deforestation and charcoal burning. The net result has been serious threat to biodiversity and wildlife habitats, ecosystem degradation and loss of ecological services (such as water availability). Conservation authorities and the government have a responsibility to conserve and protect the country's ecosystems and associated biodiversity as both a national service and contribution to global biodiversity conservation. Laxity however is compromising the Kenyan government's achievements on this (Chapman & Chapman, 1996; Rodgers, 1997).

Similarly, wetlands are also endangered ecosystems in Kenya and are in danger of being converted, drained and lost together with their biodiversity and ecological services (Cooper 1996). These ecosystems need focused attention to prevent biodiversity loss. An important initial step is to stop further land excisions of forests and wetlands for alternative land uses by enlisting the cooperation of local communities around them. Next is to outlaw any activities in these ecosystems that impact negatively on biodiversity (such as over – exploitation by the timber industry, charcoal burning, shifting cultivation, drainage and conversion of swamps for horticultural production or

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over – extraction of water resources from swamps and rivers feeding them). Appropriate legislation may be in place, but putting in place competent and efficient institutions to manage and enforce the laws is lacking. Non – governmental organizations can help to create awareness about the status of Kenya's natural resources, disseminate information, and educate rural communities on environmental ethics and conservation. This should be supplemented with a cohesive non – governmental and professional advocacy network in environmental conservation that will establish checks and balances on mismanagement of natural resources and install responsible custodianship.

One of the striking finding of this study is the fact that all marine protected areas in Kenya are threatened and highly susceptible to threat factor types. Marine protected areas face a multiplicity of threat factors. The coast is one of the hubs of tourism activities in Kenya. The sunny beaches and high class international hotels makes it a tourism heaven only comparable to Costa Rica, the Caribbean islands and Australia. Tourists dive and snorkel close to the ocean floor and enjoy observing the diversity in coral reef and marine life. But most of this marine biodiversity is very fragile and sensitive to human impacts (McClanahan, 1996). Special nesting sites for endangered species like turtles are some of the key and sensitive habitats, which could do with less tourist numbers. Estuaries and river entry points in the sea are silted as deforestation of riverine vegetation along major rivers (such as Tana and Galana) upstream becomes increasingly severe. Such rivers are also loaded with domestic and industrial pollutants from local and upstream sources that eventually get discharged into the ocean.

Another threat facing marine protected areas is pollution (sewage and litter discharges) from lodges and hotels, and over – crowding along beaches. A ring of tourist lodges and hotels and associated high human traffic to marine protected areas is a threat to marine biodiversity. Many of the protected areas are subject to disasters of the high seas such as pollution from ocean sea liners and accidental spillage of oil and petroleum products, including biological disasters such as proliferation of sea algae and micro – organisms that are fatal to fish and other marine biodiversity (McClanahan, 1996). These pollutants are washed ashore and often into marine protected areas. Collection and sale of marine biodiversity and products (such as cowry shells) is also a major threat to marine biodiversity conservation. Since many tourist towns (such as Mombasa, Lamu and Malindi) have historical and archaeological sites, business investment has been high and supports many workers and visitors. These businesses (such as tourism related industries) threaten coastal biodiversity because of associated negative impacts. Marine protected areas in Kenya are therefore faced by threats of a multiple nature and sources (from inland and sea sources, and urban communities along the beaches). A solution must be multi – faceted, targeting many sources of impacts and involving all the stakeholders.

It worthy noting that the less threatened protected areas are in arid and semi – arid parts of the country and surrounded by pastoralists whose population is relatively sparse and low. Pastoralism as a land use is more compatible with wildlife conservation compared to heavily settled areas that have both intensive subsistence and commercial agriculture, or urban centers of high industrial and human activities. Nevertheless, protected areas surrounded by pastoralists are not entirely free from threat as pastoral tribes (such as the Maasai, Samburu, Turkana) are having an influx of immigrants from other Kenyan tribes, and also their own population growth rate is increasing. These pastoral communities are also embracing previously foreign land use types such as agriculture. Where livestock and human numbers are high, then human – wildlife conflicts and environmental degradation becomes a concern, as has been the case among the Maasai of Tsavo – Amboseli ecosystem. Protected areas, when surrounded by agricultural activities and urban centers, become more threatened as human – wildlife conflicts intensify and wildlife dispersal areas and habitats become lost, converted or degraded (Okello & Kiringe 2004, KWS 1994). In Northern and North – Eastern provinces of Kenya, insecurity and remoteness makes conservation of biodiversity in that area difficulty and hence uncertain.

No control of prevalence and impact of threats to protected areas will succeed if the local communities are not socio-economically empowered and resource management policies made to include their needs and aspirations. If they do not benefit, are marginalized and are not compensated for opportunity costs and harm incurred as a result of resource conservation, then threat to biodiversity may be carried to the ultimate conclusion; extinction of species. In Kenya particularly, wildlife conservation must provide controlled and monitored user rights where tourism is non – existent for wildlife to be a credible land use in communal wildlife dispersal areas outside protected areas. Where tourism is well developed, local people need to be empowered to benefit directly from it rather than made to accept monetary tokens and hand – outs.

Lack of involvement of local communities in wildlife conservation as well as providing them economic interest in resource conservation will be reason for their continued indifference to poaching and bush meat trade, or concern for the plight of wildlife migration corridors and dispersal areas (Sarkar, 1999). On a national scale, it may be useful to formulate the a national land use plan to spell out the appropriate regional land use practices that will be compatible with the socio - economic potentials, resource base, ecological and climatic constraints within the country. Kenya is just in the processes of formulating such policies that have been lacking in the country.

A proactive strategy is needed, but must begin with identifying the threat factors as done by Okello & Kiringe (2004), formulating indices of levels and magnitude of threat (as done in this study) and then move finally into field sampling of appropriate indicators of each threat in each protected areas to quantify actual severity of threats. In this respect, this study builds on work done by Okello & Kiringe (2004) in providing information on relative threat severity and providing a ranking of Kenya's protected areas in terms of relative severity of threat. These two contributions can already help focus management actions in addressing each threat in each protected area, as well as prioritizing which protected areas need immediate attention. However, the final step is to take each protected areas and quantify levels of threat such as surrounding human density and related park encroachment issues, poaching incidences, density of snares, economic value of human crops and property destroyed, wildlife speared or poisoned by local communities around most threatened protected areas.

It is also our opinion that future protected areas should not be based blindly on the Yellowstone model, but on other alternative models that involve and enlist community support such as anthropological reserve or protected landscape model (MacKinnon *et al.,* 1986; Beresford & Phillips, 2000).

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SEASONAL DYNAMICS OF AN AQUATIC MACROINVERTEBRATE ASSEMBLY
(HYDROBIOLOGICAL CASE STUDY OF LAKE BALATON, №. 2)

CS. SIPKAY* – L. HUFNAGEL – A. RÉVÉSZ – G. PETRÁNYI

Department of Mathematics and Informatics, Corvinus University of Budapest
H–1118 Budapest, Villányi út 29-33, Hungary

e-mail: cs_sipkay@yahoo.com

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Abstract. In 2002, 2003 and 2004, we took macoinvertebrate samples on a total of 36 occasions at the Badacsony bay of Lake Balaton. Our sampling site was characterised by areas of open water (in 2003 and 2004 full of reed-grass) as well as by areas covered by common reed (Phragmites australis) and narrow-leaf cattail (Typha angustifolia). Samples were taken both from water body and benthic ooze by use of a stiff hand net. We have gained our data from processing 208 individual samples. We took samples frequently from early spring until late autumn for a deeper understanding of the processes of seasonal dynamics. The main seasonal patterns and temporal changes of diversity were described. We constructed a weather-dependent simulation model of the processes of seasonal dynamics in the interest of a possible further utilization of our data in climate change research. We described the total number of individuals, biovolume and diversity of all macroinvertebrate species with a single index and used the temporal trends of this index for simulation modelling. Our discrete deterministic model includes only the impact of temperature, other interactions might only appear concealed. Running the model for different climate change scenarios it became possible to estimate conditions for the 2070-2100 period. The results, however, should be treated very prudently not only because our model is very simple but also because the scenarios are the results of different models.
Keywords: *climate change scenarios, diversity, macrofauna, simulation model*

Introduction and aims

Lake Balaton is one of the most thoroughly studied shallow lake in the world, thus a sound knowledge is available on the macroinvertebrate fauna of its different biotopes.

The littoral region of Balaton is composed of various habitats. The natural parts of the northern shore are characterized by belts of reed, which go through a process of increasing deterioration. The most dominant species of these stands is common reed (*Phragmites australis* (Cav.) Trin.) but the expansion of narrow-leaf cattail (*Typha angustifolia* L.) at the expense of reeds is a common process typical also in some parts of Lake Balaton [10]. There are also important reed-grass associations characteristic of Lake Balaton - a most thorough research of macroinvertebrates was carried out in these communities in the past.

Research was conducted by Béla Entz on the macroinvertebrate fauna of different submerged macrophyte stands as early as in the 1940's [3]. These earlier works neglected seasonal dynamics, like the Crustacea study of Jenő Ponyi in Lake Balaton [13]. Quantitative research of the macroinvertebrate fauna of *Potamogeton perfoliatus* in Lake Balaton was first conducted by Bíró and Gulyás [2]. As they took samples only during two or three summer months, they were unable to treat seasonal changes. Using the sampling method and device described by Bíró and Gulyás, Muskó et al. took quantitative samples of macroinvertebrates in submerged macrophyte stands at the

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northern shore of Lake Balaton over a recent three year period [12]. This study also treated seasonal dynamics, having 3 or 4 samples every year.

Previous works primarily concentrated on spatial patterns and are mainly of faunistic nature. Only descriptive studies were published on temporal patterns not considering short scale seasonal changes.

Based on examinations carried out in streams, samples taken at a weekly basis proved to be appropriate to explore processes of seasonal dynamics [7]. Therefore we strove to take samples more frequently than usual.

For an investigation site we chose a part of Badacsony Bay, on the northern shore of Lake Balaton, characterised by narrowleaf cattail stands, common reed stands as well as open water areas (in the process of being populated by reed-grass). The exact sampling sites included three different microhabitat types characterised by the vegetation mentioned above. In the years 2002, 2003, and 2004 we took samples on a total of 36 occasions, from early spring to late autumn. Our data is the result of processing 208 individual samples. The spatial zoocoenological pattern of the microhabitats was described in our previous studies [19, 20, 22].

The different schools of methodology are distinctly separated from each other in ecological literature these days. Pattern descriptions based on field work constitute one of the main directions e.g. [14, 15, 16]. Modelling approaches treat either oversimplified situations or purely theoretical questions e.g. [5, 8, 10, 11]. On the other hand, experimental investigations often neglect the complexity of ecosystems e.g. [18]. Some new approaches have recently emerged to combine existing methods for the elimination of these problems [6]. In this spirit we would like our study of the processes of seasonal dynamics in Lake Balaton to provide a basis for further ecological modelling research, for designing manipulative setting of experiments and for possible research on climate change. Our aim was to construct a simulation model of the seasonal changes of a complex coenological indicator describing the whole macroinvertebrate assembly. Thus we defined a „Coenological Intensity Index" (CII) expressing the total number of individuals, biovolume and Shannon diversity.

The advantage of the simulation models is that they can provide predictions for situations where empirical examination is not as yet possible. Thus, considering the known shortcomings and conditions of validity of the given model, it will become possible to examine climate change scenarios. Of course, foreseeing the future may not be considered as a realistic goal, but we can examine the predictions of our model for data series generated by different realistic, hypothetical or other kind of simulation models. For this purpose, the internationally most comprehensively accepted climate change scenarios should be used.

In our earlier studies, we also used simulation models. In this work, by use of the Balaton database we succeeded in describing the seasonal changes of the biovolume of the crustacean *Limnomysis benedeni* with a model dependent on the daily mean temperature on the one hand and on water level values on the other [21]. We have modelled the seasonal dynamics of the dragonfly species *Ischnura pumilio* [25], the planctonic crustacean *Eudiaptomus zachariasi* and Cyclopoids [24] in artificial small ponds on the same basis, but in these models temperature was the only influential factor. We had the possibility to utilize data series longer than the Balaton database or the database of artificial small ponds: we have successfully described the seasonal changes in the abundance of *Cyclops vicinus* in the Danube with a temperature

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dependent model [23]. Instead of a species or group of species, in our present work we attempted to model a complex coenological index.

Our aims were the following:

- We wanted to explore seasonal zoocoenological patterns of macroinvertebrate assemblies, quantitative changes of significant taxon groups and temporal diversity trends.
- We wished to model the seasonal dynamics of the index describing the behaviour of the observed macroinvertebrate assembly, run the model for climate scenario data series and finally compare the predictions of the different scenarios with the help of our results.

Materials and methods

The sampling site was in Badacsony bay, where samples were taken at three nearby sampling points characterised by different kinds of vegetation, thus might be considered as three microhabitats: 1. (common) reed stand, 2. (narrowleaf) cattail stand and 3. open water. Various submerged macrophytes are typical in the area described as open water in front of the emergent macrophyte stands, but they did not form closed vegetation in the year 2002. Contiguous underwater patches of spiny naiad (*Najas marina L.*) stands appeared in the middle of the summer of 2003 and claspingleaf pondweed (*Potamogeton perfoliatus L*) was present at the same place from early summer of 2004 and densed significantly by the second half of the summer.

We took samples both from the water body and the benthic ooze of the three microhabitats, therefore 6 samples were taken on each occasion. Based on our previous research experience semiquantitative samples taken with a stiff hand net proved to be the most suitable method. The surface of the stiff hand net forms a symmetrical hemisphere. It’s maximum internal diameter is 14.8 cm, mesh size is 0.8 mm. Samples were taken by the same sampler at all three microhabitats by a standard 10 strokes of the net from the water body and by two drawings of the net from the upper layer of the benthic ooze.

In our present approach we concentrate on an investigation of the temporal changes, so we consider samples taken on the same day as one unit, without considering what microhabitat, body water or benthic ooze the specimen may come from.

We strove to take samples frequently throughout the entire growing season in each of those three years. Field work started in the spring and ended late in the autumn. In 2002, samples were taken on 16 occasions between 29th April and 16th November. Unfortunately, due to extreme weather conditions (remarkably intensive waves) we had to finish sampling in some cases, therefore some samples were left out. In 2003 we took samples on 13 occasions from 31st March to 9th November. We observed *Najas marina* stands for the first time on 13th July, yet only in smaller underwater fields and in a few places. The spiny naiad reached the surface of the water by the second half of July and it became dense during August and September. Samples were taken from the open areas between naiad patches, sweeping the plants. They sank to the bottom by October and formed an accumulated layer reaching to the borders of the emergent macrophyte stands. In 2004, samples were taken on 7 occasions between 17th April and 29th November. In June, *Potamogeton perfoliatus* was already present in the open water sampling site, in July and August, it formed a dense stand and in October, a significant amount of reed grass accumulated in front of the emergent macrophyte stands.

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Samples were taken in an extremely dry period. The decrease of the water level of Lake Balaton began in 2000 and reached its negative peak in 2003, then in 2004, it became higher again.

Maroinvertebrates were sorted into groups on the basis of taxonomic and body size categories (morphon), as well as of the states of development (e.g. larva, imago). Body size categories are important primarily from the aspect of calculating biovolume. We used five body size categories. Biovolume calculation is based on comparing the shape of an animal to a simple geometric form, the volume of which may be calculated simply. This method was described in detail in our earlier work [22].

We used the data analysis program package PAST [17] for multivariate data analysis (version 1.36, Hammer and Harper 1999-2005).

We were looking for a single index describing the whole macroinvertebrate assembly under examination. An index of this kind is the „Coenological Intensity Index" (CII) being the product of individual number, biovolume and Shannon diversity values transformed by divided by their means.

The model is based on the hypothesis that temperature is the main influential factor, in this way a pattern is defined by the daily maximum and minimum temperature values, all other factors (like trophic and other interpopulational interactions) appear concealed inside this factor. We have also presumed that the temperature reaction curve is the result of the sum of optimum curves because optimum temperature curves of the different species, of their different stages of development and perhaps their different subpopulations are summed up. In our model the value of the index changes from the first sampling day (giving the first value) according to a multiplier depending on daily maximum and minimum temperature (^{o}C) values, by following this formula:

$$CII_t = R_T \cdot CII_{t-1} \qquad \text{(Eq. 1)}$$

where CII_t is the value of the index at time „t" and R_T is the growth rate depending on temperature. The temperature reaction curve is the result of the sum of two normal distribution curves (*Fig. 1*). Standard deviation and mean value of the distributions as well as the value of an added constant are curve is the result of two normal distribution ierges of development, pherhaps their different subpopu parameters of the model. Parameters are optimized with the help of the Solver of MS Excel.

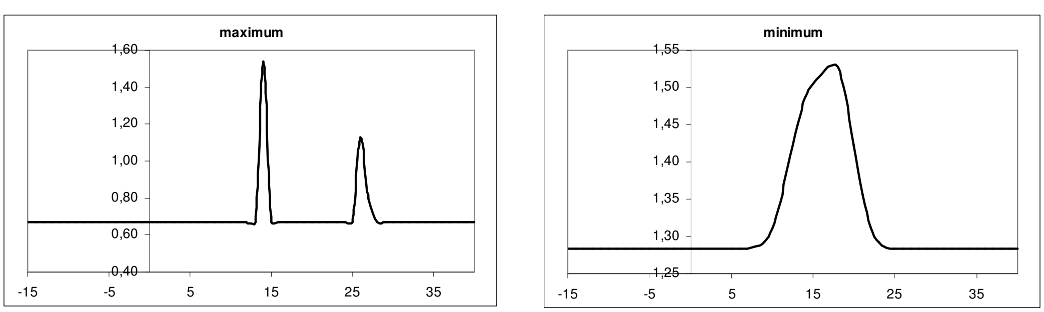

Figure 1. *The reaction curve of CII: the value of R_T multiplier plotted against maximum and minimum temperature*

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Subsequently we ran the model-system with the daily data series of climate change scenarios. The model was launched from the 90th to 135th day of the year because the samples fall into this period in all three years. OMSZ supplied us with meteorological data observed at the station of Fonyód, the station closest to the sampling site.

We used three data series of daily temperature specified for the period of 2070-2100. We employed the database of PRUDENCE EU project [4] namely A2 and B2 scenarios of „HadCM3" climate change model ran by Hadley Centre (HC) and the run results of the Max Planck Institute (MPI) for A2 scenario. The daily data series were downscaled for the region of Zalaegerszeg and Siófok, the nearest observation stations. Being the result of the run of the above mentioned three different scenarios, the daily data series include 31 run years in both three cases.

The supervention of the yearly maximum CII value was used as an indicator for the statistical analysis of the modelling results.

Results and discussion

Overview of macroinvertebrates, their seasonal patterns and diversity relations based on field observation

Considering body volume, Tubificidae were represented in the greatest amounts in benthic ooze and *Limnomysis benedeni* in the water body. Furthermore, the number of Chironomidae and Cladocera specimen were prominent. The average numbers of individuals of taxa are shown in *Table 1*.

In some cases identification at a species level is not as yet finished. Tubificidae species are mostly *Pothamotrix spp*. and *Branchiura sowerbii* Beddard. We identified the larval and juvenile stages of fishes (Cyprinidae) but identification at species level happened in a few cases only. In most cases species are presuably *Rutilus rutilus* L., *Scardinius erythrophthalmus* L. or *Alburnus alburnus* L (one specimen might be *Rhodeus sericeus amarus* Bloch).

Table 1. *Average numbers of individuals of the observed taxa in the examined years (total number of individuals divided by the number of sampling dates in the given year)*

Taxa	2002	2003	2004
HYDROZOA			
Hydra circumcincta Shulze	0.0	0.5	0.7
OLIGOCHAETA			
Tubificidae	91.1	208.2	177.4
Pristina sp.	6.1	15.7	67.3
HIRUDINEA			
Piscicola geometra L.	1.3	0.8	4.3
Glossiphonia heteroclita L.	0.0	0.2	4.1
Erpobdella octoculata L.	0.0	0.2	0.6
Helobdella stagnalis L.	0.4	0.5	1.3
BIVALVIA			
Dreissena polymorpha Pall.	2.7	1.5	3.9
Pisidium sp.	0.0	0.1	0.0
GASTROPODA			
Acroloxus lacustris L.	0.6	7.1	12.4
(other) Gastropoda	0.1	0.1	0.7
ARACHNOIDEA			
Hydrachnidae	0.0	0.8	0.7
Araneidea	0.3	0.4	0.4

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Taxa	2002	2003	2004
CRUSTACEA			
Limnomysis benedeni Czern.	112.3	34.8	186.9
Dikerogammarus sp.	4.2	2.2	3.9
Corophium curvispinum G.O.Sars	2.8	1.2	6.3
Argulus sp.	1.5	0.5	0.3
Leptodora kindtii Focke	43.3	1.7	0.4
(other) Cladocera	64.4	76.1	35.4
Asellus aquaticus L.	0.0	0.2	0.7
Copepoda	4.2	1.4	1.0
COLLEMBOLA	0.0	0.0	0.1
EPHEMEROPTERA			
Caenidae	0.0	1.5	5.3
Baetidae	0.3	11.9	24.3
ODONATA			
Ischnura sp.	0.1	5.2	6.1
Anisoptera	0.0	0.0	0.1
HETEROPTERA			
Aquarius paludum paludum Fab.	0.1	0.5	0.0
(other) Gerridae	0.1	0.5	0.1
Micronecta meridionalis Costa.	0.0	0.2	0.0
Sigara sp	0.0	1.6	0.0
Sigara striata L.	0.0	0.3	0.4
(other) Corixidae	0.0	0.3	0.0
Microvelia sp	0.9	3.0	27.9
Microvelia reticulata Scholtz.	0.0	1.0	0.0
Mesovelia furcata Mulsant & Rey.	0.1	0.1	0.3
Ranatra linearis L.	0.0	0.0	0.1
HOMOPTERA (Aphidinea)	0.0	2.0	1.1
COLEOPTERA	0.4	0.5	0.6
TRICHOPTERA			
Hydroptilidae	0.0	0.5	0.0
Polycentropodidae	0.1	0.0	0.0
Limnephilidae	0.1	0.8	1.1
(other) Trichoptera	0.0	0.0	0.1
DIPTERA			
Chironomidae	42.8	35.8	33.9
Ceratopogonidae	0.8	2.5	0.6
Tipulidae	0.0	0.2	0.1
Tabanidae	0.0	0.0	0.3
Syrphidae	0.0	0.0	0.1
"Diptera puparium"	1.1	1.2	2.6
"Diptera imago"	0.6	0.8	0.9
PISCES (Cyprinidae)	0.8	2.2	5.0

In order to explore temporal patterns we used several ordination and classification methods yielding very similar results. We present our results in ordination plots using NMDS with “Jaccard” similarity measure (*Fig. 2*).

In the ordination plots it is well observable, that the coenological states show a cyclic change throughout the growing season, thus expressing seasonality. Still, in 2002, no true trajectory could be detected. The most irregular groups fall into the period of the beginning of the season, when samples had to be taken in strong waves. In one case (138th day) only the sampling of benthic ooze was successful, this may be regarded as an error. No problem of this kind appeared in the following two years, regular trajectory and well isolated groups are typical of these years.

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In 2003, groups characterising the different periods of the year can be separated more minutely than in 2004, which might be the result of more frequent sampling.

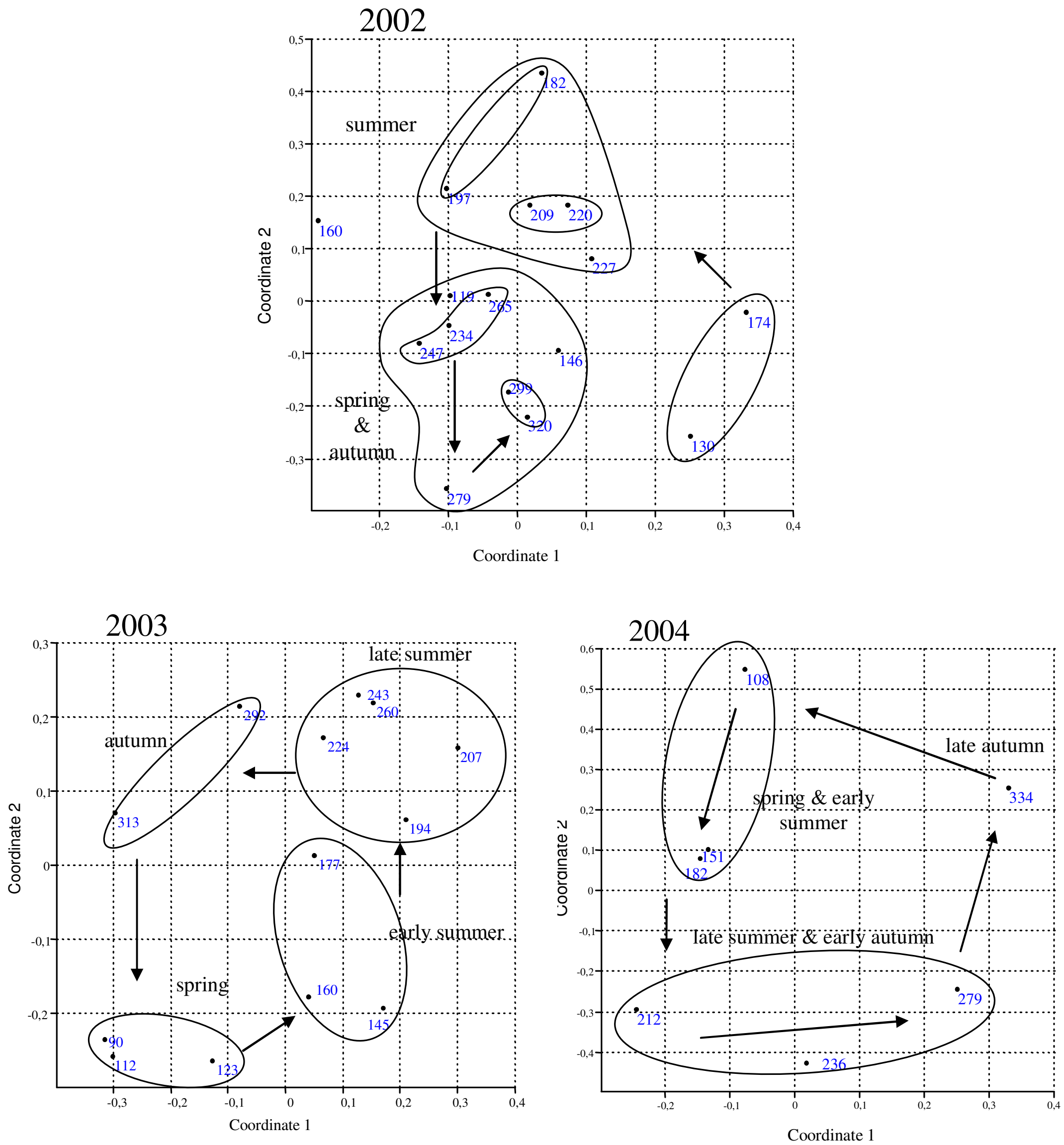

Figure 2. *Change of the coenological state of the macroinvertebrate assembly and similarity pattern of dates (ordination, NMDS, Jaccard index), in the three years examined (2002, 2003, 2004). Serial numbers of sampling dates are shown.*

The frequent and abundant morphons (all Tubificidae and Chironomidae, most body size categories of *Limnomysis benedeni* and *Dikerogammarus sp.* of medium size) were found throughout the year. Early spring proved to be the poorest in morphons in all three years, larger specimen of the frequent species characterise this period (larvae of Chironomidae and *Limnomysis benedeni*). In 2002, the early spring group is not so isolated which might be the result of the relatively late date of the first sampling (29th April) and the interfering effect of extreme weather conditions. Larval and juvenile fish are typical in the early summer. The largest numbers of morphons were observed late in

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the summer (e.g. most Heteroptera, larvae of Ephemeroptera, Odonata and Trichoptera). In the autumn again, less morphons could be detected (mainly zebra mussel and snails in most body sizes, imago of *Sigara striata*) and late in the autumn, we only found larger specimens of even less macroinvertebrate taxa (e.g. *Piscicola geometra*).

To compare diversity relations between three years, we applied Rényi's diversity ordering based on morphons (*Fig. 3*). 2002 proved to be the least diverse year but it is not possible to order because its diversity profile intersects with the one of 2003. Based on the position and shape of the profiles we may assume that 2003 was definitely richer in rare morphons but 2002 in dominant morphons. The year 2004 was the most diverse in every domain of the scale parameter, but 2003 is very close if we consider rare morphons.

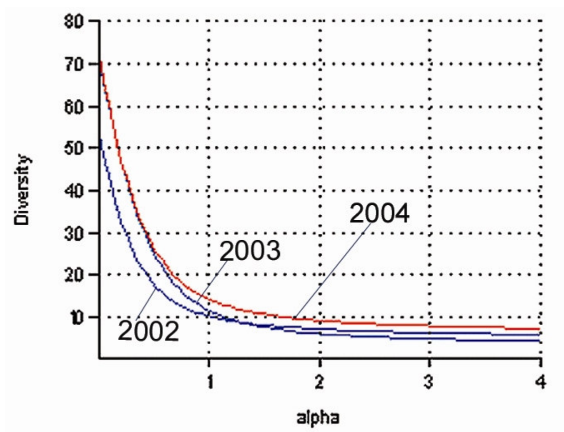

Figure 3. *Diversity profiles of the examined three years (Rényi's diversity ordering)*

For the demonstration of annual diversity trends we plotted the Shannon Diversity Index and Berger–Parker Dominance Index (*Fig. 4*). The years 2002 and 2003 are very similar in many respects: considerable decline can be observed in June (while the dominance index is increasing) and the most diverse date is in August. However, in 2002, the diversity value remains high until the end of autumn while in 2003, there is a drastic decline during this period (as the dominance index increases). Extremely high dominance values were recorded during this droughty year compared to the other years. The most diverse period in 2003 coincides with the appearance of *Najas marina* stands, reaching a higher maximum diversity value than in 2002 when reed-grass stands were not present. These observations call attention to the importance of reed-grass. Phenomena observed in 2004 confirm this theory, as high diversity values were recorded from the beginning of summer until the end of autumn, with the highest diversity value in October. *Potamogeton perfoliatus* stands were present during the whole growing season and in October a significant amount of reed-grass accumulated in front of the emergent macrophyte stands. The rule of dominant macroinvertebrates characterizes the beginning and the end of the growing season.

The seasonal dynamics of the two most abundant macroinvertebrate taxa are shown in (*Fig. 5*). The year 2002 was quite diversified: Tubificidae were more dominant until the end of June, then the quantity of the two macroinvertebrate taxa were roughly equal, that was followed by a period in which the alternate peaks of the two macroinvertebrate taxa appeared.

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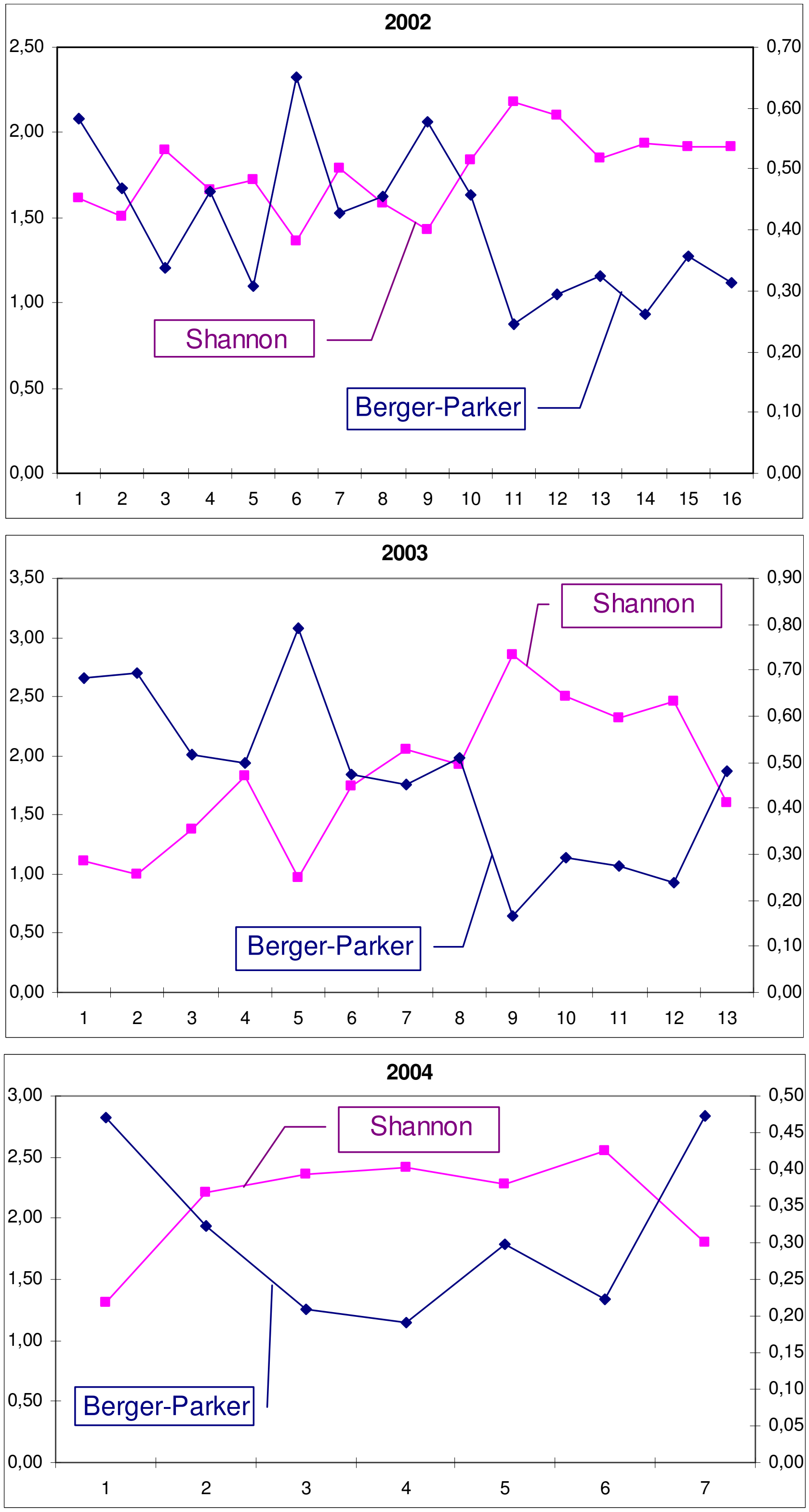

Figure 4. *Temporal change of the Shannon Diversity Index and Berger-Parker Dominance Index in the years 2002, 2003 and 2004*

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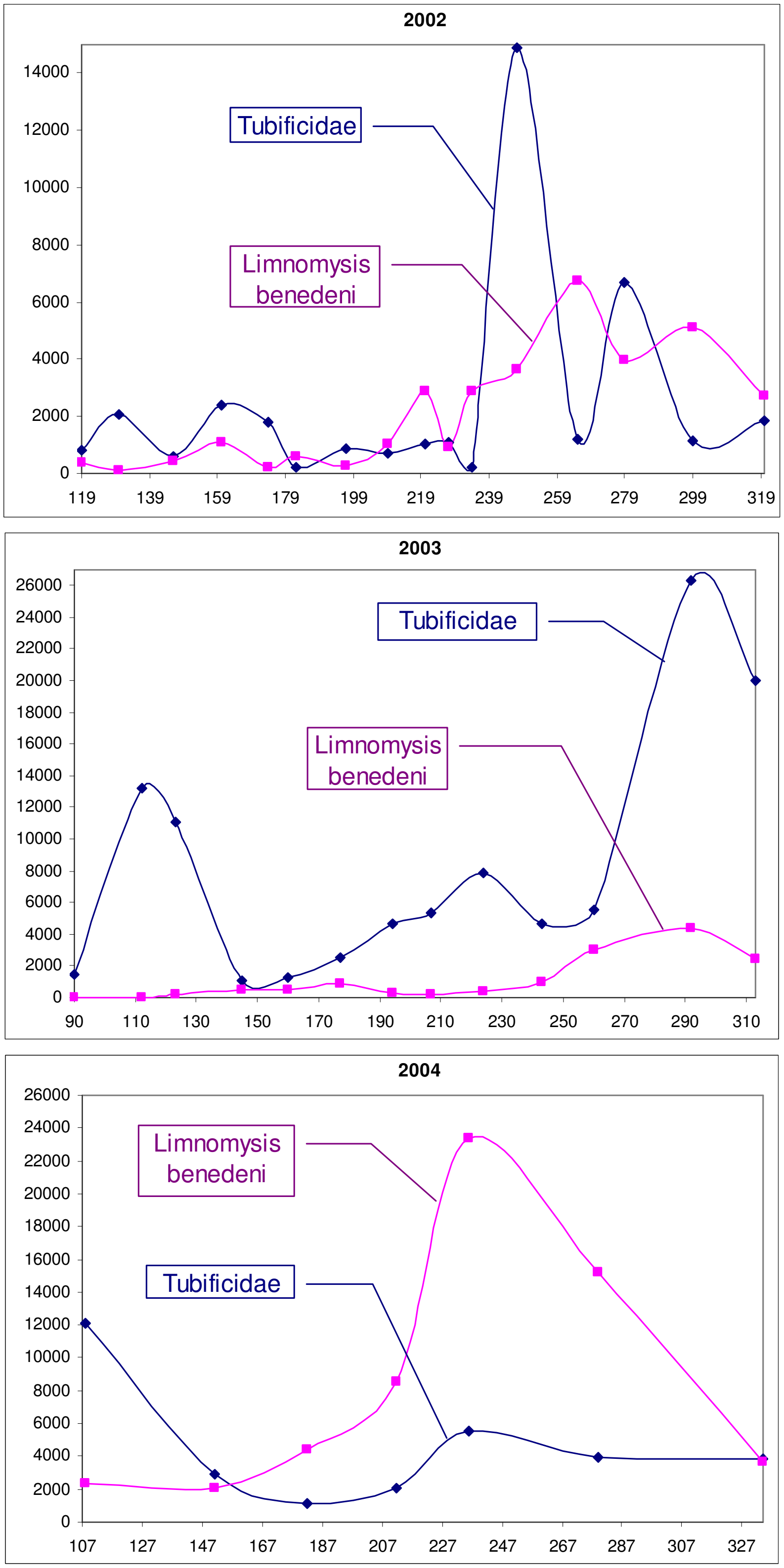

Figure 5. *Seasonal changes in the biovolume values (mm^3) of Limnomysis benedeni and Tubificidae over three years (the serial numbers of days are shown on axis x)*

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The quantity of Tubificide was larger in the extremely droughty year of 2003, while in 2004, the volume of *Limnomysis benedeni* was significantly higher from the beginning of the summer.

Based on these characteristic facts it can be presumed, that the extremely low water level is favourable primarily for benthic organisms, mainly the detritivorous Tubificidae, while a higher level is to the advantage of the zoophagous *Limnomysis benedeni* preferring waters with more plants. All this is confirmed by the fact that the highest biovolume value of Tubificidae was recorded on 19th October 2003 when the water level of Lake Balaton was the lowest.

A weather-dependent simulation model of the seasonal changes of Coenological Intensity Index (CII) and the comparison of climate change scenarios

The temporal changes of the observed and the simulated values of CII over three years are shown in (*Fig. 6*). In the figure it can be clearly seen, that the model values fit well to the observed ones. As the temperature reaction curve of the model is practically the result of the sum of two normal distributions, two theoretical populations are enough to describe the temporal trends of CII characterising the whole assembly under examination. This might be in connection with our earlier conclusion, that there were two determinant groups in the examined macroinvertebrate population: Tubificidae and *Limnomysis benedeni* [20].

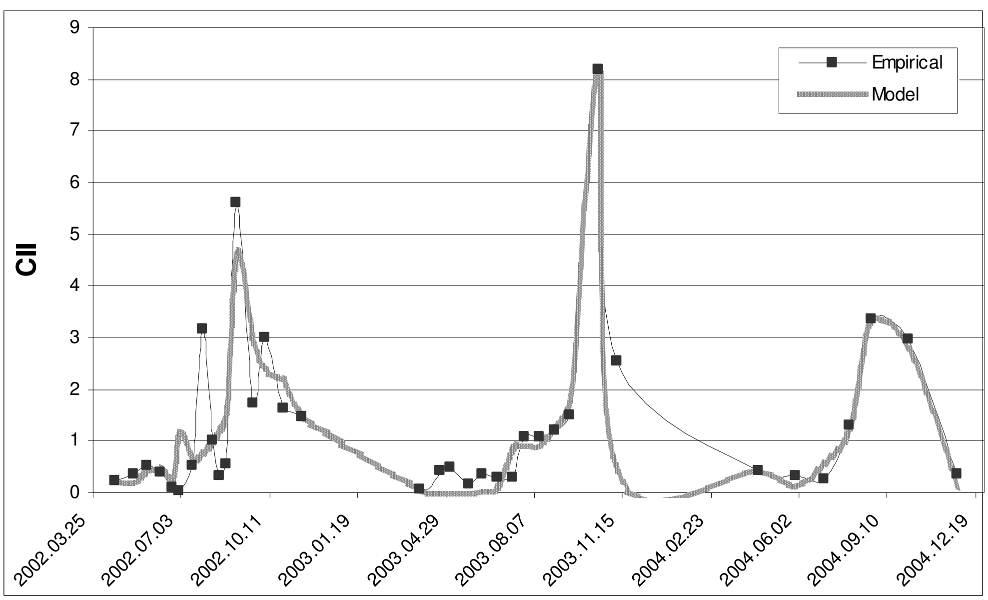

Figure 6. *The observed and the simulated values of CII during the three examined years*

We ran the model for the three available daily data series of A2 and B2 scenarios, examining 31 running year each case. We considered the date of the supervening of the maximum value of CII (on which day of the year it occurs) as an indicator and the results of a one-way ANOVA for this indicator showed a significant difference between the scenarios *(Table 2).* The results of Tukey's pairwise comparison *(Table 3)* proved the A2 scenario of the Max Planck Institute to be significantly different from the A2 and B2 scenarios of the Hadley Centre, the latter two are showing a considerable similarity.

Table 2. *The ANOVA table of the Variance Analysis made for the date of the supervening of the maximum values during the model ran for the three scenarios.*

	SS	df	MS	F	p (same)
Between Gr.	1.26E+05	2	6.31E+04	16.24	9.49E-07
Within Gr.	3.50E+05	90	3.88E+03		
Total	4.76E+05	92			

Table 3. *The results of Tukey's pairwise comparison (Tukey-test value /* ***p(same)****)*

	A2 (HC)	B2 (HC)	A2 (MPI)
A2 (HC)		**0.9817**	**0.000119**
B2 (HC)	0.2594		**0.000112**
A2 (MPI)	6.847	7.106	

CII reached it's maximum in the second half of the years under survey, between the end of August and the beginning of October (in 2002 on the 247th day in the beginning of September, in 2003 on the 292nd day in the beginning of October and in 2004 on the 236th day in the end of August). If we examine the supervening of the maximum values based on different scenarios *(Table 4)* we find these days shifting to earlier dates as much as to the beginning of the year. This phenomenon is particularly prominent in the case of the Hadley Centre scenarios, where in most of the 31 predicted years the date of maximum value falls to early spring. The A2 scenario of the Max Planck Institute also indicates a shift of this day to earlier dates, but in most cases this means dates in early summer rather than early spring.

Table 4. *Table of the attributes of the model fitted to the 31 projected years of the A2 and B2 scenarios of Hadley Centre (HC) and the Max Planck Institute (MPI). The first column indicates the intervals of the year (expressed in serial numbers of the day of the year) and intervals of CII. In the other cells the percentage of the projected 31 years falling to the given interval is shown, considering the date of the supervening of the annual maximum CII value, the value of annual maximum and the annual total CII.*

Date of Maximum CII value (Day of Year)	A2 (HC)	B2 (HC)	A2 (MPI)
under 110	62%	71%	16%
111-230	32%	23%	49%
231-300	6%	3%	16%
above 301	0%	3%	19%
Value of Annual Maximum CII	A2 (HC)	B2 (HC)	A2 (MPI)
under 2	61%	64%	13%
3-9	16%	13%	16%
10-50	13%	13%	13%
above 51	10%	10%	58%
Annual Total CII	A2 (HC)	B2 (HC)	A2 (MPI)
under 50	55%	52%	10%
51-500	29%	36%	19%
501-1000	3%	6%	6%
above 1000	13%	6%	65%

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With the help of *Table 4*. we can understand the differences between the scenarios based on an annual maximum CII and annual total CII values. It is obvious that the scenario of the Max Planck Institute definitely differs from the two scenarios of Hadley Centre regarding both indexes. The former predicts much higher maximum values then the ones observed (CII being between 3 and 10) but the latter ones forecast a shift towards regions lower then the observed values in most cases. The annual total quantity shows similar trends: the scenario of the Max Planck Institute predicts much higher values than the others.

These features may clearly be explained by the difference between the temperature data series of the scenarios. The data series of the A2 scenario of the Max Planck Institute significantly differs from those of the two scenarios of Hadley Centre regarding either maximum *(Fig. 7)* or minimum temperature values (*Fig. 8.*) while no significant difference between the A2 and B2 scenarios of the latter institute can be observed.

This means the data of the runs of the Hadley Centre contain a higher number of extremely high temperatures but also forecast lower minimum values. The data of the Max Planck Institute are comparatively less deviated.

Confronting all this with the results of our CII model we can presume that a shift towards extreme weather leads to a fall of the index values regarding either maximum or annual total quantities. Less extreme scenarios with lower summer maximum temperature values may cause an increase of index value.

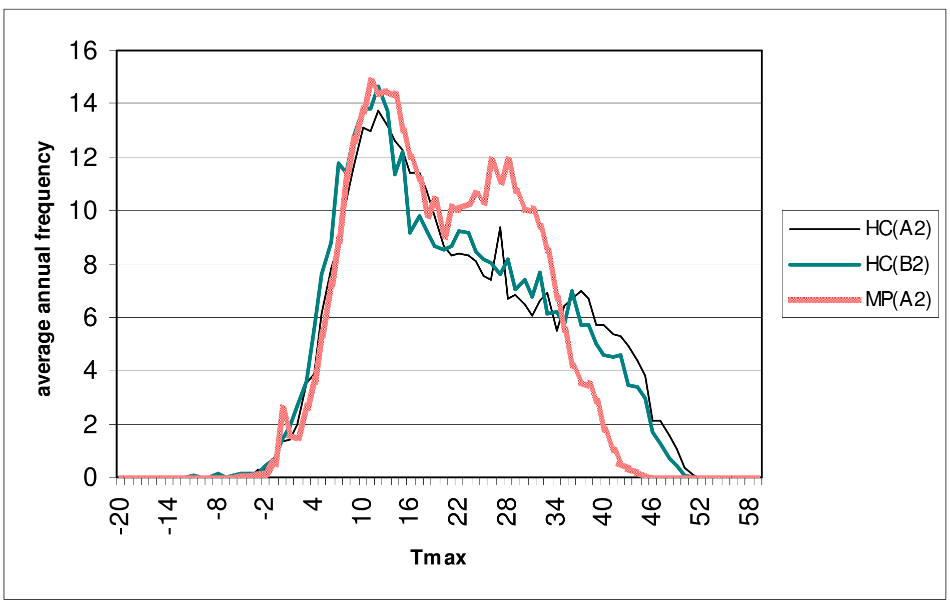

Figure 7. *Annual mean frequency of the maximum temperature values of A2 scenario of the Max Planck Institute (MPI) and for A2 and B2 scenarios of Hadley Centre (HC), downscaled for the region of the Siófok Meteorological Station*

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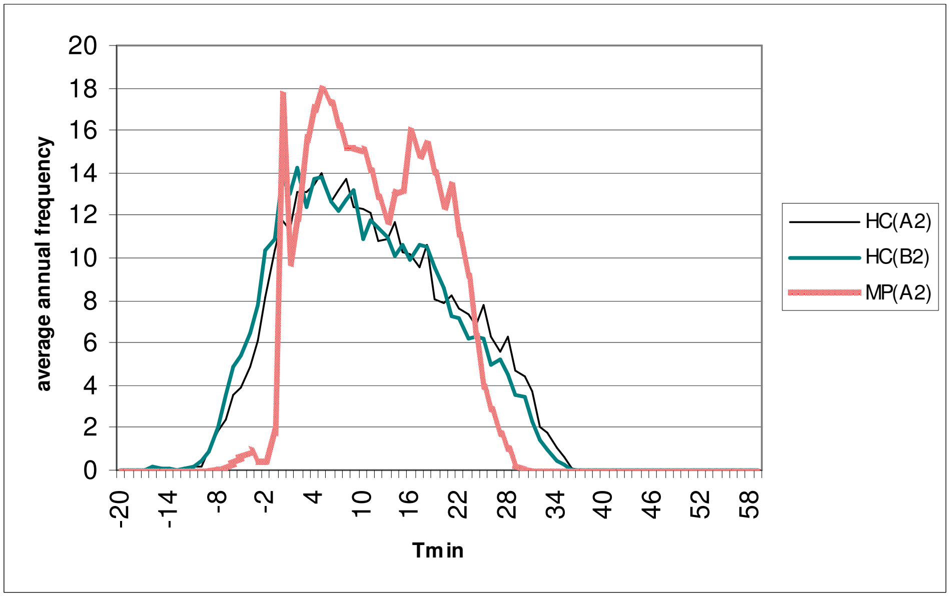

Figure 8. *Annual mean frequency of the minimum temperature values of A2 scenario of Max Planck Institute (MPI) and for A2 and B2 scenarios of Hadley Centre (HC), downscaled for the region the Siófok Meteorological Station*

Summary and conclusion

We attempted to explore seasonal patterns in a higher scale with the help of our series of samplings executed over several years at a site located in Lake Balaton, characterised by a vegetation of reed and reed-grass. The results call attention to the importance of more frequent sampling in order to explore groups characteristic for certain periods of the year in detail. In view of the final exclusion of deviations presumably caused by extreme weather events in 2002 the similarity (standardity) of the prevailing weather conditions for the time of sampling should gain greater attention. In light of all this, continued sampling shall lead us to a more detailed description of a circle of changes of states expressing seasonality, making possible a separation of morphon groups – or, using the classic term of coenology [1], aspects – characterising the different periods of the year [7].

Changes of water level and the presence of reed-grass vegetation had great influence on macroinvertebrate fauna. The effect of reed-grass was most prominent in increasing diversity, and that of decreasing water level in the stronger dominance of Tubificidae.

To model seasonal dynamics of macroinvertebrates we defined a „Coenological Intensity Index", a combined index expressing the number of individuals, body volume and diversity. We created a model based exclusively on daily temperature values, capable of producing data series fitting well to the empirical values of CII. We ran the model for the data series predicted by the available A2 and B2 scenarios for the period between 2070-2100. Our results show that CII will reach its maximum much earlier, quite in the beginning of the year instead of the early autumn dates observed in recent years. However, the scenarios give different answers to the question of whether the total CII values will increase or decrease. These differences may clearly be explained by the

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difference between the temperature data series of the institutes. We can admit, that in the case of scenarios forecasting higher maximum and lower minimum values the value of the index will decrease.

In our modelling work we use series of detailed data collected over a period of three years. The utilization of longer data series would make trophic approach possible e.g. through the modelling of trophical guilds. With the modelling of combined groups, dominant species and using the method introduced in our study we might get a much more complex picture of the possible outcomes of climate change.

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INFLUENCE OF DIFFERENT FOOD SOURCES ON GROWTH AND REPRODUCTION PERFORMANCE OF COMPOSTING EPIGEICS: *EUDRILUS EUGENIAE, PERIONYX EXCAVATUS* AND *PERIONYX SANSIBARICUS*

S. SUTHAR

Department of Zoology, J.N.V. University, Jodhpur – 342001, INDIA

Environment Biology lab, Department of Zoology, M. D. (PG) College, Sri Ganganagar 335 001, India (phone: + 91-0154-2470452 (R), fax: +91-0154-2475960)

e-mail: sutharss_soilbiology@yahoo.co.in, sutharss_biosoil@rediffmail.com

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Abstract. The impact of organic material quality on biomass production and reproduction potential of commercial composting earthworm species: Eudrilus eugeniae, Perionyx excavatus and Perionyx sansibaricus were studied, by using three different type of culture material namely Jbs: (Jowar straw (*Sorghum vulgare*) + bajra straw (*Pennisetum typhoides*) + sheep manure) (1:1:2), fym (farmyard manure), and Kw + Ll (Kitchen waste + leaf litter of *Magifera indica*) (1:1), under laboratory conditions for 150 days. The above substrate or culture materials have different palatability, particle size and physio-chemical composition. Kitchen waste (C-to-N = 26.7) as well as farmyard manure (C-to-N = 27.4) is a high quality material with fast decomposition rates, while crop residues are low quality materials with slow decomposition rates (C-to-N = 45.6). All the three studied earthworm species showed maximum biomass production rate in Kw + Ll culture (*E. eugeniae* = 9.80 ± 0.01 mg $worm^{-1}$ day^{-1}, *P. excavatus* = 3.75 ± 0.01 mg $worm^{-1}$ day^{-1}, and *P. sansibaricus* = 3.77 mg $worm^{-1}$ day^{-1}). Individual cocoon production rate varied drastically, and maximum value ($worm^{-1}$ $week^{-1}$) of it was observed in Kw + Ll for *E. eugeniae* (1.88 ± 0.15) and *P. sansibaricus* (1.77 ± 0.14), while *P. excavatus* showed maximum cocoon production rate in Fym (1.79 ± 0.17). The hatchling success of cocoons obtained from different beddings was also observed and cocoon obtained from Kw + Ll culture exhibited maximum hatchling success (%) in all the three species studied. The cocoons of both *E. eugeniae* and *P. sansibaricus*, obtained from Fym culture showed the highest number of hatchlings ($cocoon^{-1}$) i.e. 1.59 ± 0.04 and 1.81 ± 0.10, respectively, whereas cocoons of *P. excavatus* showed the highest hatchling number (1.77 ± 0.06) in Kw + Ll. In this present study, there was a consistent trend of decreasing individual biomass as well as cocoon production rate, followed by their peak values with ageing of the culture materials. The relationship between different earthworm parameters and N-content or C-to-N of culture material was also evaluated. The biomass production rate and hatching success in all the three species studied showed direct correlation ($p<0.05$) with N-content of the culture material. However, beddings' N-content did not affect the individual cocoon production rate, except to *P. sansibaricus* ($r = 0.987$, $P<0.001$). The number of hatchlings per cocoon for *P. sansibaricus,* showed good correlation ($r = 0.935$ $p<0.01$) with N-content of organic material used for worm culture. Results clearly show a possible relation between hatchling success/number of hatchlings per cocoon, and chemistry of culture substrate.
Keywords: o*rganic waste, vermiculture, Eudrilus eugeniae, Perionyx excavatus, Perionyx sansibaricus, Cocoon, Hatchlings success, Hatchling number*

Introduction

Vermicomposting is a biooxidation and stabilization of organic material involving the joint action of earthworms and microorganisms [1], where negligible organic waste resources are converted into nutrient rich plant growth media i.e. vermicompost. Studies reveal that epigeics seem well fitted for such kind of operations, because of their surface activity, their ability to colonize organic material quickly, and in the process to convert it into useful compost and their ability to minimize malodour formation. However, involvement of earthworms in composting process decreases the time of stabilization of the waste and produces an organic pool with energy reserves as vermicompost [38]. The composting biology and ecology of earthworms in waste resources are controlled by several abiotic and biotic factors: temperature, moisture, pH, substrate, feed and its palatability, particle size, earthworm density and fecundity, which directly or indirectly influence the composting potential of the composting species.

Several earthworm species e.g. *Eisenia fetida, Eisenia andrei, Eudrilus eugeniae, Perionyx excavatus* have been identified as potential candidates for managing organic waste resources. World-wide spread *E. fetida* was and still remains a favoured earthworm species for vermicomposting operations due to their wide range of tolerance for environmental variables. The growth patterns *E. fetida* in number of different organic waste resources have been investigated by various authors in laboratory culture [9,12,18,17]. However, in tropical and sub-tropical conditions earthworm: *P. excavatus*, *P. sansibaricus*, and *E. eugeniae* appeared as best vermicomposting species. The life cycle parameters of above tropical species has been investigated in respect to their environmental requirement like temperature [37], moisture [13], fecundity and density pressure [16], pH [31], but influence of culture material or substrate quality on their life cycle parameters is less considered. Although, animal dung is recognized as a suitable earthworm culture media, but other organic waste material from agriculture and industry have also proved successful. The chemical structure of given culture media for large scale vermiculture practices is always important. Besides to high concentrations of nutrient in plant origin wastes, some secondary metabolites e.g. polyphenols are also important, which directly or indirectly influence the composting potential as well as growth patterns of given composting species during vermicomposting practices. The impact of such kind of wastes on life cycle parameters of composting worms must be of a research priority.

The aims of this study were three-fold. First, to establish whether types of substrate or feeding material effect the biomass and cocoon production patterns in composting earthworm species: *Eudrilus eugeniae* Kinberg, *Perionyx excavatus* (Perrier, 1872) and *Perionyx sansibaricus* (Perrier, 1872), which are very popular for commercial vermitechology programs in many part of the world. Second, to confirm the fact that earthworm shows species-specific sensitivity for C-to-N ratio or for other chemical substances presented in the substrates [27]. Does all earthworm species showed specific behaviour for physio-chemical quality of feeding stuff, or they are more sensitive for N-content or some other content of it? Third, to investigate, by means of a cocoon hatchling experiments, whether the quality of organic material is important factor for production of viable cocoons and for hatchling numbers per cocoon. Knowledge about the effect of feed quality on hatchling success and number of hatchlings per cocoon is very scared in scientific literature. In present study three different types of culture materials: crop residues with animal dung, farm yard manure, and kitchen waste with leaf litter were selected for experimentation, mainly due to their different chemical, microbial and physical nature.

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Review of literature

The biology of composting earthworms had been investigated in terms of their certain fundamental requirements, for instance the temperature [17,23,28,37], pH [17,31], density or stocking rate [16,30], substrate [4,7], and moisture content of the substrate [13,29]. The effect of bedding substrate on biological parameters such as biomass production, cocoon numbers, hatchling success in popular composting earthworms is still unanswered. A review by Lowe and Butt [24] demonstrated the recent research on the development of earthworm culture techniques for temperate, soil dwelling (anecic and endogeic) species: *Allolobophora chlorotica, Aporrectodea caliginosa, Aporrectodea longa* and *Lumbricus terrestris.* In this paper they summarized the optimum requirements of these species for laboratory-based culture. Nevertheless, for commercial based vermicomposting practices much of the research have been focused on *Eisenia fetida* [8,14,18,22,25,36]; *Lumbricus rubellus* [5,19] and *Dendrobaena veneta* [10,20,21]. Some tropical composting earthworms namely *Eudrilus eugeniae, Perionyx excavatus* and *Perionyx sansibaricus* had been getting much attention among the commercial worm growers in both tropical and sub tropical countries. Unfortunately, little is known about their nutrition for mass rearing. However, among the different variables necessary for raising earthworms, it seems that the type of feeding material or substrate is most important. According to Edwards [9] the type, quality and quantity of the organic wastes were very important to determining the rates of growth of earthworms. In general, for large-scale vermiculture practices the knowledge of biological requirement of candidate species must be pre-determined and their optimum requirement concerning nutritional factors might be an active field of research in earthworm biotechnology [3, 24]. However, different earthworm species are impacted differently by nutritional status for a specific earthworm species [33, 34], and a specific feed mixture [35]. Recently Singh et al. [31] demonstrated the effect of initial substrate pH on vermicomposting potential of *P. excavatus.* They concluded that the earthworm species *P. excavatus* performs well in a wide range of substrate pH, but neutral initial pH of substrate was found to be suitable for stabilization of waste with minimal processing time. It is well understand that the quality and amount of food material influences not only the size of population but also the species present and their rate of growth and cocoon production [12, 23, 35]. However, earthworm biomass and cocoon production rate can acts useful biomarkers to measure the efficiency of earthworm in vermiculture operations. Hendricksen [15] suggested that C-to-N ratio and particularly polyphenols concentration are the most important factor determining litter palatability in detrivorus earthworms. It is very important to evaluate the influence of feed stocks on life cycle of tropical earthworms.

Materials and methods

Preparation of feeding material

Three different type of feeding materials namely Jbs (Jowar straw + bajra straw + sheep manure) (1:1:2), fym (farmyard manure) and Kw + Ll (Kitchen waste + leaf litter of *Magifera indica*) (1:1) was used during present study. Jbs was prepared from the dry straw of *Sorghum vulgare* (Jowar straw); *Pennisetum typhoides* (bajara straw) and sheep

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manure, mixed in 1:1:2 ratios. All plant materials were obtained from a local agriculture field, Jhalamand village, Jodhpur, India. Urine free sheep manure was obtained from a local sheep farm and 1-week-old farmyard manure was collected from Jhanwarangarh Agriculture Farm, Kakani Road, Jodhpur. Kitchen waste was collected from P.G. Boys Hostel, New Campus, J.N.V. University, Jodhpur.

All the three prepared culture beddings were kept for prior microbial composting and thermostabilization for 15 days in large closed container with small drain holes drilled in the bottoms. The moisture content of the beddings was maintained 60 – 70 % by sprinkling water regularly.

Earthworm culture

100 gm of pre-composted organic material was taken in plastic containers (20 cm height, 28 cm diameter) with plastic lids pierced by aeration holes. Five juveniles (one week old) of *Eudrilus eugeniae*, *Perionyx excavatus* and *Perionyx sansibaricus,* each in the range of 9.9 – 14.6 mg fresh weight, were collected from stock culture in laboratory. These juveniles were pre weighed and inoculated in experimental containers. Each experiment was replicated five times. The earthworm were counted and weighed every week for 22 weeks. The total number of cocoons produced in different experimental containers was counted during each week.

For hatchling experiment fresh cocoon of all the three species were collected from each experimental bedding type. For this ten cocoon of each species were placed in Petri dished, containing the parental bedding material (material where cocoons were laid down originally). Petri plates of each culture type were replicated five times for each earthworm species studied. Petri plates were observed regularly for hatchling of cocoons. Simultaneously number of hatchlings produced from each cocoon was also calculated. The hatchling success was calculated in percent by counting total number of hatched cocoons in each Petri plate. Newly emerged earthworms (juveniles) were transferred in separate containers contained the same parental material and the total earthworm production was calculated at the end of the experiment. The experimental containers were incubated in dark and humid palace at room temperature (28.9 ± 0.53°C).

Chemical analysis

The organic bedding material was analyzed for organic C, total N, total phosphorus, potassium, C-to-N ratio *(Table 1).* Organic C was determined by Walkley – Black method. Nitrogen was determined by following the microkjeldhal method. Phosphorous was estimated by using Tecator model 5012 auto analyzer. The amount of exchangeable potassium was analyzed at Perkin Elmer model 3110 double beam atomic absorption spectrophotometer.

Table 1. *Chemical properties of different culture materials.*

Culture Material	Carbon (g kg^{-1})	Nitrogen (g kg^{-1})	Phosphorous (g kg^{-1})	Potassium (g kg^{-1})	C to N ratio
Jbs	477.14 ± 0.05	10.44 ± 0.05	4.05 ± 0.01	4.18 ± 0.02	45.57 ± 0.16
Fym	348.56 ± 0.08	12.62 ± 0.22	6.73 ± 0.04	9.86 ± 0.03	27.44 ± 0.56
Kw + Ll	389.62 ± 0.24	15.56 ± 0.03	5.76 ± 0.10	12.42 ± 0.03	26.76 ± 0.04

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Statistical analysis

Data were subjected for analysis of variance (ANOVA) followed by Duncan's multiple-ranged tests to differentiate the statistical difference between results of earthworm growth, cocoon production rate, hatchling success, number of hatchlings cocoon^{-1}, and total earthworm population in different culture material. Pearson's correlation coefficient was also calculated between chemical parameters of culture material and different parameters of earthworms.

Results

Earthworm growth

The earthworm biomass production in different feeding mixture is illustrated in *(Fig 1 (a, b, c))*. Statistically both feed mixtures (or culture material) and species type showed significant (P<0.01) difference in biomass production patterns *(Table 2)*.

Table 2. *ANOVA table*

	df^b	F	P
Cocoon production			
Species	2	10.61	< 0.001
Feeding material	2	77.98	< 0.001
Species × Feeding material	4	24.81	< 0.001
Biomass production			
Species	2	19378.58	< 0.001
Feeding material	2	1615.55	< 0.001
Species × Feeding material	4	326.5936	< 0.001
Hatchling success			
Species	2	2.781372	0.088
Feeding material	2	34.13211	< 0.001
Species × Feeding material	4	2.237684	0.105
Hatchlings cocoon^{-1}			
Species	2	0.725048	0.497
Feeding material	2	8.986634	0.001
Species × Feeding material	4	10.97509	< 0.001

b Error *df*= 18

Table 3. *Earthworm biomass (mg) (mean ±SEm; n=5) production in different culture material*

Culture Material	Eudrilus eugeniae			Perionyx excavatus			Perionyx sansibaricus		
	At start	At end	Growth Rate*	At start	At end	Growth Rate*	At start	At end	Growth Rate*
Jbs	14.6 ± 0.16	1129.5 ± 3.12[a]	7.24 ± 0.02[a]	9.9 ± 0.22	358.4 ± 1.81[a]	2.26 ± 0.02[a]	10.4 ± 0.05	476.8 ± 2.36[b]	3.03 ± 0.01[b]
Fym	14.0 ± 0.12	1151.4 ± 3.28[b]	7.39 ± 0.02[a]	10.3 ± 0.09	539.7 ± 1.56[b]	3.44 ± 0.03[b]	9.8 ± 0.09	445.5 ± 2.37[a]	2.83 ± 0.03[a]
Kw + Ll	14.1 ± 0.05	1522.9 ± 1.10[c]	9.80 ± 0.01[b]	10.2 ± 0.05	587.3 ± 4.46[c]	3.75 ± 0.01[c]	9.5 ± 0.12	589.6 ± 3.63[c]	3.77 ± 0.02[c]

* (mg worm^{-1} day^{-1})
The mean values followed by different letters are statistically different (ANOVA, Duncan's multiple-ranged test; p< 0.05)

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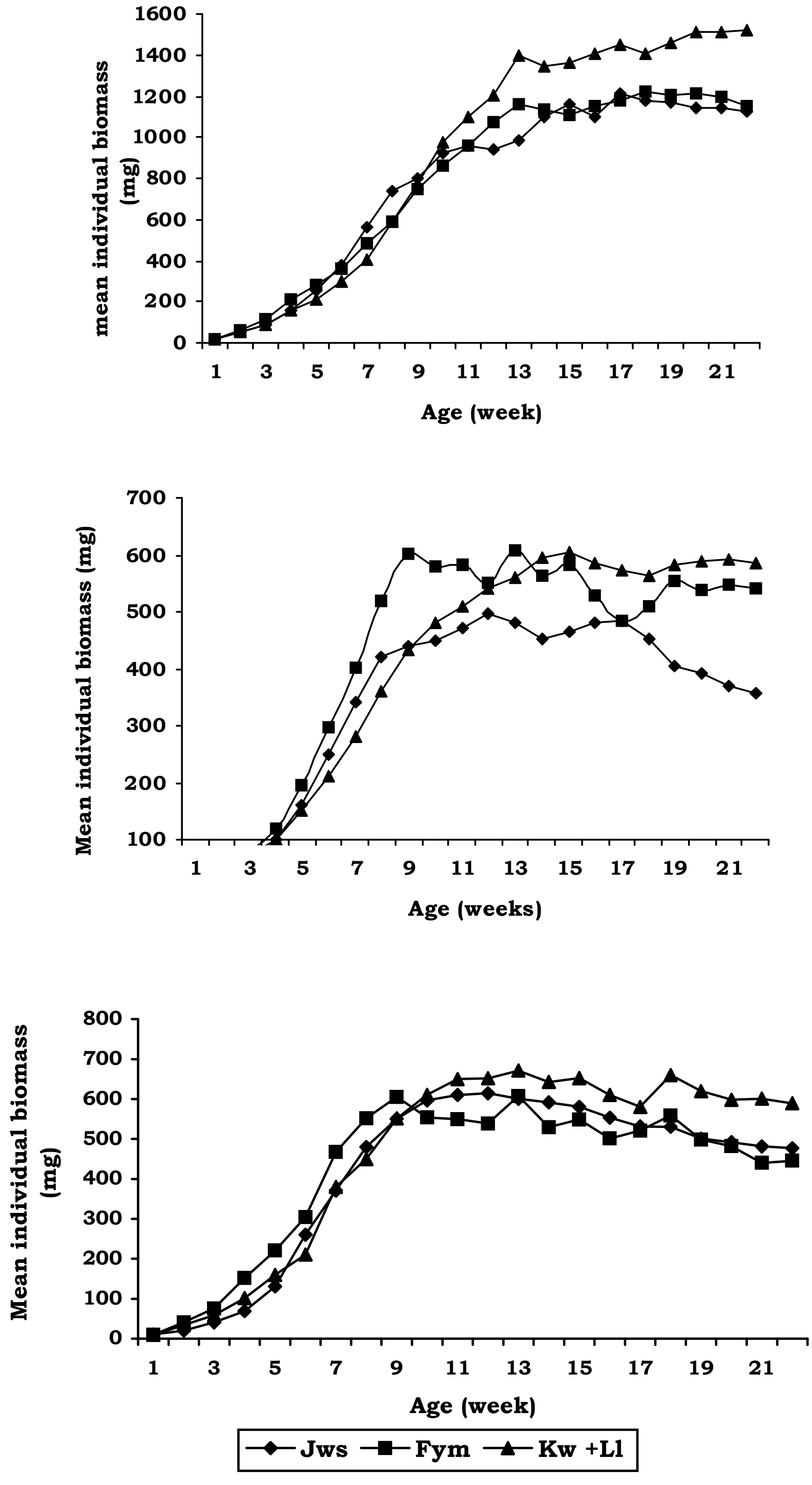

Figure 1. *The growth of E. eugeniae (a), P. excavatus (b) and P. sansibaricus (c) in different waste materials.*

E. eugeniae showed maximum mean individual biomass (1522.9 ± 1.10 mg) on Kw + Ll followed by Fym, and Jbs culture. The maximum individual biomass of *E. eugeniae* on Kw + Ll was 34.8 and 32.3 % higher than Jbs and Fym culture, respectively *(Table 3).* In *E. eugeniae* maximum mean individual biomass was reached after 17 week (1210.6 ± 1.01 mg) on Jbs, after 18 week (1220.4 ± 4.15 mg) on Fym, and after 22 week (1522.9 ± 1.10 mg) on Kw + Ll *(Fig. 1 (a)).* At the end of experiment the maximum mean individual biomass in *P. excavatus* was registered with Kw + Ll (587.3 ± 4.46 mg) followed by fym, and Jbs *(Table 3).* The maximum mean biomass of *P. excavatus* was 63.9 % and 8.8 % higher than the individual biomass on Jbs and fym, respectively. *P. excavatus* achieves its' peak of individual biomass after 12 week, 13 week, and 15 week on Jbs (495.7 ± 4.67 mg), Fym (608.6 ± 2.94 mg), and Kw + Ll (603.3 ± 3.97 mg), respectively *(Fig. 1(b)).* In both Fym and Kw + Ll cultures, *P. excavatus* showed excellent biomass increase up to achieving the peak, afterward a clear stabilization was observed, while in Jbs a drastic decline in biomass was noted up to end of the experiment. *P. sansibaricus* showed maximum mean individual biomass on Kw + Ll (589.6 ± 3.63 mg) followed by Jbs and Fym at the end of the experiment *(Table 3).* The maximum mean biomass of *P. sansibaricus* was 23.7 and 32.3 % higher than Jbs and Fym, respectively. *P. sansibaricus* showed more or less stabilized pattern for biomass production after achieving peak values of it in Fym and Kw + Ll, but on Jbs a gradual linear decline was noted up to end of the experiment *(Fig. 1 (c)).*

Cocoon production

The cocoon production patterns of all the three studied species in different culture material have been illustrated in *(Fig. 2 (a, b, c)).* There was consistent pattern of influence of the culture material ($P < 0.001$), as well as species type on total cocoon production ($P < 0.001$) *(Table 2).* Earthworm in most of the culture beddings started to produce cocoons after 7-8 weeks. In *E. eugeniae* maximum cocoon numbers (worm^{-1}) and reproduction rate (cocoon worm^{-1} week^{-1}) was recorded on Kw + Ll culture (150.67 ± 5.51 and 1.88 ± 0.15, respectively) followed by Fym and Jbs *(Table 4).* The highest cocoon number in Kw + Ll was, respectively 54.3 and 18.3 % higher from that of Jbs and Fym culture. The peak value of cocoon production rate (cocoon worm^{-1} week^{-1}) in *E. eugeniae* was recorded on Jbs, Fym, and Kw + Ll in 18th week (1.87 ± 0.20), 18th week (2.53 ± 0.22), and 16th week (2.80 ± 0.25) of culture, respectively *(Fig. 2 (a)).*

P. excavatus, exhibited maximum cocoon numbers and cocoon production rate on Fym (143.0 ± 4.33 and 1.79 ± 0.17, respectively) followed by Jbs, and Kw + Ll *(Table 4).* The maximum cocoon production on Fym culture was 34.5 and 37.9 %, higher than that of Jbs and Fym, respectively. Similarly, *P. excavatus* exhibited peak value of reproduction rate in Jbs (2.27 ± 0.20), Fym (2.67 ± 0.24), and Kw + Ll (2.07 ± 0.14) in 18th, 16th and 19th week of culture *(Fig. 2 (b)).* The maximum number of cocoons and individual reproduction rate in *P. sansibaricus* was recorded in Kw + Ll (141.67 ± 2.33 and 1.77 ± 0.14, respectively) followed by Fym and Jbs culture. The greater cocoon numbers on Kw + Ll was 108.3 and 22.8 % higher than that of Jbs and Fym culture, respectively. *P. sansibaricus* showed its' peak reproduction rate on Jbs (1.33 ± 0.10), Fym (2.27 ± 0.05), and Kw + Ll (2.67 ± 0.05) in 16th & 19th, 17th and 18th week of culture *(Fig. 2 (c)).* However, there was a tendency for the mean cocoon production rate to decrease relatively to the ageing of the bedding materials.

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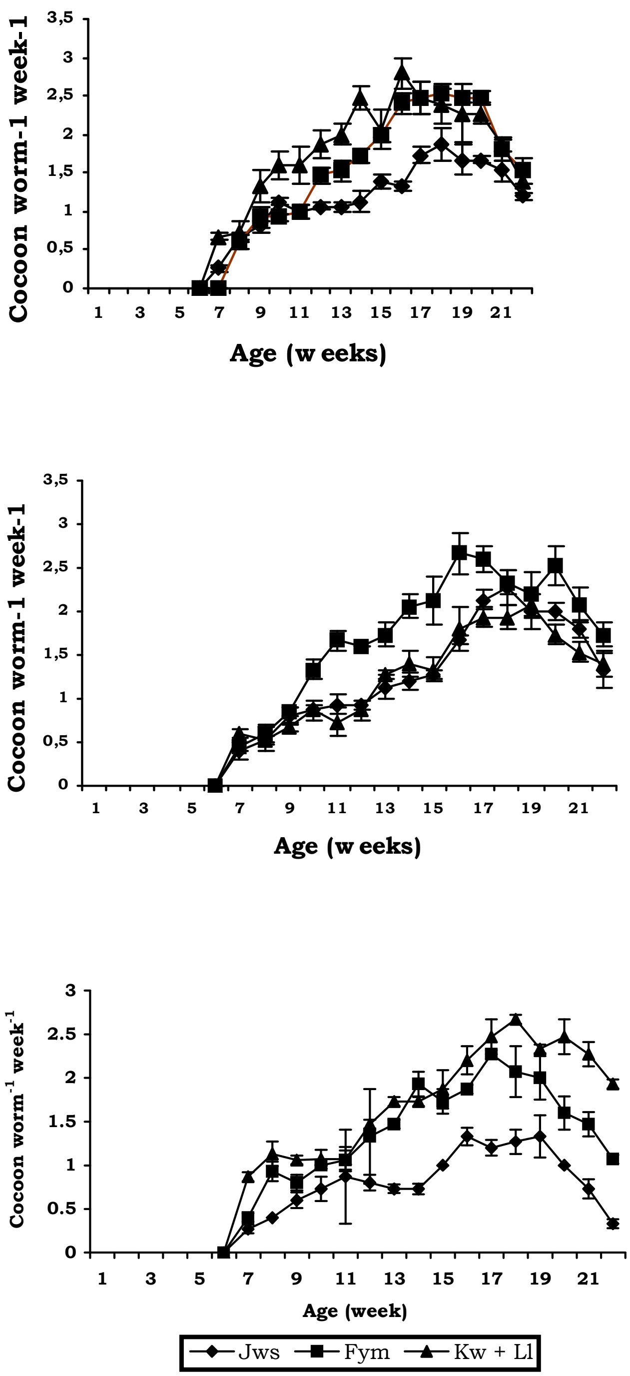

Figure 2. *The cocoon production by E. eugeniae (a), P. excavatus (b) and P. sansibaricus (c) in different waste materials.*

Table 4. *Cocoon (mean ± SEm; n=5) production in different culture material*

Culture Material	Eudrilus eugeniae			Perionyx excavatus			Perionyx sansibaricus		
	At start	At end	Production Rate*	At start	At end	Production Rate*	At start	At end	Production Rate*
Jbs	0	97.66 ± 4.38[a]	1.22 ± 0.10[a]	0	106.33 ± 4.46[a]	1.33 ± 0.14[a]	0	68.0 ± 1.89[a]	0.83 ± 0.08[a]
Fym	0	127.33 ± 3.07[b]	1.72 ± 0.16[b]	0	143.00 ± 4.33[b]	1.79 ± 0.17[b]	0	115.3 ± 2.38[b]	1.38 ± 0.13[b]
Kw + Ll	0	150.67 ± 5.51[c]	1.88 ± 0.15[b]	0	103.67 ± 3.14[a]	1.29 ± 0.13[a]	0	141.67 ± 2.33[c]	1.77 ± 0.14[c]

* Cocoons $worm^{-1}$ $week^{-1}$

The mean values followed by different letters are statistically different (ANOVA, Duncan's multiple-ranged test; p< 0.05)

Cocoon hatchling success and number of hatchlings per cocoon

Statistically the percent of hatchling success was not different (P=0.088) among the different species, whereas feeding material or/ substrate type clearly influenced (P<0.001) the cocoon hatchling success in presently studied earthworms. The cocoons collected from Kw + Ll culture showed highest hatchling success (%) for *E. eugeniae* (73.19 %), *P. excavatus* (74.39 %), and *P. sansibaricus* (73.16 %) *(Table 5).* The cocoons of *E. eugeniae, P. excavatus* and *P. sansibaricus* showed lowest hatchling success, which were collected from Jbs, Fym and Fym culture, respectively (Table 5). The largest numbers of hatchlings ($cocoon^{-1}$) in *E. eugeniae* were produced on Fym (1.59 ± 0.04) followed by Jbs, and Kw + Ll culture *(Table 5).* In *P. excavatus* the largest numbers of hatchlings were obtained in Kw +Ll (1.77 ± 0.06) followed by Fym, and Jbs culture. While, cocoons of *P. sansibaricus* exhibited largest hatchling numbers ($cocoon^{-1}$) in Fym (1.81 ± 0.10) followed by Jbs, and Kw + Ll culture. The hatchling numbers per cocoon did not show statistical difference among different species (P=0.497), whereas, feeding or/ substrate type (P<0.001) clearly affects the numbers of hatchling per cocoon in presently studied earthworm species *(Table 2).*

Table 5. *Cocoon hatchling success and hatchling per cocoon (mean ± SEm, n = 5) in different culture material.*

Culture material	Eudrilus eugeniae		Perionyx excavatus		Perionyx sansibarius	
	Hatchling success (%)	Hatchling $cocoon^{-1}$	Hatchling success (%)	Hatchling $cocoon^{-1}$	Hatchling success (%)	Hatchling $cocoon^{-1}$
Jbs	61.62 ± 4.51[a]	1.55 ± 0.12[a]	56.0 ± 2.34[a]	1.14 ± 0.04[a]	59.19 ± 1.48[a]	1.30 ± 0.03[a]
Fym	66.17 ± 1.53[a]	1.59 ± 0.04[a]	54.84 ± 0.90[a]	1.46 ± 0.14[b]	54.59 ± 0.85[a]	1.81 ± 0.10[b]
Kw + Ll	73.19 ± 2.80[a]	1.41 ± 0.04[a]	74.34 ± 2.38[a]	1.77 ± 0.06[c]	77.16 ± 1.15[b]	1.19 ± 0.02[a]

The mean values followed by different letters are statistically different (ANOVA, Duncan's multiple-ranged test; p< 0.05)

Discussion

In this study effort has been made to evaluate the recycling process of some crop residues and growth patterns of *P. excavatus* during vermicomposting process. The substrate material used in this study includes: crop residues of *Sorghum vulgare* and *Pennisetum typhoide* amended with sheep manure (Jbs), farmyard manure (Fym), and kitchen waste mixed with leaf litter of *Mangifera indica* (Kw + Ll). The substrate combinations namely Jbs, Fym and Kw + Ll have different palatability, particle size, microbial structure and chemical composition. Kitchen waste as well as farmyard manure is high quality material with fast decomposition rates, while crop residues are low quality material with slow decomposition rates. All the three composting species showed drastic variations for growth and cocoon production rates among the different culture material. Earthworm showed maximum bio-potential in kitchen waste (discarded vegetables, peel off, green leaves, fermented food etc.) as well as in farmyard manure. According to Fayolle et al. [10] among the different variables necessary for earthworm production, it seems that the type of food is most important. In general the proportion of N or even C-to-N ratio of the feeding material appeared to be an important factor for influencing the worms' activity. Cortez and Bouche [6] studied the effect of litter quality on growth patterns of *Nicodrilus meridionalis*, and reported maximum biomass gain in composted litter treatments. They concluded that this weight increase was possibly related to the C-to-N ratio of the litters, which was lower in composted litters. Hendrikson [15] observed that the growth of earthworm was correlated negatively with C-to-N ratio of the feeding material. In present study there was also a negative relation between biomass production and C-to-N ratio of the culture beddings for *E. eugeniae* and *P. excavatus*, which further strongly, supports the above hypothesis. Dramatically biomass production in *P. sansibaricus* did not show the same relation in respect to C-to-N ratio of the culture material.

Overall in present study there was a constant trend of better biomass production in N rich beddings. More over, the positive correlations recorded between N-content of substrate material and growth rate (mg worm^{-1} day^{-1}) further supports the hypothesis *(Table 6)*.

Table 6. *Pearson correlation coefficients between chemical parameters of culture material and biological parameters of different composting species.*

Culture material	Growth rate	Reproduction Rate	Hatchling Succes	Number of Hatchlings Cocoon^{-1}	Total Population
E. eugeniae vs. N	0.868**	0.470	0.679*	-0.328	0.463
E. eugeniae vs. C-to-N	-0.572	-0.932**	-0.554	0.161	-0.305
P. excavatus vs. N	0.952**	0.007	0.721*	-0.082	-0.693*
P. excavatus vs. C-to-N	-0.986**	-0.400	-0.358	-0.161	-0.417
P. sansibaricus vs. N	0.727*	0.987**	0.775*	0.935**	0.951**
P. sansibaricus vs. C-to-N	-0.356	-0.946**	-0.464	-0.820**	-0.970**

P<0.05, ** P<0.01

The final individual biomass of all studied composting species did not show significant variation between Kw + Ll and Fym treatments, even thus these substrates were not differ statistically for their C-to-N ratio (P=0.292). So here it can be hypothesized that other characters of the substrate such as particle size, palatability,

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microbial composition and presence of special substances were rather more important than its' N-content or C-to-N ratio. In general, different earthworm species are impacted differently by C-to-N ratio of the substrates. Unfortunately, in literature there are no more published reports regarding the impact of substrate or feeding material on growth patterns of the presently studied tropical composting earthworms. Therefore, pilot studies are necessary to establish the optimal C-to-N ratio for a specific earthworm species [27], and a specific feed mixture for specific earthworm for better vermiculture practices. However, on the basis of present results it is concluded that the decomposition efficiency of earthworms in plant derived materials could be retarded due to presence of some chemical substances (e.g. polyphenols). The behaviour of composting earthworm in such kind of wastes must be of a research priority. Comparatively, in present study the earthworm showed least biological potential on Jbs (mainly consist of crop residues), and it could be due to presence of some polyphenols and related substances [2, 26] in this culture. Garcia and Fragoso [11] reported influence of feeding source on growth and reproduction performance of *Pontoscolex corethrurus* and *Amynthas cortices.* They concluded that despite of C-to-N ratio of the feeding material the concentration of polyphenols and related compounds were more important for controlling earthworm activities.

However, cocoon production rate in presently studied species did not correlate significantly with N-content of the substrate material except to *P. sansibaricus.* Nevertheless, C-to-N ratio showed close negative relationship with cocoon production rate for *E.eugeniae* and *P. sansibaricus (Table 6).* It is clear that earthworm exhibited different patterns of cocoon production on presently studied substrates. Studies revealed that *E. fetida* showed drastic variations in cocoon production efficiencies in respect to nature of the waste materials [12, 36]. Fayolle et al. [10] pointed out that feeding source played an important role on cocoon production patterns in *Dendrobaena veneta*. However, the difference in cocoon production patterns in different treatment suggesting a physiological trade-off [32] related to N-limitations [11]. Moreover, the cocoon production behaviour of earthworms' could be interpreted with the chemical, physical and biological properties of waste material. Studies reveal that microbial population of substrate material clearly influences the concentrations of metabolites that consequently affect biological activities of earthworms [10]. Garcia and Fragoso [11] concluded that the presence of some growth-retarding substances in waste material is also important to determine the earthworms' cocoon producing efficiency.

The hatchling success of cocoons collected from different substrate martial also varied statistically. Since, there are no more published reports in literature regarding the effect of substrate quality on cocoon hatchling success. However, it is very hard to define the direct impact of feeding material on such kind of biological activities, so it can be hypothesized that nutrients especially N-content in substrate might be of primary determinant. The significant correlation observed between N-contents of substrate and cocoon hatchling success in *E. eugeniae* ($r = 0.679$, $p < 0.05$), *P. excavatus* ($r = 0.721$, $p < 0.05$) and *P. sansibaricus* ($r = 0.775$, $p < 0.05$) further strong the proposed hypothesis. Similarly, number of hatchlings ($cocoon^{-1}$) in different substrate material also varied drastically. The broad categories of optimal physico-chemical requirement for composting earthworms on organic waste were confirmed but the nutritional element of their needs remain unclear. There are few other such statements in the literature. The number of hatchlings per cocoon in *P. sansibaricus* was significantly related with N-content ($r = 0.935$, $p < 0.01$) as well as with C-to-N ratio ($r = - 0.820$,

$p < 0.01$) of the substrate. However, in this study the expected relation between number of hatchlings ($cocoon^{-1}$) and substrate N-content and/or C-to-N ratio was not observed for *E. eugeniae* and *P. excavatus* species. Therefore, cocoon hatchling success and number of hatchlings per cocoon could be related with the chemical quality of the substrates in which cocoons were laid down. In general, it has been well established that nitrogen content of the culture media affects the cocoon production rate and their further development through influencing the dietary need of the protein for earthworms. It can hypothesize that in nitrogen rich culture media earthworm produce more viable cocoons due to efficient protein supply in their diets. However, in past studies cocoon hatchling success has been studied extensively in terms of their environmental need (temperature, moisture, pH etc.). In present study effort has been made to evaluate the dietary impact on hatchling success and number of hatchlings per cocoon in composting worms. Clearly evaluating the some degree of optimal chemical composition of substrate material (N-content, C-to-N ratio, concentration of polyphenols and related compounds) may be beneficial for rapid vermiculture, but more work is still needed to define the hypothesis.

It can be concluded that: better results of biomass as well as reproduction potential of composting earthworm species could be interpreted with the physio-chemical, palatability, and microbial composition of their feeding or substrate materials. Our data demonstrated that quality of the waste material used for vermiculture could drastically influence the biomass production and reproduction performance of composting earthworms. However, tropical composting earthworms: *E. eugeniae, P. excavatus,* and *P. sansibaricus* had contributed potentially in commercial vermitechnology operations in both tropical and temperate parts of the world. So, using appropriate substrate and/or feeding material for earthworm culture could optimize vermicomposting practices. There are vast opportunities to study the influence of substrate quality interrelating with environmental variables in the field of earthworm biotechnology. Still a great work is required to establish the optimal conditions for culturing of tropical earthworms for sustainable vermiculture operations.

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CARBON SEQUESTRATION POTENTIAL IN ABOVEGROUND BIOMASS OF THONG PHA PHUM NATIONAL FOREST, THAILAND

J. TERAKUNPISUT[1] – N. GAJASENI[2]* – N. RUANKAWE[2]*

[1]*Zoology Major, Faculty of Liberal Arts and Science, Kasetsart University Kamphaeng Saen Campus, Nakornpratom 73140, Thailand*
(phone: +66-1-362-8696, fax: +66-2-942-8010 ext 4613)

[2]*Biology Department, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand*
(phone: +66-2-218-5360, fax: +66-2-218-5386)

e-mail: tjiranan@hotmail.com

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Abstract. This study assessed the potential of carbon sequestration on aboveground biomass in the different forest ecosystems in Thong Pha Phum National Forest, Thailand. The assessment was based on a total inventory for woody stem at ≥ 4.5 cm diameter at breast height (DBH). Aboveground biomass was estimated using allometric equation and aboveground carbon stock was calculated by multiplying the 0.5 conversion factor to the biomass. As the results, carbon sequestration showed varied in different types of forests. Tropical rain forest (Ton Mai Yak station) higher carbon stock than dry evergreen forest (KP 27 station) and mixed deciduous forest (Pong Phu Ron station) as 137.73± 48.07, 70.29± 7.38 and 48.14± 16.72 tonne C/ha, respectively. Habitat variability caused differences of biomass accumulation, species composition and the allometric relationships of forests. In the study area, all forest had a similar pattern of tree size class, with a dominant size class at ≥ 4.5-20 cm. The ≥ 4.5- 20 cm trees potentially provided a greater carbon sequestration in tropical rain forest and dry evergreen forest while the size of > 20- 40 cm gave potentially high carbon sequestration in mixed deciduous forest. Due to the trees have the lowest carbon sequestration but they considerably grow up to the further size classes. Apparently, they will be able to increase more biomass accumulation and store more carbon. In conclusion, the greatest carbon sequestration potential is in mixed deciduous forest and followed by tropical rain forest and dry evergreen forest in Thong Pha Phum National Forest. Finally, the appropriate forest ecosystem management can be an alternative solution for carbon dioxide reduction in terms of carbon sink role.
Keywords: *carbon stock, biomass, allometric equation, diameter at breast height*

Introduction

Increasingly convincing evidences show that the Earth is getting warmer and in the future warming could have serious effects on affect human [13]. Atmospheric concentration of carbon dioxide (CO_2), the primary and best studied greenhouse gas, has increased by about 30 % from the start of the industrial revolution to 1992 due to fossil fuel combustion and change in land use [14]. The ultimate objective of The United Nations Framework, in which Thailand is a member, is to stabilize the atmospheric greenhouse gas concentrations at the level that will not cause dangerous anthropogenic interference with the climate system. The emission reduction of greenhouse gases from a member of industrialized countries called for in Kyoto Protocol. Thailand has ratified Kyoto Protocol since August 28, 2002; therefore, the country will voluntarily participate in CO_2 reduction. There are two alternatives to reduce CO_2: decreasing carbon source and increasing carbon sink.

The world's forests are prominent sites to study of climate change, not only in terms of total net carbon emissions but also in terms of global storage capacity, important for climatic regulation. Under the processes of nutrient uptake and cycling in forest ecosystems and thus highly influenced by changes in temperature or precipitation regimes as well as by changes in the atmospheric CO_2 concentration.

Therefore, this study is focusing on carbon sequestration, specifically in terms of aboveground biomass and carbon stock. The estimates of carbon stock are also important for scientific and management issues such as forest productivity, nutrient cycling, and inventories of fuel wood and pulp. In addition, aboveground biomass is a key variable in the annual and long term changes in the global terrestrial carbon cycle and other earth system interactions. It is also important in the modelling of carbon uptake and redistribution within ecosystems. Of most interest is live wood biomass, which is involved in the regulation of atmospheric carbon concentrations. Thus, its dynamics must be understood if annual spatial variations are to be related to spatial weather and climate variables. Other computations, which require an accurate estimate of biomass along with carbon emission and carbon sequestration rates, are defining the carbon status and flux in a given geopolitical unit for the assessment, for example carbon taxes and similar international CO_2 mitigation measures.

Materials and methods

Study area

The study was located at Thong Pha Phum District, Kanchanaburi Province, Thailand which can be classified into three forest types as tropical rain forest, dry evergreen forest and mixed deciduous forest. Three sampling sites were selected, one from each of three forest types. The geographical characteristics of the sampling sites were recorded in *(Table 1.)*

Aboveground biomass assessment was carried out in three natural forests from November 2002 to April 2003. Average annual rainfall is 1,650 mm, which rainy season normally started from April to October [18]. Average annual temperature is 25^{o} C that distributed the range of 9.3^{o} C to 42.2^{o} C in the natural forest. In the study area, the species area curves of all three forests were available at different densities and a square mesh of one plot. Each plot in tropical rain forest, dry evergreen forest and mixed deciduous forest had a square plot with 80 x 80, 80 x 80, and 50 x 50 m^2, respectively. The replications of plot in tropical rain forest at Ton Mai Yak were 3 plots, dry evergreen forest at KP 27 were 4 plots, and mixed deciduous forest at Phong Phu Ron station were 5 plots.

Table 1. *Geographical coordination of the study area and forest types at Thong Pha Phum National Forest.*

Name	Location	Forest type	No. sampled plot
Ton mai yak station	1609720 N and 0470402 E	Tropical rain forest	3 (80x80 m^2)
KP 27 station	1613596 N and 0470585 E	Dry evergreen forest	4 (80x80 m^2)
Phong phu ron station	1619296 N and 0474970 E	Mixed deciduous forest	5 (50x50 m^2)

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Sampling methods

Three different forests were selected on the basis of total inventory for woody stems DBH ≥ 4.5 cm. SILVIC Program was used for tree height estimation (H_t) by using a minimum of 40 randomly trees in various sizes in the sample plots as following the equation by [15]:

$$1/ H_t = 1/ A (DBH)^h + 1/ H^* \qquad \text{(Eq. 1)}$$

Where Ht = height of tree (m), DBH = diameter at breast height (cm), A, h, H*= constant

After the trees were harvested, diameter and height were estimated with SILVIC Program, were applied to these data allometric regression equations to estimate the total aboveground biomass. Aboveground biomass was calculated by summing the stem, branches and leaf mass of individual trees, using allometric equations of [19] for tropical rain forest and dry evergreen forest, and [16] for mixed deciduous forest, as the following:

Stem (Ws) $= 0.0509^*(D^2 H)^{0.919}$……… [19]
Branch (Wb) $= 0.00893^*(D^2 H)^{0.977}$
Leaf (Wl) $= 0.0140^*(D^2 H)^{0.669}$

And Stem (Ws) $= 0.0396^*(D^2 H)^{0.9326}$……… [16]
Branch (Wb) $= 0.003487^*(D^2 H)^{1.027}$
Leaf (Wl) $= ((28.0/ WS + WB) + 0.025)^{-1}$

Where Ws = stem mass (kg/individual tree), Wb = branches mass (kg/ individual tree), Wl = leaf mass (kg/individual tree)

The carbon content was calculated by multiplying the 0.5 conversion factors to aboveground biomass [1, 3, 12, 8, 5]

Results and discussion

Aboveground biomass was estimated at the different forest types in order to indicate the proportion of biomass. It was found that DBH and height of trees were distributed different size classes. The characteristics of size class of three different forests were compared in *(Figure 1)*, showing the relationship between DBH and tree density in each size class. This would tend to make the biomass differences even greater. The frequency distribution curves of DBH were all L-shaped, the frequency patterns were more or less exponentially toward larger diameter classes with a maximum at the left- end or smallest DBH size classes.

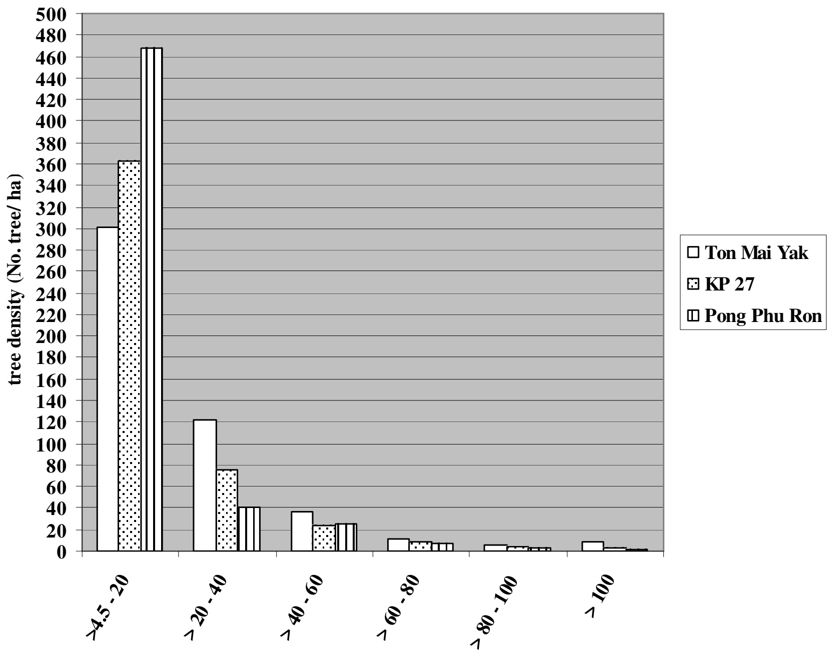

Figure 1. *Tree density in different size classes at Ton Mai Yak station, KP 27 station, and Pong Phu Ron station sampling sites.*

Aboveground biomass accumulation was highest in tropical rain forest *(Figure 2);* while the aboveground biomass in dry evergreen forest was lower than mixed deciduous forest at DBH size class over 100 cm. Although mixed deciduous forest had the high number of tree and species, but most of trees were smaller than 20 cm in a typical uneven – aged stand and caused the lowest individual volume and biomass. The main conclusion showed an opposite relationship between biomass and tree size class. The most aboveground biomass accumulation was found in big trees of size class at ≥ 80 – 100 and ≥ 100 cm. Because these trees had the highest stem volume and large diameter, they also had the lowest number of tree densities.

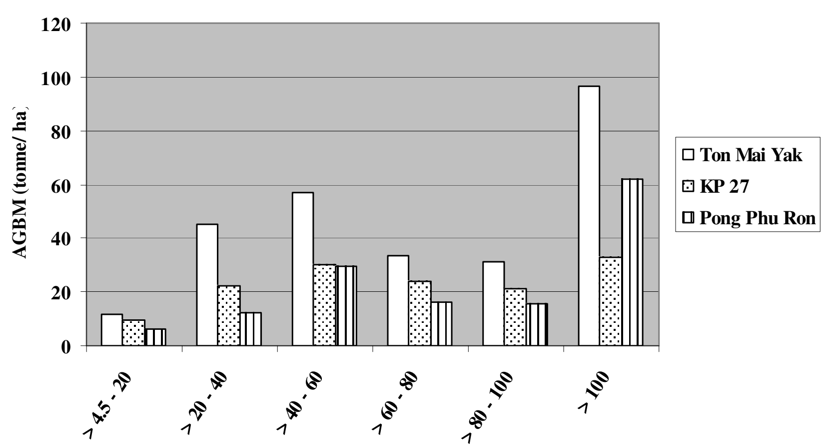

Figure 2. *Aboveground biomass in different tree size classes in Ton Mai Yak station, KP 27 station, and Pong Phu Ron station sampling sites.*

The percentage data of tree density and aboveground biomass were presented in *Table 2* and showed the similar pattern of tree density and aboveground biomass in each size class. In the sample plot, all forests had a tree size class, with the dominant size class at ≥ 4.5 – 20 cm, were accounted for 85.88, 76.22 and 61.98 % at Pong Phu Ron station, KP 27, and Ton Mai Yak station, respectively. On the other hand, the size class of all forests had the lowest aboveground biomass accumulation that comprised approximately ranging from 4.17 – 6.71% of the total biomass density in this study, due to low stem volume, low basal area and short trees with small diameters.

Comparison of the size class distribution and aboveground biomass showed some evidences of biomass reduction in larger size classes, > 60 – 80 and > 80 – 100 cm, resulting from selective logging in this area. Logging in excess of regrowth was also a significant cause of loss, particularly in Asian forests [17] and usually destroyed the small size of tree during the tree felling and log dragging process [10], which reflected the reduction of classes > 20 – 40 and > 40 – 60 cm size classes in the mixed deciduous forest. In the sample plot, all forests had a similar pattern of tree size class, with a dominant size class at ≥ 4.5 – 20 cm.

Table 2. *A comparison of the percentage of tree density and carbon sequestration potential in each size class in the different study sites.*

Size class (cm)	**Tropical rain forest**		**Dry evergreen forest**		**Mixed deciduous forest**	
	Ton Mai Yak station		(KP 27 station)		(Pong Phu Ron station)	
	Tree density (%)	C-storage (%)	Tree density (%)	C-storage (%)	Tree density (%)	C-storage (%)
≥4.5 – 20	62.0	4.2	76.22	6.71	85.88	4.49
>20 – 40	25.2	16.5	15.74	16.05	7.50	8.82
>40 – 60	7.4	20.7	5.01	21.42	4.56	20.83
>60 – 80	2.4	12.1	1.64	17.03	1.18	11.31
>80 – 100	1.3	11.4	0.82	15.19	0.59	10.89
>100	1.7	35.2	0.58	23.61	0.30	43.67

Carbon sequestration potential in the different forest types to be correlated to DBH size class (*Table 2*). In tropical rain forest and dry evergreen forest, the main tree size classes that had a great potential in carbon sequestering from small up to medium tree size at ≥ 4.5–20 up to >40–60 cm. While, the main tree size classes that had the highest potential in carbon sequestering in mixed deciduous forest from small up to medium tree size at >20–40 cm up to > 40–60 cm.

For example, in Ton Mai Yak station, the smallest tree in size class ≥4.5–20 cm had biomass accumulation or carbon sequestration potential only 4.2 %. When trees considerably grow up to further size class at >20–40 cm, these trees had a highest carbon sequestration potential. For the size class at >40–60 cm, trees had a high carbon sequestration potential but not as much as in size class at >20–40 cm. In accordance with dry evergreen forest, trees in size class ≥ 4.5–20 cm were able to grow fast and store more carbon. While trees in mixed deciduous forest had potential to grow fast and store more carbon at size class of > 20–40 cm.

The results of aboveground biomass and carbon sequestration in *Table 3* that showed the average aboveground biomass in Ton Mai Yak station (tropical rain forest), KP 27 station (dry evergreen forest) and Pong Phu Ron station (mixed deciduous forest) were 275.46±96.15, 140.58±14.76 and 96.28±33.44 tonne/ha, respectively. Aboveground biomass varied from plot to plot in forest area due to different stage of forest growth cycle, habitat variation, and tree density. The stem weight, especially tree biomass of bigger trees, is the largest component of a forest biomass [16].

In this study, the results included only the tree components of aboveground biomass. In general, root biomass is approximately 25 % of aboveground biomass [7], so the calculated root biomass in Ton Mai Yak station, KP 27 station and Pong Phu Ron station are about 68.87, 35.15, and 24.07 respectively.

Carbon content was calculated from aboveground biomass with the method used by [1, 3, 12, 8, 5]. Carbon content would be about 50 % of the amount of total aboveground biomass. Therefore, the aboveground carbon sequestration of three forest types are calculated, the carbon is stored at Ton Mai Yak station as 137.73±48 and follow by KP 27 and Pong Phu Ron station are 70.81, 70.29±7.38 and 48.14±16.72 tonne C/ha respectively (*Table 3*).

Table 3. *Aboveground biomass of tree and carbon sequestration at three study sites.*

Study sites	Tree density (No./ha)	Stem mass (tonne/ha)	Branch mass (tonne/ha)	Leaf mass (tonne/ha)	Total AGBM (tonne/ha)	Carbon sequestration (tonne C/ha)	Calculate root biomass* (tonne C/ha)
Ton Mai Yak station	745 ± 142.3	217.241± 52.62	54.667± 40.960	3.554± 0.790	275.46± 96.15	137.73± 48.07	34.43
KP 27 station	560 ± 68.9	103.391± 11.16	34.911± 30.487	2.297± 0.493	140.58±1 4.76	70.29±7.38	17.57
Pong Phu Ron station	544 ± 98.3	110.256± 50.63	30.657± 29.96	0.151± 0.005	96.28±33. 44	48.14±16.72	12.03

*Note: root biomass is approximately calculated as 25 % of aboveground biomass [7]

Data on carbon sequestration in the different forest types showed that the highest amount of carbon was stored in the biomass of tropical rain forest at Ton Mai Yak station. Because tree sizes at Ton Mai Yak station were quite large when compared to other stations so calculated carbon sequestration are the highest in this station. It does not mean that other forest types are not important, because the mainly groups of small tree sizes at ≥ 4.5 – 20 cm will grow to bigger size in the near future. They will have greater potential for future sequestration if the forests are under appropriate management without human disturbance. Huston and Marland [11] showed that carbon sequestration depended not only on rates of productivity but also on the size of the tree. Disturbance of landscapes can result in rapid release of large amount of carbon that will be recaptured slowly as forest regrowth.

In *(Table 4),* the comparison of biomass accumulation and carbon sequestration in various forest types showed the largest biomass in the tropical rain forest and the lowest biomass in the mixed deciduous forest. The results from this study showed the range of aboveground biomass in tropical rain forest, dry evergreen forest and mixed deciduous forest as 275.46, 140.48, and 96.28 tonne/ha, with calculated carbon sequestration as

137.73, 70.29, and 48.14 tonne C/ha. Ogawa *et al.* [16] reported aboveground biomass data of different forests in Thailand such as tropical rain forest, dry evergreen forest and mixed deciduous forest at 358, 126 and 311 tonne/ha, with calculated carbon sequestration as 179, 60.30, and 155.50 tonne/ha, based on direct measurement by destructive method of tropical rain forest in the Forest Reserve of Khao Chong, Trang Province of peninsular Thailand, as well as dry evergreen forest and mixed deciduous at Ping Kong, Chaing Mai Province. As the results of this study, carbon sequestration was considerably lower than the Ogawa *et al.* study, which may suggest that these forests were more disturbed and affected to change in forestland due to different initial time study, site qualities, carbon sequestering carrying capacities and reflected that the tropical rain forest in this study was an immature forest. Flint and Richards [9] studied that carbon sequestration was estimated in Southeast Asia including India, Thailand, Cambodia, Malaysia and Indonesia ranging from 17.5 tonne C/ha or less in severely degraded tropical dry forest to almost 350 tonne C/ha in relatively undisturbed mature tropical rain forest. The lower biomass values often reflected an immature forest.

Brown and Lugo [3] summarized the total carbon sequestration estimates of tropical forest in three countries including Malaysia, Cameroon and Sri Lanka, ranging from 76.50 tonne C/ha in disturbed tropical rain forest to 223 tonne C/ha in relatively undisturbed mature tropical rain forest based on direct measurement was the highest in Malaysia (112.5–223 tonne C/ha), followed by Cameroon (119–170.5 tonne C/ha), and Sri Lanka (76.5–110.5 tonne C/ha). The ranges of biomass lower than the other forest areas often reflected an immature forest, which may suggest that it due to human population pressure.

Table 4. *A schematic of aboveground biomass and carbon sequestration in different forest types between this study and other studies.*

	Tropical rain forest		**Dry evergreen forest**		**Mixed deciduous forest**		**Source**
	AGBM (tonne/ha)	**C-stock (tonne C/ha)**	**AGBM (tonne/ ha)**	**C-stock (tonne C/ha)**	**AGBM (tonne/ha)**	**C-stock (tonne /ha)**	
Thailand	275.46	137.73	140.58	70.29	96.28	48.14	This study
Thailand	358	179	126	60.30	311	155.50	[16]
Thailand	-	-	252	126	-		[10]
Thailand	-	-	-	-	31.95–175.50	15.97–87.75	[20]
Malaysia	225–446	112.5–223	-	-	-		[3]
Cameroon	238–341	119–170.5	-	-	-		
Sri Lanka	153–221	76.5–110.5	-	-	-		

By comparison of the carbon sequestration of tropical rain forest between this study and the study by [3], the result showed that the average total aboveground biomass in Thailand was 137.73 tonne C/ha, which is in the range of carbon sequestration in

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Malaysia and Cameroon. From annual precipitation data of Thailand, Malaysia and Cameroon as 1400, 2000 and 3000 mm/yr., respectively this possibly caused the carbon sequestering capacity [4].

Another factor that possibly caused of sequestered carbon lower than the other forest areas is tree height. Ogawa *et al.* [16] reported the calculated carbon sequestration of tropical rain forest at Khao Chong Forest Reserve, Thailand was 179 tonne C/ha that lower than calculated biomass from Malaysia because of the difference in tree height. The tallest tree actually measured there was only 36 m in height, whereas the maximum tree height of tropical rain forest in Malaysia often reaches 60 m. Therefore, plant biomass in Malaysia was greater than here. Thus, the accuracy to estimate biomass by used allometric equations with containing both diameter and total height was better than diameter alone.

Regarding to Chittachumnonk *et al*. [6] studied on carbon sequestration of Teak plantation in Thailand, there were four study areas located in northern and western regions included Mae Mai Plantation at Muang District, Lampang, Thong Pha Phum Plantation at Thong Pha Phum District, Kanchnaburi, Sri Satchanalai Platation at Sri Satchanalai District, Sukhothai, and Khao Kra Yang Plantation, Wong Thong District, Phitsanulok. The study showed that all aboveground biomass of Teak plantation was equal to 78.15 tonne/ha or equivalent to 646,997.19 tonne of total aboveground biomass of area, which total study area are 8,278.50 ha. In the estimate of carbon sequestration of Teak plantation were 39.08 tonne C/ha. The carbon sequestration in Teak plantation was seemingly near by the natural mixed deciduous forest (48.14 tonne C/ha).

Viriyabuncha *et al.* [20] studied the evaluation system for carbon storage in forest ecosystems in Thailand. The result showed that the carbon sequestration at Doi Suthep – Pui National Park, Chiang Mai, evergreen forest and mixed deciduous forest were in the range 15.97–87.75 tonne C/ha. The maximum biomass was found in dry evergreen forest because it was old forest and have been strictly controlled the illegal logging. The minimum carbon sequestration was found in dry dipterocarp forest, which was a young forest. The study also showed carbon storage of mixed deciduous forest was in the range 15.97–87.75 tonne C/ha. Comparison of the carbon sequestration from this study and Viriyabuncha *et al.* [20] indicated the similar range and pattern that tropical rain forest sequestered carbon higher than dry evergreen forest and mixed deciduous forest as 137.73, 70.29 and 48.14 tonne C/ha, respectively. It indicated that carbon sequestration varies from forest types and age of forest and carbon sequestration potential was rely on tree size class. Mixed deciduous forest, tree sizes at > 40–60 cm had trend of carbon sequestration more than other size classes, while size class at > 20–40 and > 40–60 cm in dry evergreen forest and tropical rain forest had more carbon sequestration than other size classes.

In general conclusion from biomass and carbon sequestration studies, under the different disturbance, old growth forest had more carbon sequestration than logged forest and secondary forest, respectively. Each size class had a different carbon sequestration potential. Almost small up to medium sizes of trees had a greater potential for carbon sequestering than big trees due to the forest type because the growth rate will slowly in bigger trees. Therefore, to conserve and manage the small tree at ≥ 4.5–20 and > 20–40 cm can considerably increase carbon sequestration potential in the near future. If the forest is deforested and changed by human activities, it will potentially cause the severe carbon loss to atmosphere from terrestrial ecosystems in relation to deforestation. In the summary, the estimation of aboveground biomass is based on data sets that consider only live trees, and do not consider litter or standing dead trees. Tropical forests tend to carry their biomass in the standing crop relatively more than temperate forests. Therefore, tropical forest inventories, which ignore dead matter, will be a small loss of proportion to total aboveground biomass than similar inventories in the temperate zone. According to carbon sequestration potential, it is clear that tropical forests have more effective in carbon sequestering than temperate forest due to net productivity differences [2]. Then tropical forest can play a major role in carbon dioxide reduction as carbon-sink.

Conclusions

Carbon sequestration varies from forest types and age of forest and carbon sequestration potential is rely on tree size class. Tropical rain forest has the highest potential of carbon sequestration and following by the dry evergreen forest and the mixed deciduous forest respectively. Tree sizes in mixed deciduous forest at > 40–60 cm has trend of carbon sequestration potential more than other size classes, while size class at > 20–40 and > 40–60 cm in dry evergreen forest and tropical rain forest has more carbon sequestration potential than other size classes. This evidence indicates the potential for growth to reach the climax stage of succession in the near future. These smaller trees are not the highest carbon sequestration potential but they are relevant in terms of their future potential to grow up. With high carbon sequestration potential in Thong Pha Phum National Forest, the Ministry of Natural Resources and Environmental must urgently consider to strictly protect and conserve these forests for sequestering atmospheric CO_2, which can increase carbon sink into the natural forest. Thailand can contribute to reduce the problem of greenhouse effects regarding global warming and climate changes.

The problem in this study was that the available data on carbon sequestration in tropical forest were extremely limited and incomplete. In some cases, inappropriate field measurements, as a result forest biomass may be significantly under or overestimated. To resolve these uncertainties will require both improved practices with current field methods and new techniques for measuring processes to understanding of the carbon dynamics of the world's forests.

Acknowledgement. The authors gratefully acknowledge the financial support by Department of Biology, Chulalongkorn University Graduate School, and especially the BRT foundation for supporting this study at Thong Pha Phum Natural Forest. Thank for all researchers of Biodiversity Research and Training Program (BRT) and foresters at Thong Pha Phum District, Kanchanaburi Province, for all their supports assistance in the data collection, especially guiding appropriate forest inventories under the difficult field conditions. Also thank Mr. Chingchai Viriyabuncha at Forest Research Office, Royal Forest Department for training and providing useful data from the research of Teak plantation. The Development and Promotion of Science and Technology Talents Project provided support for Terakunpisut.

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SOIL PH AND ITS ROLE IN CYANOBACTERIAL ABUNDANCE AND DIVERSITY IN RICE FIELD SOILS

S. NAYAK – R. PRASANNA*

Centre for Conservation and Utilization of Blue-Green Algae, Indian Agricultural Research Institute (IARI), New Delhi-110012, India
(phone: +91-011-25848431; fax : +91-011-25741648)

e-mail: *radhapr@gmail.com*

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Abstract. The influence of soil pH was evaluated on the abundance and generic diversity of cyanobacteria in soil samples collected from diverse rice soil ecologies of India. Qualitative and quantitative studies of the 52 soil samples collected from nine agroecologies was carried out using enrichment, MPN (Most Probable Number) techniques and diversity indices were measured. A total of 166 forms, including 130 heterocystous and 36 non-heterocystous isolates were isolated and the highest percentage of abundance of heterocystous forms was observed at pH of 8.1. Highest Shannon's diversity index was recorded at a pH of 6.9, followed by pH of 7.4, while indices of richness and evenness (J and E) were highest in soil samples of pH of 9.3. This study highlighted the successful colonization of cyanobacteria in rice field soils of diverse pH and the need for enrichment of the native flora as a means of exploiting the full potential of cyanobacterial biofertilizers in agriculture
Keywords: *abundance; cyanobacteria; diversity indices; rice ecologies; soil pH*

Introduction

Cyanobacteria represent cosmopolitan prokaryotes, which can be found in almost every conceivable habitat, including soil, on rocks, in fresh water, and in salt water [6,11]. In aquatic environments, cyanobacteria often form thick microbial mats, serving as crucial components in such ecosystems, as they are the primary producers at the base of the microbial food web. Cyanobacteria also increase the oxygen concentration and improve other physico-chemical parameters of the environment, in which they grow and flourish [13]. They are also found in the deserts, where they remain dormant for most of the time, taking advantage of the occasional rains, although this is not the most common [7]. Because they secrete polysaccharides that bind soil, cyanobacteria help to control stability, erosion, runoff, and site availability for germination by higher plants. In nature, cyanobacteria are abundant in places where there is a major nitrogen-deficiency.

Among soil properties, pH is a very important factor in growth, establishment and diversity of cyanobacteria, which have generally been reported to prefer neutral to slightly alkaline pH for optimum growth [23,10]. Acidic soils are therefore one of the stressed environments for these organisms and they are normally absent at pH values below 4 or 5; eukaryotic algae, however, flourish under these conditions. Soil pH is also known to have a selective effect on the indigenous algal flora, especially cyanobacteria and their succession and abundance in soil. Species of *Nostoc, Anabaena, Tolypothrix, Aulosira, Cylindrospermum, Scytonema, Westiellopsis* and several other genera are widespread in Indian rice field soils and are known to contribute significantly to their fertility [29,9'15]. There are very few reports on the existence of cyanobacteria at low pH (acidic range) as they are in general, intolerant to low pH conditions [1,8,5]

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Among the diverse habitats, rice fields constitute one of the favourable ecologies for the growth and proliferation of cyanobacteria [30,26,14]. In the 1970s, algalization or the enrichment of soil via inoculation of selected cyanobacterial strains led to the promotion of these biofertilisers among the farming community in South East Asia [26,28]. However, in recent years, an urgent need has felt to address inherent deficiencies, which have limited their extensive exploitation in diverse rice ecologies and soil types. In a country such as India, rice is grown under diverse ecologies with

Therefore, this investigation was aimed at quantification of the cyanobacterial diversity – in terms of population counts, relative generic abundance, and their correlation with pH of the various soil samples collected and isolation of dominant genera from diverse rice ecologies of India.

Materials and methods

Collection of soil samples and isolation and enumeration of cyanobacteria

Soil samples collected from diverse agroecological regions and soil types *(Fig. 1 and Table 1)* were measured for their EC and pH range and utilized for enrichment studies in BG –11 medium with/ without nitrogen supplementation. Enumeration of populations was carried out by MPN (Most Probable Number) technique and tabulated for each site under the various locations. The enrichment flasks and MPN tubes were regularly monitored for growth and observed microscopically. Standard plating / streaking techniques were used for isolation and purification of cyanobacterial strains [24].

Identification and purification of cyanobacteria

The growth pattern and morphological examination of the cyanobacterial strains was carried out at different stages of growth in nitrogen-free liquid and solid (agar) BG-11 medium. The strains were viewed under a Nikon (Microphot-FX) microscope and the nature of filaments and the shape and size of vegetative cells, heterocysts and akinetes, were analysed and assigned to different genera , using the keys of Desikachary [4].

Measurement of soil EC and pH

The soil samples (soil : water = 1: 2.5) were analysed with respect to their EC (Electrical Conductivity) and pH range following the methodology outlined by Black [2].

Measurement of acetylene reducing activity

Gas chromatographic quantification of ethylene formed (acetylene reduction activity, ARA) was utilized as an index of nitrogen fixation. The vials with log phase cultures (14d) were injected with acetylene (10% gas phase) after removing an equal amount of air, using airtight syringes and incubated for 90 minutes under optimal conditions of temperature and illumination [18]. The samples (1 ml) were injected into a Gas Chromatograph (Chemito, model GC 1000), fitted with an oven containing a 2m long column of stainless steel (2mm internal diameter) packed with Poropak N (80-100 mesh). Nitrogen gas flowing at the rate of 35ml min^{-1} was used as the carrier, while hydrogen and air were used to produce the flame in the Flame Ionisation Detector. The oven, injector and detector were maintained at 100-120°C to allow for ionization and detection of ethylene produced.

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Commercially available standard ethylene was utilized for quantification and vials with an equivalent volume of water served as controls [18]. The ARA values were expressed per mg chlorophyll. Spectrophotometric estimation of the chlorophyll content of cells was carried out following the method of Mackinney [12]. All values presented are means of triplicate measurements.

Statistical analyses

The diversity indices (Shannon's diversity index and Simpson's index of diversity) were calculated using the standard formulae.

Correlation coefficients were calculated using Microsoft Excel package and analysed for their significance using Pearson's tables

Results and discussion

The trophic independence from carbon and nitrogen, together with a great adaptability to environmental variations, enables cyanobacteria to be ubiquitous. Their structural-functional flexibility provides them with not only great versatility, but also makes them among the most successful in extreme environments including high temperatures, high levels of UV light, and high salinity and inhabit a wide range of environments and niches. Their role in the soil ecosystem is manifold, the most important consequences being the fixation of nitrogen and carbon, besides promoting release of nutrients and reducing the rate of loss of water and soil through erosion. In paddy fields, their relative occurrence varies within large limits, ranging from 0 to 76-85%. However, contrary to the general belief, nitrogen fixing forms are not invariably present in tropical rice soils. All India survey showed that out of 2,213 soil samples from rice fields, only about 33% harboured nitrogen-fixing forms [27], and limited systematic analyses on their limited distribution has been undertaken in relation to major environmental factors [19,21,22].

Among the soil properties, pH is certainly the most important factor determining the flora and fauna composition. In culture media, the optimal pH for the growth of cyanobacteria ranges from 7.5 – 10, with a lower limit of 6.5 – 7.0. However, in soil-culture experiments, soils having slightly alkaline reaction were more favourable, while in natural environments cyanobacteria prefer neutral to alkaline pH [3,20]. The development of soil acidity is generally believed to be associated with the base unsaturation caused by leaching out of bases and genesis from base-poor acidic rocks. The dissolved or free acidic substances, such as sulphuric acid and ferric and aluminium sulphate, accentuate acidity in acid sulphate soils [5].

In the present investigation, soil samples were collected from nine locations *(Fig.1),* differing in their EC and pH values and evaluated for cyanobacterial abundance and generic diversity. A total of 166 forms, including 130 heterocystous and 36 heterocystous isolates were recorded. A predominance of heterocystous forms (68 – 95%) was observed at all locations, while non-heterocystous forms exhibited 5-32% abundance in the various locations *(Table 1).* Highest % abundance of heterocystous forms was observed at pH of 8.1, followed by 7.9 (83 and 80% respectively). In terms of non-heterocystous forms, soil samples with pH of 7.4 and 9.3 recorded highest % abundance.

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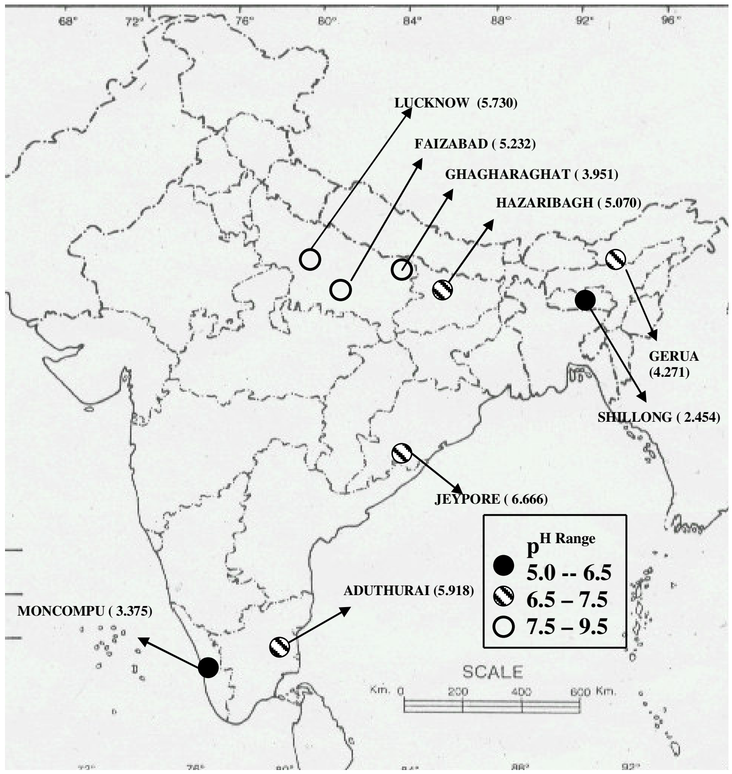

Figure 1. *Map of India depicting the various locations sampled, along with their pH range and Simpson's Reciprocal diversity indices (given in parentheses)*

Table 1. *Occurrence and distribution of cyanobacteria in various locations in India ,along with selected diversity indices*

Locations	EC (dS/m)	Total no of Genera	Non Heterocystous forms	Heterocystous forms	Shannon H	J	E
Aduthurai (Tamil Nadu)	3.4	19	6 (32%)	13 (68%)	1.909	0.918	0.843
Jeypore (Orissa)	2.7	20	5 (25%)	15 (75%)	2.038	0.927	0.853
Hazaribagh (Bihar)	2.9	17	4 (23%)	13 (77%)	1.785	0.903	0.828
Lucknow (Uttar Pradesh)	4.4	19	5 (26%)	14 (74%)	1.836	0.943	0.896
Faizabad (Uttar Pradesh)	3.5	15	3 (20%)	12 (80%)	1.859	0.894	0.802
Ghagraghat (Uttar Pradesh)	2.9	18	3 (17%)	15 (83%)	1.541	0.860	0.778
Gerua (Assam)	2.9	31	7 (23%)	24 (77%)	1.691	0.813	0.678
Shillong (Meghalaya)	2.5	09	1 (5%)	8 (95%)	0.964	0.878	0.874
Moncompu (Kerala)	4.2	18	2 (11%)	1 (89%)	1.442	0.804	0.704

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Simpson's Reciprocal indices *(Fig.1)* were highest in Jeypore soil samples, followed by those from Aduthurai. Shannon's diversity index was highest at a pH of 6.9, followed by pH of 7.4, indicative of the higher number of genera recorded in these soil samples. Indices of richness and evenness (J and E) were highest in soil samples of pH of 9.3 *(Table 2).* Shannon's indices are strongly biased towards richness, as it is calculated from proportional abundances of the species. On the other hand, Simpson's index is a measure of diversity, which takes into account both richness and evenness, although it gives more weight to the more abundant species in a sample.

Table 2. *Major genera and their relative abundance in the samples*

Genus	Total number of strains	Relative abundance
Anabaena	46	100 %
Nostoc	51	100 %
Calothrix	14	89 %
Scytonema	3	22 %
Westiellopsis	3	22 %
Hapalosiphon	8	89 %
Aulosira	1	11 %
Cylindrospermum	5	56 %
Oscillatoria	9	78 %
Phormidium	20	89 %
Lyngbya	1	11 %
Aphanocapsa	5	22 %

The extensive diversity in pH and EC led to a significant effect on the abundance of cyanobacterial species, depicted as log values of MPN *(Fig. 2 and 3).*

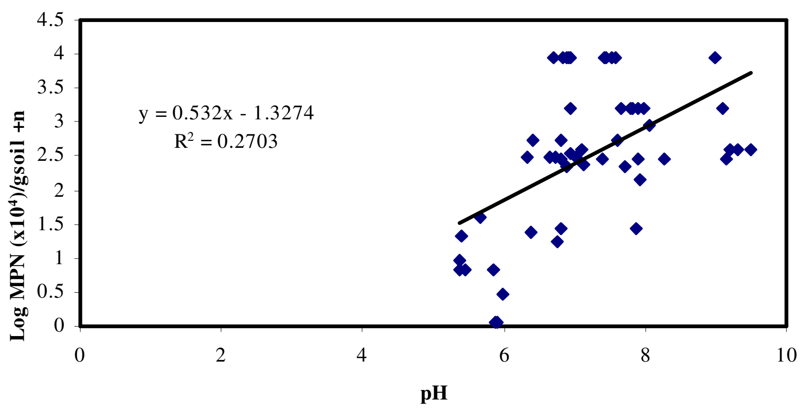

Figure 2. *Abundance of cyanobacterial populations (log MPN/g soil) as a function of pH ($r = 0.52$; $P < 0.01$)*

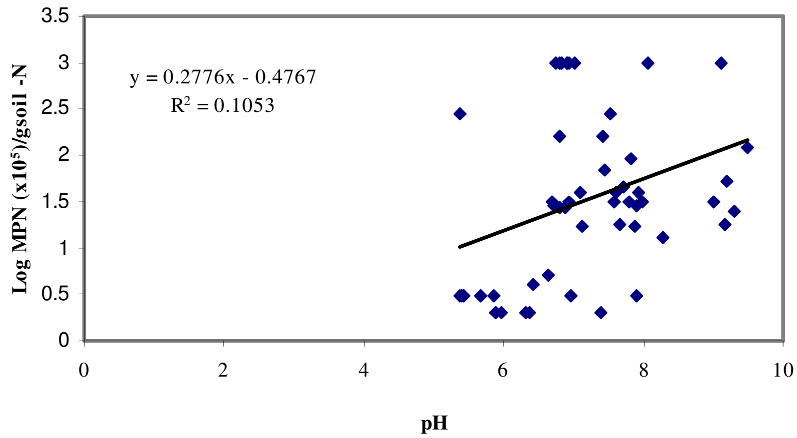

Figure 3. *Abundance of cyanobacterial populations (log MPN/g soil) as a function of EC (r =0.32; P < 0.01)*

The pH values and to a lesser extent EC of the various locations showed a significant positive correlation with MPN (expressed as log values), especially in relation to the enrichment studies in nitrogen supplemented media. The correlation between EC and pH was observed to be positive, but Pearson's coefficient was not significant *(Fig.4).*

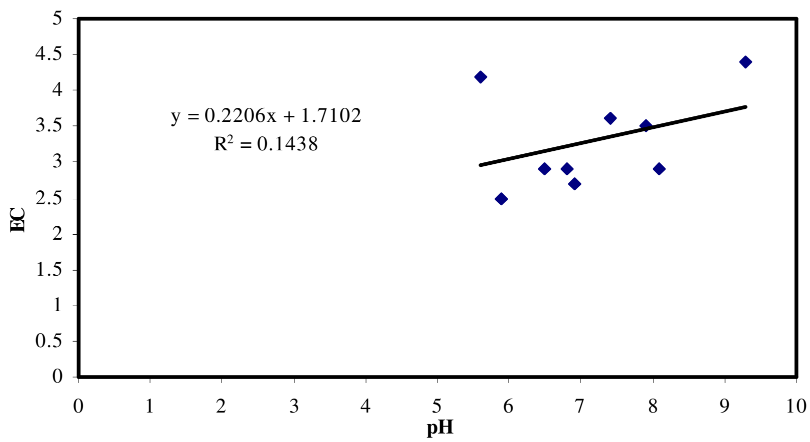

Figure 4. *Correlation between EC and pH*
(average over all locations; r=0.38, not significant)

Cyanobacteria belonging to 12 genera were isolated which included 8 heterocystous forms: Anabaena, Nostoc, Westiellopsis, Calothrix, Scytonema, Aulosira, Hapalosiphon, Cylindrospermum and 4 non-heterocystous forms: Phormidium, Oscillatoria, Lyngbya and Aphanocapsa. The genera-wise distribution of the five dominant cyanobacterial forms at various pH levels is illustrated in *Figure 5.*

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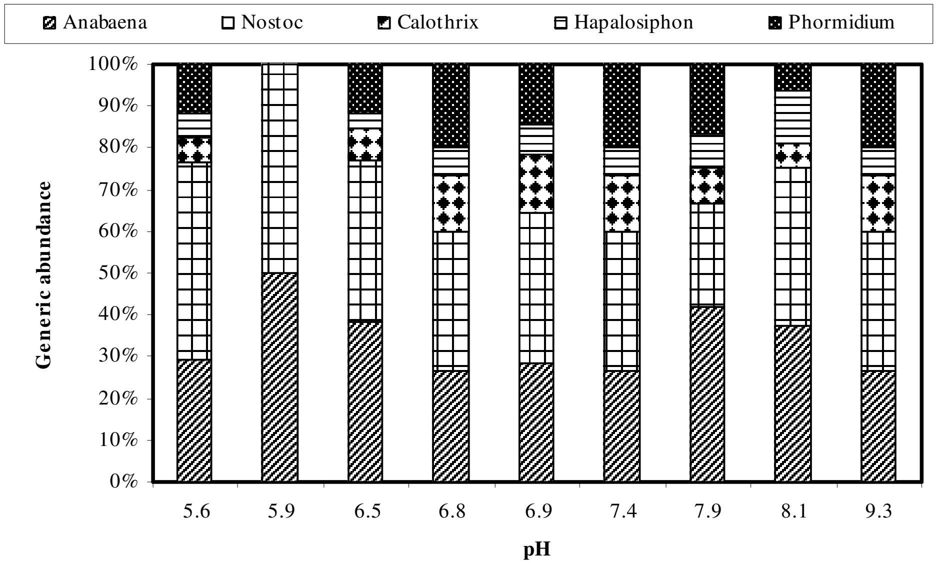

Figure 5. *Distribution of the selected dominant genera as a function of soil pH.*

In general, *Nostoc* and *Anabaena* recorded maximum number of isolates i.e. 10 at pH of 6.5. The number of isolates belonging to the genera *Anabaena* and *Nostoc* also showed the highest relative abundance of 100%, while lowest values were recorded for *Aulosira* and *Lyngbya,* as they were isolated only from one of the 9 locations. The relative abundance of cyanobacteria in rice soils and biofertilizer inocula from four countries revealed that significant correlation could be made with respect to pH and available P content of soils [21]. Algalization, when effective, is generally associated with increase in yield but the success of algalization is dependent on a number of factors that include flooding due to rains, simultaneous use of inorganic fertilizers, animal manures, pesticides and amount of light available to the cyanobacteria as the plant grows.

Earlier studies on the distribution pattern of cyanobacteria in soils of Andhra Pradesh, Haryana, Delhi, Rajasthan, Uttar Pradesh and Punjab revealed that although recurrent combination of forms were discernable, there appeared to be a localized distribution of cyanobacteria depending upon the soil pH, electrical conductivity and exchangeable sodium. Species of *Nostoc, Calothrix, Scytonema, Hapalosiphon* and *Wetiellopsis* were recorded in salt-affected soils of Maharashtra and Andhra Pradesh (where pH of the soil varied from 6.0 to 9.0), although the species of *Nostoc* and *Calothrix* were predominant. On analyzing the total cyanobacterial flora, it was observed that out of the total 37 species, 50 percent were nitrogen- fixing strains, including the non-heterocystous nitrogen fixers. Unicellular and colonial forms, with extensive mucilage like *Aphanocapsa, Aphanothece, Chroococcus, Gloeocapsa* and *Gloeothece* and filamentous species of *Scytonema, Lyngbya* and *Tolypothri* were also very common in Maharashtara soils. A significant reduction in soil salinity (12-35%) due to repeated cultivation of *Anabaena torulosa* in soils rendered saline owing to bad farm management has also been reported Cyanobacteria have been found not only to grow in highly saline-alkali soils, but also improve the physico-chemical properties of the soil by enriching them with carbon, nitrogen and available phosphorus [9,10]. Successive cultivation of BGA makes the environment more favourable and after a few years it may help to produce a reasonably good yield of crops, as observed by Singh [23] for sugarcane after 3 years of reclamation with BGA. Although infrequent at pH below 6.0, their ability to grow in diverse pH ranges and modify their environment makes them successful in any niche. Acidic soils, in general do not support their growth, although a few reports on their presence in soils with pH values between 5 and 6 are available [1,16,17].

Correlation between the number of isolates from each of the dominant genera and pH of the location sampled also showed a positive correlation for *Calothrix, Phormidium* and *Hapalosiphon (Fig. 6).* The numbers of isolates belonging to the genera *Nostoc* and *Anabaena*, however, exhibited a negative correlation, indicating that although these two genera show highest relative abundance quantitatively, their relative tolerance to pH is low. *Nostoc* and *Anabaena*, therefore, exhibited superior establishing and adaptive traits, although in terms of numbers, they showed an uneven distribution at different pH. However, the correlation was not statistically significant in any of these cases. Among the non-heterocystous cyanobacteria, *Phormidium* was the most cosmopolitan –20 isolates as against 9 and 5 belonging to *Oscillatoria/Lyngbya.* A similar trend was observed on correlating EC values and number of isolates belonging to the dominant genera (data not shown). Species of *Calothrix* and *Aulosira* have been reported to be ubiquitous in rice fields of Kerala where pH ranges from 3.5 – 6.5 [1]. Also, the number of spore producers is known to show a positive correlation with soil pH. Enrichment of such soils with the indigenous cyanobacterial isolates may help in ameliorating the land and making them suitable for obtaining higher yields as benefits, other than nitrogen fixation, include solubilisation of phosphorus, improved soil structure and synthesis of growth promoting substances are also known.

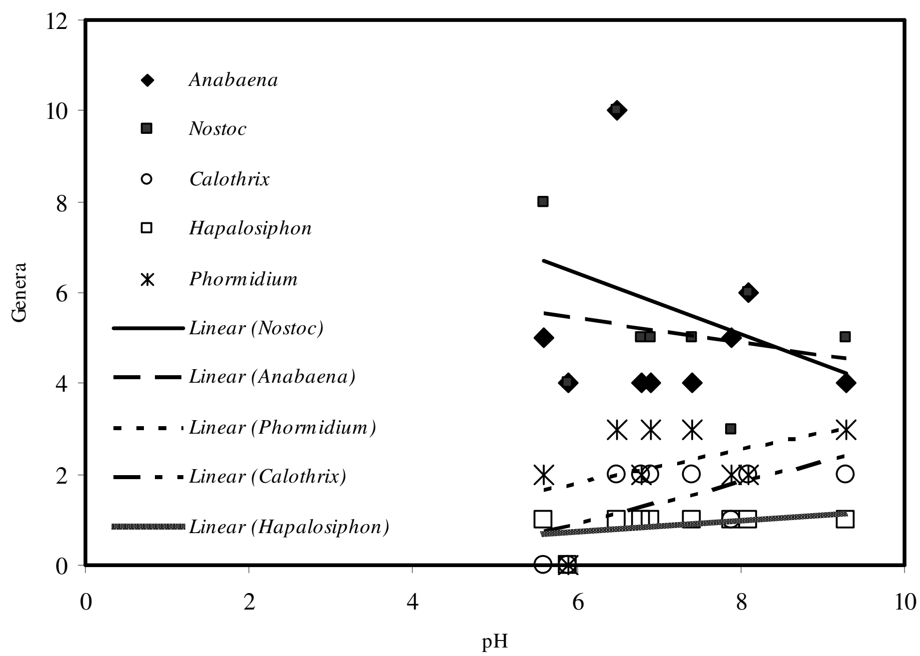

Figure 6. *Correlation of number of isolates of dominant genera with soil pH.*

The diversity within a genus was also further analysed by measuring the nitrogen fixing potential (using acetylene reducing activity as an index) of 46 isolates of

Anabaena (Fig.7). Highest values were recorded in the isolates from Assam (A43 and 44), followed by the isolate from saline-alkali soil samples from Lucknow (A15).

The effect of pH on algal flora is generally difficult to evaluate as it is often correlated with other factors, for *e.g.* arid soils are almost universally alkaline and many continuously wet soils acidic. Among correlations between the relative abundance of the individual groups of heterocystous cyanobacteria and soil physico-chemical properties, only the correlation between pH and the relative abundance of *Nostoc* was found to be statistically significant, but a degree of bias was introduced when dry and wet samples were tested separately [21]. Contradictory reports regarding the occurrence in acid and very acid environments are available. However, one of the most acid lakes (pH 2.9) was observed to be inhabited by *Oscillatoria/Limnothrix* and *Spirulina* [25]. Despite the preference for neutral-highly alkaline environments, acidic soils do exhibit low diversity and abundance of cyanobacteria [11].

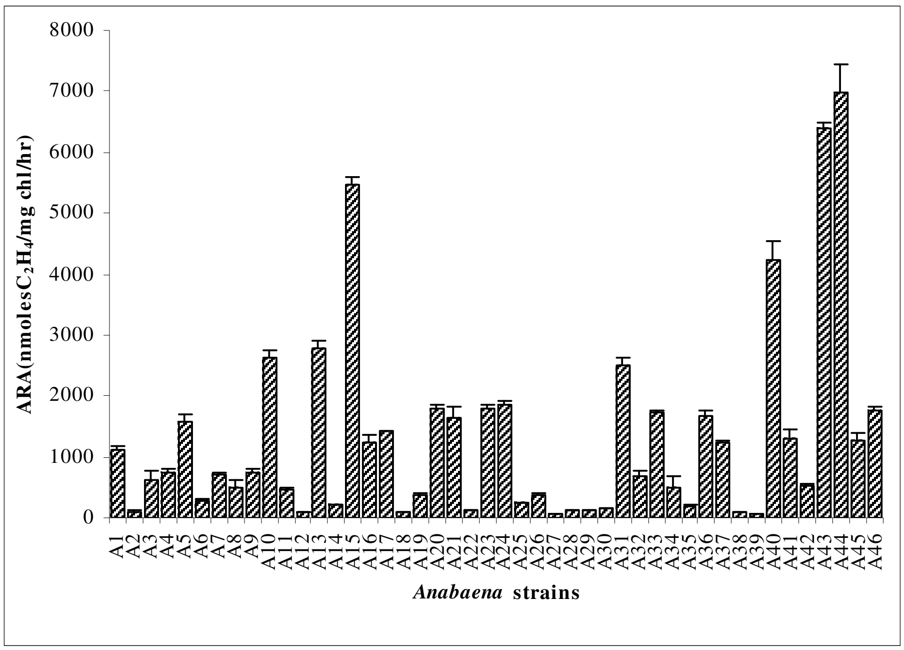

Figure 7. *Diversity in nitrogen fixing potential (measured as acetylene reducing activity, ηmoles C_2H_4 mg $chl^{-1}h^{-1}$) among the isolates of genus Anabaena*

Therefore, efforts need to be focused towards enrichment of indigenous cyanobacterial populations, which are better adapted to the specific niche, through development of multiple inocula preparations on a regional basis. Research programs should be oriented towards agricultural practices, including application of biofertilizers, which enhance the growth and proliferation of indigenous strains.

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MICROBIOLOGICAL QUALITY AND SAFETY ASSESSMENT OF CAMEL MILK (*CAMELUS DROMEDARIES*) IN SAUDI ARABIA (QASSIM REGION)

M. G. EL-ZINEY[1,2*] – A. I. AL-TURKI[3]

[1]*Department of Dairy Science and Technology, Faculty of Agriculture-Al Shatby, Alexandria University, Alexandria, Egypt*
[2]*Department of Food Science and Human Nutrition*
[3]*Department of Plant Production and Protection, College of Agriculture and Veterinary Medicine, Qassim University, P.O. Box 1482, 51431 Buraidah, Saudi Arabia*
(phone: +966-6-3800050 ext 2361, fFax: +966-6-3801360)

e-mail: elziney@yahoo.com

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Abstract. The microbiological quality and safety of raw camel milk from different farms in Qassim region (middle Saudi Arabia) were examined. Milk samples (n=33) were aseptically collected from the milking bowls. Samples were analyzed for several microbial quality attributes including aerobic total plate count (ATPC), psychrotrophs (PC), aerobic mesophilic sporeforming bacteria (AMSC), *Enterobacteriaceae*, total coliforms, faecal coliforms and moulds and yeasts. Furthermore, the presence of selected pathogens such as *Staphylococcus aureus* and Salmonella was detected. The mean log counts per ml for ATPC, psychrotrophs, aerobic mesophilic spore former, *Enterobacteriaceae*, and moulds and yeasts were 5.0, 3.8, 2.1, 2.7, and 1.9, respectively. Coliform group was found in 45.5 % of samples while 12% were faecal coliform positive as revealed by MPN method. *S. aureus* was located in 70% of the samples and the mean count was 2.7 log cfu per ml. Meanwhile, salmonella was detected in 24% of the samples. Results indicate the potential health risk of consuming raw camel milk under the present production conditions.

Keywords: *camel milk, aerobic total count, psychrotrophs, coliforms, aerobic spore former, S. aureus, Salmonella*

Introduction

Nowadays, public health concern associated with microbial food safety has arisen. Numerous epidemiological reports have implicated non-heat treated milk and raw-milk products as the major factors responsible for illnesses caused by food-borne pathogens [9, 19]. Cross-contamination with pathogenic microorganisms can gain access to milk either by faecal contamination or by direct excretion from the udder into milk.

Camel meat and milk are the key foods in arid and semi-arid areas of the African and Asian countries, especially in Saudi Arabia. Food Agriculture Organization has reported that more than 18 million camels around the world support the survival of millions of people [14]. Camel milk not only contains more nutrients compared to cow milk [1], but also it has therapeutic and antimicrobial agents [4, 12]. Saudi Arabia produced over one percent of world stocks of camels (425,000 head). In regard to camel milk production, Saudi is globally ranked at the seventh position (89,500 cubic metres) [14].

In fact, most of camel milk is consumed in the raw state without any heat treatments or acid fermentation and kept at high ambient temperature coupled with lack of refrigeration facilities during milking and transporting. These conditions turn the milk to be unsafe, capable of causing food-borne diseases and it even spoil fast.

In Qassim area, as in many regions around the kingdom, camel milk is produced in traditional way by hand milking, handled and transported under low hygienic measures. However, there is no reports documented any outbreak related to unpasteurized (raw) camel milk. Furthermore, there is a limited data on the microbial assessment of raw camel milk [2, 29, 5]. Furthermore, in view of its health benefits, there is a fast growing demand for raw camel milk in Saudi Arabia and further it is expected to be introduced as a new functional food in the European market. Therefore, there is a high necessity to find out about the present hygienic situation regarding the raw camel milk in Qassim area.

The aim of the present study were (1) to assess the microbial quality of raw camel milk in Saudi Arabia (Qassim area) using several microbial quality attributes including aerobic total plate count, aerobic mesophilic spore count, psychrotrophic count, and moulds and yeasts (2) to study the prevalence of a variety of indicator organisms (Nitrobacteria, and total and faecal coliforms) and food-borne pathogens, with reference to *Staphylococcus aureus* and *Salmonella* spp.

Materials and methods

Milk samples

Between February and May 2005, a total of thirty-three-bulk camel milk samples were collected from different locations in Qassim area (middle Saudi Arabia). Milk was collected from camels by hand milking as normally practiced by the farmers in except of the experimental station of animal production of the college of agriculture and veterinary medicine, Qassim University, which introduced the mechanical milking of camels. The samples were collected in sterile screw bottles kept in cool boxes until transported to the laboratory. The samples were analyzed within 24 h.

Microbiological analysis

Milk samples (25 ml) were diluted in buffered peptone saline (225 ml, 0.5% w/v; peptone; 0.85% w/v; NaCl), mixed in stomacher bag and stomached in Seward stomacher (Seward 400, England) for 2 minutes. In order to quantify the various microbial groups, appropriate dilutions were surface plated. Aerobic total plate count (ATPC) was carried out on plate count agar (PCA), incubated at 32°C for 72h [23]. For aerobic mesophilic spore count (AMSC), the milk was heat-shocked at 80°C for 10 min to destroy vegetative cells. After being cooled in an ice bath, the milk was immediately plated on plate count agar and incubated at 32 °C for 48h [23]. Psychrotrophic count (PC) was performed by incubation of appropriate dilutions on PCA kept at 7°C for 10 d [23].

For enumeration of members of the family of *Enterobacteriaceae*, eosine methylene blue agar (modified) Levine (EMB) was used (35°C for 24h). Total and faecal coliforms were determined by MPN method according to US standard method [15]. The enumeration of moulds and yeasts was done on potato dextrose agar (PDA) acidified by lactic acid 10% (Oxoid, SR21).

Staphylococcus aureus was enumerated on Baird Parker agar supplemented with egg yolk enrichment at 37°C for 48h. Black shiny colonies surrounded by hello zone were

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examined microscopically and tested for catalase, coagulase and staphylase production using Oxoid reagents according to the manufacturer's instructions.

Salmonella spp. was detected as it is previously described by Andrews and Jacobson [3]. A portion of 25 ml of milk was pre-enriched in 225 ml of buffered peptone water at 37°C for 24h. Then, 1 ml of pre-enrichment sample was incubated in 10 ml cystine selenite broth and Rappaport-Vassiliadis broth at 37°C for 24h. Selective enrichments were then streaked onto bismuth sulphite, xylose lysine desoxycholate (XLD) and Hekton entreic agars. All selective media were incubated at 37°C for 24h. Typical colonies were examined by microscope, characteristics of growth on lysine iron agar, negative of urease production and then tested with *Salmonella* polyvalent O antiserum (Salmonella latex test, Oxoid FT0203). Isolates with typical reactions for salmonella were then confirmed by using API 20E identification kit (BioMérieux, France). Unless otherwise stated, all the media and supplements used throughout the present study were purchased from Oxoid (Oxoid, Basingstoke, Hampshire, England)

Statistical analysis

Descriptive and correlation analysis between the different microbial parameters were performed using SPSS software (Version 10, SPSS Inc., Chicago)

Results and discussions

The presence of the various microbial groups found in raw camel milk from the Qassim area is presented in *(Figure 1 and Table 1.)* The profile of total aerobic mesophilic bacteria in milk samples is shown in *(Fig. 1A).* The mean of TAPC in collected samples was 5 log cfu/ml with a maximum of 7.15 log cfu/ml *(Table 1).* These results are in agreement with those reported for Saudi (i.e., 5.4 log cfu/ml in average) and Ethiopian (i.e. 5.6 log cfu/ml in average) camel milk by Al Mohizea [2] and Semereab and Molla [29], respectively. It is worth to mention that there are no microbiological standards concerning camel milk. Therefore, the microbiological limit values for cow milk was used to assess the quality of camel milk. In our study, 54.5% (n=18) of ATPC results were within the accepted limits (5.3-5.6 log cfu/ml) of APHA [23] and Directive 92/46/EEC [10].

The count of psychrotrophic bacteria was varied between samples. Approximately 30% of the samples had a psychrotrophic count (PC) of ≅ 1 log cfu/ml, with a mean value of 3.8 log cfu/ml while the maximum was 6.82 log cfu/ml *(Fig 1B and Table 1).* The results of psychrotrophs are comparable with average counts (3.3-3.7 log cfu/ml) reported for raw cow milk by Boor et al., [6] and Chye et al., [7]. Further, no information in the literature documented the content of psychrotrophs in camel milk. Psychrophilic bacteria are responsible for an increased production of proteinases and lipases, which can survive heat treatments (i.e. pasteurization) thus affecting the shelf-life and quality of milk [18].

In terms of residual spore forming bacteria, approximately 60% of the samples had >50 aerobic mesophilic spore-formers/ml, with mean value of 2.1 log cfu/ml *(Fig 1C and Table 1).* These results are full within the ranges (i.e., 1.7 log cfu/ml as a mean) for cow's milk found by Boor et al. [6]. No data in the in the literature reported the level of this group of organisms in raw camel's milk. Spore-forming bacteria are known to, apart from causing spoilage, cause food-poisoning by producing heat labile enterotoxins [13, 28].

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Yeasts and moulds were only detected in 19 samples (57%) with the mean and maximum values of 1.9 and 5.65 log cfu/ml, respectively *(Fig 1D and Table 1).* The yeast and moulds content in Moroccan camel's milk was found to be high with an average raised to 4.6 log cfu/ml [5]. In agreement with our results it is reported that the high counts of yeast and moulds in milk is rather uncommon as a result of natural milk pH, causing bacteria to predominate [16, 27].

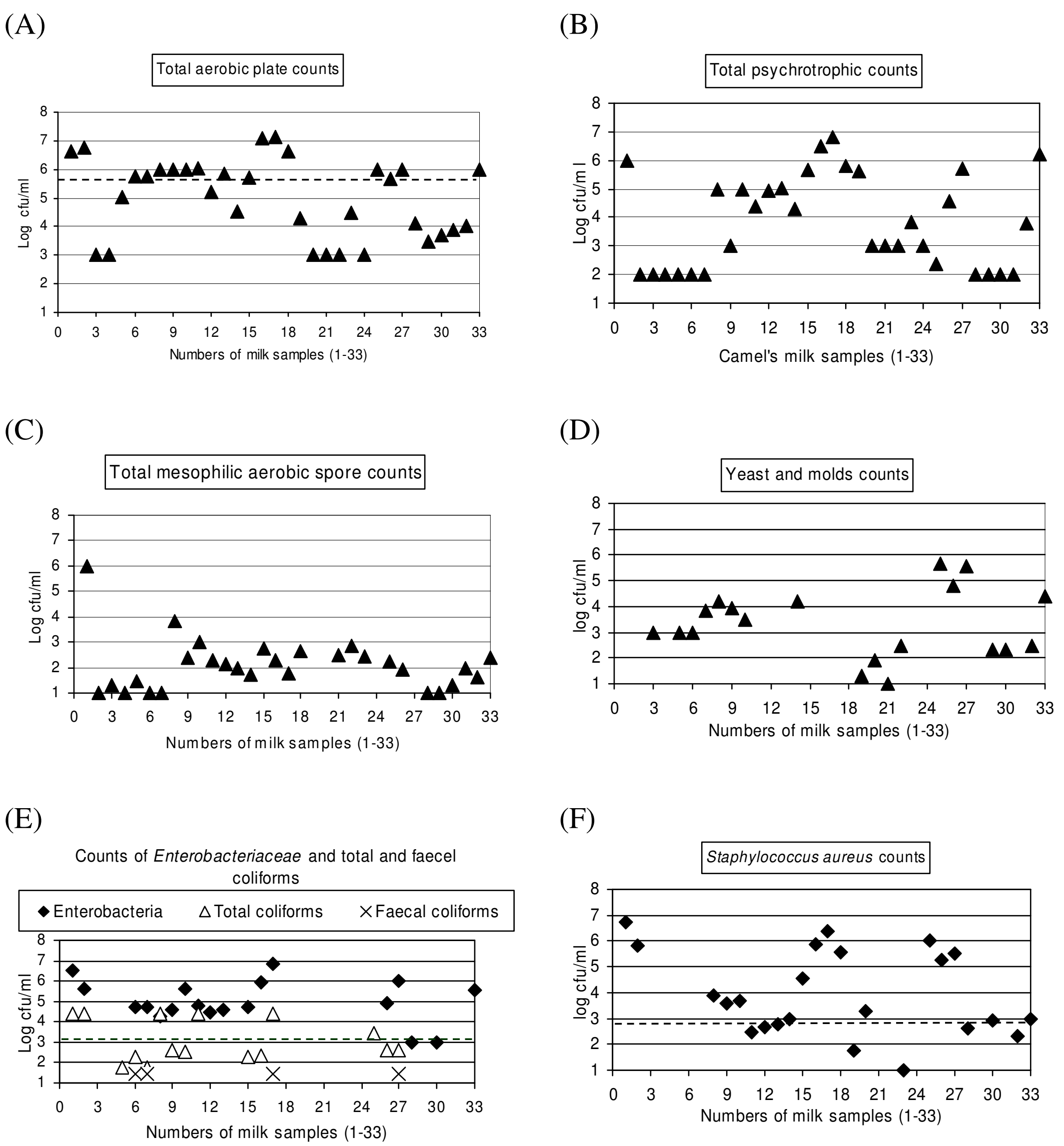

Figure 1. *Patterns of total aerobic plate counts (A), psychrotrophs (B), aerobic mesophilic spore former (D), yeast and moulds (E), Enterobacteriaceae and total and faecal coliforms (F) and Staphylococcus aureus in raw camel's milk (n=33) collected from Qassim region. Dash line (------) represents the EU standards.*

Table 1. *Selected statistical values (log cfu/ml) of different microbial parameters detected in raw camel's milk.*

Microbial parameter	Mean	Std. Dev	Maximum	Minimum
Total aerobic plate counts	5.0	1.36	7.15	3.0
Psychrotrophic counts	3.8	1.64	6.82	2.0
Aerobic mesophilic spore formers	2.1	0.98	6.0	1.0
Enterobacteriaceae	2.72	2.63	6.82	0
Total coliforms	1.4	1.7	4.38	0
Yeast and moulds	1.9	1.93	5.65	0
Staphylococcus aureus	2.7	2.29	6.72	0

Enterobacteriaceae were detected in 18 samples (54.5%). The mean count value of EMB plates was 2.7 log cfu/ml, with a maximum of 6.82 log cfu/ml *(Fig 1E and Table 1)*. The total coliform determined by MPN technique showed a positive result in 15 samples (45.5%) with a maximum value of 4.2 log cfu/ml *(Fig 1E).* Out of total coliforms positive samples (15), only four samples were positive for faecal coliforms which identified as *E. coli* by the growth on MacConkey plates and IMViC tests. The occurrence of total coliforms, in our study, was much lower than reported for Ethiopian raw camel's milk (100%) by Semereab and Molla [29]. Further, Benkerroum et al. [5] demonstrated high total coliforms counts for Moroccan camel's milk (i.e., 6.8 log cfu/ml in average). In our study, six samples (18%) were over the coliform limits fixed by the EC regulations for raw cow milk [10]. The *Enterobacteriaceae* family has earned a reputation placing them among the most pathogenic and most often encountered organisms in food. *Enterobacteriaceae* family includes coliform group (*Escherichia*, *Enterobacter*, *Citrobacter* and *Klebsiella*) in addition to many other genera (*Salmonella*, *Shigella*, *Morganella*, *Providencia*, *Edwardseilla*, *Proteus*, *Serratia* and *Yersinia*) which are isolated from animal intestine [8, 20]. The existence of coliform bacteria may not necessary indicate a direct faecal contamination of milk, but precisely as an indicator for poor sanitary practices during milking and further handling processes. Moreover, the presence of faecal coliforms, i.e. *E. coli* implies a risk that other enteric pathogens may be present in the sample.

Nearly 70% (n=23) of the collected samples were contaminated by *S. aureus*, with a mean count of 2.74 log cfu/ml, while the highest level of contamination reached to 6.72 log cfu/ml *(Fig 1F and Table 1).* The existence rate of *S. aureus*, in the present study, was relatively high, however, the organism has been detected in all tested samples (n=12) in Morracan camel milk [5] with an average of 5.1 log cfu/ml. Semereab and Molla [29] reported that *S. aureus* isolates represent 15% of the total bacteria isolated from composite camel udder milk. The incidence of mastitis in camel herds (19.5%) and the high frequency of *S. aureus* (31.5%) as the casual agent may explain these results [25]. According to the EC standards for raw cow's milk intended for direct consumption [10], 51% (n=17) of the samples were found to have *S. aureus* counts higher than the fixed limit (2.7 log cfu/ml). An overview of the annual reports of food-borne diseases from seven countries indicated that milk and milk products implicated in 1-5% of the total bacterial outbreaks. *S. aureus* was by far the most frequent pathogen associated with these outbreaks (85.5%), followed by *Salmonella* (10%) [9].

The incidence of *Salmonella* spp. was high as 8 (24%) out of 33 milk samples were found to be positive for this organism. The reported isolation rate of this organism for raw cow milk was found to be within the range of 3-9% [22]. However, sixteen percent of organ and faecal samples collected from healthy slaughtered camels were positive for *Salmonella* spp. [24]. Moreover, Huston et al. [21] reported that in 31% of the study dairy herds was shedding *Salmonella* spp.

Salmonella spp., are an infrequent cause of mastitis in dairy animals but several species of *Salmonella* have documented to colonize udders and shed at levels of up to 2000 cells/ml [17]. In addition, camel herds rarely benefit from veterinary care [25] with lack of using appropriate sanitizers between milking intervals, which could enhance the microbial colonization. These organisms pose a health risk to consumer if milk is consumed without any heat treatment. De Buyser et al. [9] reported that *Salmonella* spp is one of the most etiologic agents responsible for several outbreaks associated with the consumption of raw milk and milk products.

In the present study, the correlation analysis between pairs of different microbiological parameters was conducted in order to evaluate the correlate degree among it. The results showed no correlation coefficients above 0.8. The highest positive correlation were found between ATPC and enterobacterial counts (0.77), ATPC and total coliforms (0.73), enterobacterial counts and total coliforms (0.668), ATPC and PC (0.58) and PC and aerobic mesophilic spore counts (AMSC, 0.544). All other correlation coefficients were below 0.5.

The correlation value between ATPC and PC was low compared with those (i.e., 0.74 in average) reported by Peeler el al. [26] and Boor et al. [6], but a weak correlation (0.42) was found by Chye et al. [7]. It is suggested that these results might be affected by the differences between the climatic conditions between the countries involved in those studies and consequently reflected on the PC levels. The good correlation between PS and AMSC established in our study indicates the possibility of the wide spread of psychrotrophic *Bacillus* spp. These organisms have the ability to survive the pasteurization, grow secreted enzymes or metabolites and affected the milk quality during the cold storage. Correlations between ATPC and total coliforms (0.74), ATPC and faecal coliforms (0.38) and total coliforms and faecal coliforms (0.66) suggest that the contamination is likely to be not originated from faecal origin.

Conclusion

The outcome of the present results suggests that approximately 50% of the examined raw camel milk samples were produced and handled under poor hygienic conditions with high health risk to the consumers. Based on these findings, it is strongly recommended that large-scale research studies regarding the quality of raw camel milk, milking protocols and sanitizing programs should be conducted. Such studies will help to understand the behavioural risk factors associated with raw milk production, consumption and that educational programs will be developed to address issues connected to consumption of raw camel milk. The characteristics and especially the behaviour of isolated microorganisms show that the pathogens must be studied to explore the cycle of contamination and how these organisms are able to survive under severely arid conditions.

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ASSESSMENT OF PLANT DIVERSITY AND PRIORITIZATION OF COMMUNITIES FOR CONSERVATION IN MORNAULA RESERVE FOREST

S. PANT – S.S. SAMANT*

G.B. Pant Institute of Himalayan Environment and Development, Himachal Unit, Mohal-Kullu 175 126, Himachal Pradesh, India
(phone: +91-1902-225329 Ext. 21 (O); fax: +91-1902-226347)

e-mail: samantss2@rediffmail.com; samant62@yahoo.com

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Abstract. Assessment of plant diversity of the Reserve Forests of the west Himalaya and prioritization of communities for conservation have not been given much attention. Therefore, the study has been conducted in a biodiversity rich Mornaula Reserve Forest between 1500-2200m to analyse the structure, composition of the forest communities including richness of economically important, native, endemic and rare-endangered species, and prioritize communities for conservation. A total of 123 sites were sampled. For each site, habitat characteristics, altitude and dominant species have been given. From the sampled sites, 289 species (37 trees; 37 shrubs; and 215 herbs) and 31 forest communities have been recorded. The density of trees ranged from 340-2438 Ind ha^{-1} and TBA from 19.52-234.31 Ind m^2. The densities of saplings ranged from 340.00-2277.00 Ind ha^{-1} and seedlings 266.00-1571.00 Ind ha^{-1}; shrubs 357-1156 Ind ha^{-1} and herbs 21.73-431.04 Ind m^{-2}. The richness of the trees ranged from 3-27, shrubs, 8-36, herbs, 17-145, seedlings, 3-22, and saplings, 2-21. Species diversity for trees ranged from 0.99-2.93, seedlings, 0.86-2.65, saplings, 0.44-2.78, shrubs, 1.94-4.43 and herbs, 1.42-4.66. These recorded values were almost comparable with the studies conducted in sub-tropical, temperate and sub-alpine regions of the west Himalaya. In some cases the values were slightly higher than the reported values. The communities have been prioritized for conservation based on the species richness, nativity, endemism, economically important and rare-endangered species. Among, all the prioritized communities, *Rhododendron arboreum* community supports maximum species including native, endemic, economically important and rare-endangered species. In view of the high socio-economic and conservation values of the identified communities, monitoring of these communities at least for a period of five years and development of appropriate strategy and action plan for the conservation and management have been suggested.

Keywords: *Reserve Forest, communities, diversity, native, rare-endangered, socioeconomic, prioritization, conservation*

Introduction

The Indian Himalayan Region (IHR) is very well known throughout the globe due to its representative, unique, natural, and socio-economically important flora and fauna [1]. Due to this peculiar feature, the eastern Himalaya has been identifies one of the biodiversity Hot Spots [2]. This rich biodiversity is being utilized by the inhabitants of the region for medicine, as wild edible (food), fodder, fuel, timber, in making agriculture tools, religious and various other purposes [3, 4, 1]. With the increasing human population, the demand of the economically important biodiversity is increasing fast. Collection of fodder and fuel species from the forests has been identified one of the chronic problems in the IHR for the degradation of forest [5]. The anthropogenic pressures including heavy grazing coupled with the natural calamities have lead the degradation of natural habitats of many species to a great extent. Such practices are discoursing the moisture loving species and promoting the hardy and spiny species having least value for the society. This loss of biodiversity and changing pattern of

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vegetation has necessitated assess the biodiversity of the region and prioritize habitats, communities and species for conservation.

In general, structural and functional diversity of the some parts of the IHR have been evaluated by various workers [6-33]. However, the protected areas of the IHR including Reserve Forests have been very poorly evaluated for the structural and functional diversity [34] except a few studies carried out in Nanda Devi Biosphere Reserve (NDBR) and Askot Wildlife Sanctuary (AWLS) [35-39]. Further, studies integrating compositional, structural and functional diversity, native, endemic, economically important and rare-endangered species, and prioritization of community for conservation have been attempted in a few protected areas [37, 38]. Therefore, the attempt has been made to; (i) study the site/habitat characteristics; (ii) assess the diversity and distribution pattern of the species; (iii) delineate forest communities; (iv) study the distribution pattern of economically important, native, endemic and rare-endangered species within the identified communities; and (v) prioritize communities for conservation.

Materials and methods

Identification and selection of transects, sites and habitats

Four transacts *i.e.,* (i) Harinagar to Nartola; (ii) Bercheula-Lohanigaon-Mornaula; (iii) Khakar-Bheutania-Tarani; (iv) Dole-Damar-Mornaula were selected along the trails of the villages on account of typical topography and inaccessibility of the area. The sites were selected on each and every accessible aspect along transacts between 1500-2200m. The habitats were identified on the basis of physical characters and dominance of the vegetation. Sites having closed canopy with high percent of humus and moisture were considered as moist habitats whereas low percent of the same as dry habitats. The sites having >50% boulders of the ground cover had been considered as bouldary habitat, and the sites facing high anthropogenic pressures considered as degraded habitats.

Survey, sampling, identification and analysis of data

The field surveys and samplings were conducted between 2002 and 2004 within the selected sites along the transacts. In each site, a plot of 50x50m was laid. Trees, saplings and seedlings were sampled by randomly placed 10, 10x10m quadrates; shrubs by 10, 5x5m quadrates; herbs by 20, 1x1m quadrates in each plot. The size and number of quadrates was determined following [40]. For the collection of data from these quadrates standard ecological methods [41, 42, 40, 43, 35, 39, 38] were followed. From each site, samples of each species were collected and identified in the Institute with the help of florulas and research papers [44, 45, 46, 29].

For trees, basal area and Importance Value Index (IVI) have also been computed. IVI has been calculated as the sum of relative frequency, relative density and relative basal area. The abundance data of different sites were pooled to get community averages in terms of density, total basal area and IVI. Communities were identified based on the IVI. A species contributing 50 % or >50 % of the total IVI in a particular site/habitat is considered as a single species dominated community, <50 % of the total IVI is named as a mixed community.

Species diversity (H') and Concentration of dominance (Cd)

Species richness is the total number of species in a particular community. Species diversity was determined by Shannon Wiener's information statistic (H') [47] and concentration of dominance by [48]

Identification of native, endemic and rare-endangered species

Native species were identified following [49, 1, 50, 28, 29]. The species having their origin in the Himalayan region have been considered as natives. Endemism is based on the distribution range of the species [51, 1, 36]. The species restricted to the IHR have been considered as endemic whereas those with extended distribution to neighbouring countries/States as near endemic. Rare-Endangered species has been identified based on habitat specificity, population size, distribution range and anthropogenic pressures [52, 53, 36].

Prioritization of communities

Based on the occurrence of number of species (species richness), economically important, native, endemic and rare-endangered species, within the communities, prioritization of the communities for conservation has been done.

Results and discussion

Site and habitat characteristics

Site/habitat characteristic, dominant species, altitude, slope and aspect of all four transects are presented *(Table 1)*. The altitude ranged between 1500-2200m and majority of the study sites fall in northeast aspect. In all five habitats *i.e.*, shady moist, dry, riverine, bouldary, and degraded were represented in the area *(Table 1)*.

Community diversity and distribution pattern

Thirty-one forest communities have been identified between 1500-2200m in the Mornaula Reserve Forest (MRF). The community types, altitudinal distribution, sites and habitat representation and major tree associates are presented in *(Table 2)*. *Rhododendron arboreum* community represented maximum sites (26), followed by *Quercus leucotrichophora* (18 sites), and *Pinus roxburghii* (16 sites), and the remaining communities showed less representation of sites. Among the communities *Rhododendron arboreum*, *Quercus leucotrichophora*, *Pinus roxburghii*, *Quercus floribunda*, *Cupressus torulosa*, and *Quercus leucotrichophora-Rhododendron arboreum* mixed, showed comparatively wide altitudinal range of distribution.

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Table 1. *Physical characteristics of sites in the MRF*

Transact 1: Nartola to Harinagar

S.No.	Altitude (m)	Slope (°)	Habitat (s)	Aspect	Dominant Species
1	2130	10	D	E	*Quercus floribunda* & *Quercus leucotrichophora*
2	2130	20	B	E	*Quercus leucotrichophora* & *Quercus floribunda*
3	2130	5	B	SW	*Quercus floribunda* & *Quercus leucotrichophora*
4	2130	15	B	S	*Rhododendron arboreum* & *Quercus floribunda*
5	2130	20	B	N	*Rhododendron arboreum* & *Quercus floribunda*
6	2130	25	B	NE	*Quercus floribunda* & *Betula alnoides*
7	2120	20	B	SE	*Betula alnoides*, *Quercus floribunda*, *Lyonia ovalifolia*
8	2120	25	B	SE	*Rhododendron arboreum*, *Quercus floribunda*, *Alnus nepalensis*
9	2120	45	B	NE	*Quercus leucotrichophora*, *Rhododendron arboreum*, *Betula alnoides*
10	2125	25	D	S	*Quercus leucotrichophora*
11	2120	15	B	NE	*Quercus leucotrichophora* & *Quercus floribunda*
12	2115	10	C	NE	*Quercus floribunda*
13	2115	30	B	NE	*Quercus floribunda*
14	2120	30	B	NW	*Rhododendron arboreum*
15	2125	15	B	SE	*Rhododendron arboreum*
16	2125	20	B	E	*Quercus leucotrichophora* & *Rhododendron arboreum*
17	2125	35	B	SE	*Rhododendron arboreum* & *Quercus leucotrichophora*
18	2070	45	B	NE	*Abies pindrow*
19	2070	40	B	NW	*Abies pindrow* & *Quercus leucotrichophora*
20	2125	40	B	NW	*Quercus leucotrichophora* & *Rhododendron arboreum*
21	2125	40	B	E	*Rhododendron arboreum* & *Quercus leucotrichophora*
22	2120	35	B	SW	*Abies pindrow*
23	2125	5	D	NW	*Rhododendron arboreum* & *Quercus leucotrichophora*
24	2120	20	C	SE	*Persea duthiei* & *Rhododendron arboreum*

Transact 2: Bercheula – Lohanigaon-Mornaula

S.No.	Altitude (m)	Slope (°)	Habitat (s)	Aspect	Dominant Species
1	1900	40	A	S	*Pinus roxburghii*
2	1860	10	C	E	*Rhododendron arboreum*, *Daphniphyllum himalayense*
3	1870	40	B	N	*Rhododendron arboreum*, *Aesculus indica*, *Quercus floribunda*
4	1870	40	A	NE	*Pinus roxburghii*
5	1890	20	C	NE	*Rhododendron arboreum*
6	1900	40	A	NE	*Pinus roxburghii*
7	1890	20	C	NW	*Alnus nepalensis*
8	1970	45	B	NE	*Rhododendron arboreum*
9	1970	40	B	NE	Rhododendron arboreum
10	1960	40	D	NW	*Pinus roxburghii*
11	1960	40	B	W	*Cedrus deodara*
12	1950	50	B	E	*Cupressus torulosa*
13	2050	20	B	S	*Cedrus deodara*
14	2060	30	C	SE	*Rhododendron arboreum*
15	2040	20	C	SE	*Alnus nepalensis*
16	2070	40	A	S	*Quercus leucotrichophora*
17	2090	35	A	S	*Pinus roxburghii*
18	2095	45	B	NW	*Rhododendron arboreum*

Transact 2: Bercheula – Lohanigaon-Mornaula

S.No.	Altitude (m)	Slope (°)	Habitat (s)	Aspect	Dominant Species
19	2090	40	E	SW	*Pinus roxburghii*
20	2090	40	A	W	*Pinus roxburghii*
21	2100	20	B	W	*Rhododendron arboreum*
22	2090	35	B	N	*Rhododendron arboreum* & *Myrica esculenta*
23	2095	35	B	SE	*Cupressus torulosa*
24	2070	35	C	NW	*Rhododendron arboreum* & *Alnus nepalensis*
25	2070	35	A	W	*Pinus roxburghii*
26	2095	45	B	E	*Myrica esculenta*
27	2095	25	B	SW	*Cupressus torulosa*
28	2095	35	B	SE	*Myrica esculenta*
29	2080	45	B	SW	*Cupressus torulosa*
30	2105	60	B	N	*Rhododendron arboreum*
31	2105	50	B	NW	*Rhododendron arboreum*
32	2110	40	B	NE	*Rhododendron arboreum*
33	2110	25	B	W	*Rhododendron arboreum*
34	2120	35	C	SE	*Persea duthiei* & *Litsea umbrosa*
35	2115	20	C	NE	*Betula alnoides* & *Rhododendron arboreum*
36	2115	20	B	N	*Rhododendron arboreum* & *Lyonia ovalifolia*
37	2105	25	B	NE	*Betula alnoides* & *Rhododendron arboreum*
38	2105	20	B	NE	*Rhododendron arboreum*
39	2100	5	C	SE	*Acer cappadocicum* & *Persea duthiei*
40	1990	10	C	SE	*Quercus floribunda*
41	1985	40	B	NE	*Quercus leucotrichophora*
42	1990	15	B	S	*Rhododendron arboreum*
43	2105	15	B	E	*Quercus floribunda* & *Quercus leucotrichophora*
44	2110	15	D	SW	*Quercus floribunda*

Transact 3: Khakar-Bheutania-Tarani

S.No.	Altitude (m)	Slope (°)	Habitat (s)	Aspect	Dominant Species
1	2010	15	C	E	*Quercus floribunda*
2	2020	45	A	S	*Quercus floribunda*
3	2030	35	A	E	*Quercus floribunda* & *Quercus leucotrichophora*
4	2060	45	B	NE	*Rhododendron arboreum*
5	2080	35	C	E	*Rhododendron arboreum* & *Betula alnoides*
6	2100	35	A	SE	*Quercus floribunda*
7	2100	50	B	NE	*Rhododendron arboreum*
8	2075	35	C	NE	*Rhododendron arboreum*
9	2100	25	D	N	*Rhododendron arboreum*
10	2100	45	C	NE	*Rhododendron arboreum*
11	2100	40	B	NW	*Rhododendron arboreum*
12	2110	25	B	NW	*Quercus leucotrichophora*
13	2115	25	B	NW	*Quercus leucotrichophora*
14	2110	5	B	E	*Persea duthiei* & *Rhododendron arboreum*
15	2015	50	A	S	*Rhododendron arboreum* & *Quercus floribunda*
16	2010	40	C	N	*Rhododendron arboreum*
17	2030	40	A	E	*Pinus roxburghii*
18	1940	30	B	N	*Quercus floribunda*
19	1960	45	D	N	*Pinus roxburghii*
20	1960	50	D	SW	*Pinus roxburghii*
21	1940	35	D	E	*Quercus floribunda*

Transact 3: Khakar-Bheutania-Tarani

S.No.	Altitude (m)	Slope (°)	Habitat (s)	Aspect	Dominant Species
22	1960	55	A	SW	*Pinus roxburghii*
23	1950	50	A	E	*Pinus roxburghii*
24	1940	10	C	SE	*Daphniphyllum himalayense*
25	1650	50	B	N	*Quercus leucotrichophora*
26	1650	70	B	SW	*Quercus leucotrichophora*
27	1720	40	A	SW	*Quercus leucotrichophora*
28	1790	45	B	NW	*Quercus leucotrichophora*
29	1790	60	B	SW	*Myrica esculenta* & *Quercus leucotrichophora*
30	1790	30	C	NE	*Quercus leucotrichophora* & *Rhododendron arboreum*
31	1800	65	B	NW	*Quercus leucotrichophora*
32	1840	50	B	W	*Quercus leucotrichophora*
33	1860	70	A	S	*Pinus roxburghii*

Transact 4: Dol-Damar-Mornaula

S.No.	Altitude (m)	Slope (°)	Habitat (s)	Aspect	Dominant Species
1	1960	15	A	E	*Quercus leucotrichophora*
2	1950	50	B	N	*Cedrus deodara*
3	1910	15	C	N	*Cedrus deodara*
4	2030	30	A	S	*Pinus roxburghii*
5	2030	65	B	SE	*Cupressus torulosa*
6	2025	40	C	SE	*Quercus leucotrichophora*
7	2025	35	A	SE	*Quercus leucotrichophora*
8	2070	50	B	S	*Quercus leucotrichophora*
9	2060	70	A	SW	*Quercus leucotrichophora*
10	2080	50	B	SW	*Rhododendron arboreum*
11	2080	50	B	NW	*Myrica esculenta*
12	2085	45	A	SW	*Pinus roxburghii*
13	2100	40	B	N	*Rhododendron arboreum*
14	2100	10	B	N	*Quercus leucotrichophora* & *Rhododendron arboreum*
15	2105	15	D	NE	*Quercus leucotrichophora* & *Rhododendron arboreum*
16	2110	30	B	NE	*Rhododendron arboreum*
17	2120	15	C	NE	*Rhododendron arboreum*
18	2110	20	C	NE	*Persea odoratissima*
19	2110	15	B	E	*Litsea umbrosa* & *Rhododendron arboreum*
20	2110	15	B	E	*Rhododendron arboreum* & *Quercus floribunda*
21	2110	10	C	SE	*Persea odoratissima*
22	2110	45	B	E	*Rhododendron arboreum*

Abbreviations used: SR= Site representation; A= Dry habitat; B= Moist habitat; C= Riverine habitat; D= Degraded habitat; and E= Bouldary habitat; E=East; N=North; W=West; S=South; NE=North east; SE=South east; NW=North west; SW=South west; and SE=South east

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Table 2. *Community types, distribution and major tree associates*

Community types	SR	Altitud. range (m)	Habitat (s)	Density (Ind/ha)					TBA (Ind m^2)	Major associate species
				Trees	Seedlings	Saplings	Shrubs	Herbs		
Abies pindrow	3	2120-2200	B	1128	964	1644	9522	72.19	135.49	*Litsea umbrosa*, *Quercus floribunda*
Acer cappadocicum-Persea duthiei-Quercus floribunda mixed	1	2100	C	1100	1348	1290	9240	51.54	125.20	*Symplocos chinensis*, *Aesculus indica*, *Rhododendron arboreum*
Aesculus indica-Litsea umbrosa-Quercus leucotrichophora mixed	1	1985	B	710	1285	1310	8100	38.83	46.21	*Quercus floribunda*, *Acer cappadocicum Carpinus viminea*
Alnus nepalensis	2	1890-2040	C	1215	693	1265	4525	131.65	229.76	*Rhododendron arboreum*, *Lyonia ovalifolia*, *Litsea umbrosa*, *Daphniphyllum himalayense*, *Carpinus viminea*
Betula alnoides	3	2105-2115	B, C	1282	1556	1470	10528	55.47	155.71	*Persea odoratissima, Quercus floribunda, Daphniphyllum himalayense, Ulmus wallichiana, Rhododendron arboreum*
Cedrus deodara	4	1910-2050	B, C, D	1033	712	758	8863	103.65	122.89	*Quercus leucotrichophora*, *Ilex dipyrena*, *Myrica esculenta*
Cupressus torulosa	5	1695-2095	B, C	973	947	692	8710	181.23	112.83	*Quercus leucotrichophora, Rhododendron arboreum*
Daphniphyllum himalayense	1	1940	C	1010	710	690	5630	75.35	82.52	*Litsea umbrosa*, *Aesculus indica*, *Ilex dipyrena*
Litsea umbrosa-Rhododendron arboreum-Quercus leucotrichophora mixed	1	2110	B	1010	837	370	8500	61.85	124.27	*Stranvaessia naussia*, *Acer cappadocicum*, *Quercus floribunda*
Myrica esculenta	1	2080	B	760	697	1580	9490	21.73	81.78	*Rhododendron arboreum*, *Quercus leucotrichophora*
Myrica esculenta-Quercus leucotrichophora-Rhododendron arboreum mixed	1	1790	B	590	635	860	5300	32.30	35.33	*Myrica esculenta*, *Quercus leucotrichophora*, *Rhododendron arboreum*
Myrica esculenta-Rhododendron arboreum mixed	3	2060-2095	B, C	1543	884	1125	5315	102.80	189.54	*Quercus leucotrichophora*, *Quercus floribunda*
Persea duthiei	2	2120	C	1055	983	2120	8515	94.55	99.67	*Persea odoratissima*, *Daphniphyllum himalayense, Litsea umbrosa, Quercus leucotrichophora*
Persea duthiei-Rhododendron arboreum mixed	1	2110	B	1060	960	777	2070	40.05	109.69	*Ilex dipyrena*, *Viburnum mullaha*, *Pyrus pashia*
Persea odoratissima	3	2110	B, C	846	914	2277	5807	109.18	132.42	*Litsea umbrosa*, *Quercus floribunda*, *Meliosma pungens*

Community types	SR	Altitud. range (m)	Habitat (s)	Density (Ind/ha)					TBA (Ind m^2)	Major associate species
				Trees	Seedlings	Saplings	Shrubs	Herbs		
Pinus roxburghii	16	1840-2090	A, B, C, D, E	1453	1488	625	8930	243.78	138.73	*Rhododendron arboreum*, *Quercus leucotrichophora*, *Acer oblongum*, *Quercus floribunda*
Pinus roxburghii-Quercus leucotrichophora mixed	1	2070	A	1200	732	830	357	76.48	142.39	*Rhododendron arboreum*, *Symplocos chinensis*
Quercus floribunda	9	1940-2130	A, B, C, D	1907	983	2242	9856	431.04	190.35	*Quercus leucotrichophora*, *Myrica esculenta*, *Daphniphyllum himalayense*
Quercus floribunda-Quercus leucotrichophora mixed	2	1990-2130	B	1240	572	1378	4265	36.20	127.57	*Rhododendron arboreum*, *Lyonia ovalifolia*
Quercus floribunda-Rhododendron arboreum mixed	3	1940-2105	B, D	1222	747	747	7263	128.18	117.75	*Quercus leucotrichophora*, *Persea duthiei*, *Symplocos chinensis*, *Lyonia ovalifolia*
Quercus leucotrichchophora-Quercus floribunda mixed	1	2130	B	1250	960	960	704	28.88	133.89	*Lyonia ovalifolia*, *Symplocos chinensis*, *Pyrus pashia*
Quercus leucotrichophora	18	1650-2130	A, B, C, D	1930	1371	722	10153	182.79	158.31	*Pinus roxburghii*, *Quercus floribunda*, *Myrica esculenta*, *Rhododendron arboreum*
Quercus leucotrichophora-Rhododendron arboreum mixed	3	1790-2125	B, C	1607	1571	1078	6603	61.75	156.76	*Myrica esculenta*, *Betula alnoides*, *Quercus floribunda*, *Litsea umbrosa*
Rhododendron arboreum	26	1860-2125	B, C, D	2438	1171	657	11056	264.25	234.31	*Pinus roxburghii*, *Myrica esculenta*, *Acer oblongum*, *Aesculus indica*, *Quercus floribunda*
Rhododendron arboreum - Quercus floribunda mixed	6	1990-2130	B	1977	1362	1003	10580	110.99	175.43	*Lyonia ovalifolia*, *Persea odoratissima*, *Quercus leucotrichophora*
Rhododendron arboreum-Alnus nepalensis mixed	1	2070	C	1080	674	830	4630	43.28	195.35	*Quercus floribunda*, *Persea duthiei*, *Betula alnoides*
Rhododendron arboreum-Betula alnoides mixed	1	2080	C	1420	910	350	9310	69.50	221.66	*Persea duthiei*, *Alnus nepalensis*, *Stranvaessia naussia*, *Quercus leucotrichophora*
Rhododendron arboreum-Myrica esculenta mixed	1	2090-2100	B	1290	1066	888	4460	330.75	154.12	*Quercus floribunda*, *Cedrus deodara*, *Cupressus torulosa*, *Quercus leucotrichophora*
Rhododendron arboreum-Persea odoratissima mixed	1	2110	B	920	1319	1880	5450	42.03	104.69	*Quercus leucotrichophora*, *Litsea umbrosa*
Quercus floribunda-Rhododendron arboreum- Pinus roxburghii-Quercus leucotrichophora mixed	1	2015	A	1110	266	340	4530	91.95	85.64	*Ilex dipyrena*, *Lyonia ovalifolia*
Rhododendron arboreum-Quercus leucotrichophora mixed	1	2125-2160	C, D	340	557	1510	6710	43.48	19.52	*Pinus roxburghii*, *Symplocos chinensis*

Abbreviations used: SR= Site representation; A= Dry habitat; B= Moist habitat; C= Riverine habitat; D= Degraded habitat; and E= Bouldary habitat

Vegetation composition

Species richness

In all, 289 species (37 trees; 37 shrubs; and 215 herbs) were recorded. Richness of trees ranged from 3-27; shrubs from 8-36, herbs from 17-145, seedlings from 3-22, and saplings from 2-21. The values for trees were higher than the earlier reported values [54, 20, 22] but comparable to the values reported by [36, 38, 39] from high altitude areas, and also comparable to the sub-tropical and temperate regions (*i.e.,* 9-28) [14, 33]. For shrubs, the values were slightly higher than earlier records, (4-22) from subtropical and temperate forests [22, 23, 15]. For herb layer, the values were higher than previous records [54]. The high richness of trees and shrubs may be due to diverse habitats and suitable edaphic and climatic factors supporting growth and survival of the species.

Richness of native and endemic species

Of the total 289 species, 206 species were native to the Himalayan Region; 83 species were non-natives; 117 species were near endemic; and only two species *i.e., Goldfussia dalhoussiana*, and *Onychium fragile* were endemic. Of the natives, 29 species were trees, 26 species were shrubs and 151 species were herbs. The high percentage of native species in the area may be due to unique topography, inaccessibility and distance from road heads.

Among all the communities, *Rhododendron arboreum* community supports maximum, native and endemic species, followed by *Quercus leucotrichophora*, *Quercus leucotrichophora*, *Quercus floribunda*, *Rhododendron arboreum*-*Quercus floribunda* mixed, *Pinus roxburghii*, *Quercus floribunda*-*Rhododendron arboreum*, *Cupressus torulosa*, *Betula alnoides*, *Persea duthiei*, *Cedrus deodara*, *Myrica esculenta*-*Rhododendron arboreum* mixed, and *Alnus nepalensis*, communities *(Table 3).*

Table 3. *Richness of economically important, native, endemic, near endemic and rare endangered species in prioritized communities*

Community types	No. of Species				
	Native	Economically important	Endemic	Near endemic	Rare endangered
Rhododendron arboreum	119	127	2	33	6
Quercus leucotrichophora	97	98	2	25	6
Quercus floribunda	73	85	1	21	5
Rhododendron arboreum-Quercus floribunda mixed	70	90	1	18	4
Pinus roxburghii	67	59	1	16	3
Quercus floribunda-Rhododendron arboreum mixed	55	104	1	14	5
Cupressus torulosa	55	69	1	19	2
Betula alnoides	51	67	1	20	2
Persea duthiei	51	52	1	18	4
Cedrus deodara	46	59	1	11	1
Myrica esculenta-Rhododendron arboreum mixed	45	68	1	18	2
Alnus nepalensis	43	58	1	10	4

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Structural pattern

In general, density, TBA, and IVI of trees and density of saplings, seedlings, shrubs and herbs have been presented *(Table 2).* The tree density in the communities ranged from 340-2438 Ind ha^{-1}, TBA ranged from 19.52-234.31 Ind m^2, sapling density from 340.00-2277.00 Ind ha^{-1} and seedling density from 266.00-1571.00 Ind ha^{-1}; shrub density ranged from 357-1156 Ind ha^{-1} and herb density from 21.73-431.04 Ind ha^{-1}; Tree density and TBA were slightly higher than the earlier reported values (320-1670 Ind ha^{-1} and 360-1787.50 Ind ha^{-1}) from low and high altitude forests of west Himalaya [10, 8, 11, 16, 54, 22, 36]. The total shrub density for MRF is lower than the reported range for the Pindari area [20], Kedarnath Wildlife Sanctuary [30] from sub-tropical and temperate zone in Kumaun Himalaya [18]. Total herb density was ranged from (21.73-431.04 Ind m^{-2}) for MRF, which was higher than from the reported value (0.3-17.70 tiller m^{-2}) [55] but lower when compared to the Pindari and NDBR [36]. This may be due to the diversity in habitats and mild climatic conditions supporting diversity of herbaceous species and also high density of trees in the MRF. A positive correlation between and the total basal area and richness ($r=0.34$, $p<0.05$, $n=31$) (*Fig. 1*) was observed. This indicates that the increase in the species number increases the total basal cover.

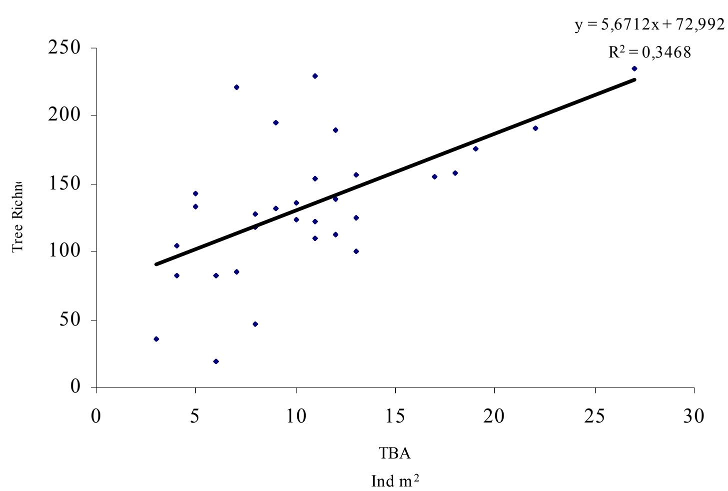

Figure 1. *Correlation between Total Basal Area and Richness*

Species diversity (H')

Species diversity of trees ranged from 0.99-2.93, seedlings from 0.86-2.65, saplings from 0.44-2.78, shrubs from 1.94-4.43 and herbs from 1.42-4.66. The diversity of trees was highest in the *Rhododendron arboreum* community (2.93), followed by *Quercus floribunda* (2.90), *Rhododendron arboreum-Quercus floribunda* mixed (2.68), and *Quercus leucotrichophora* (2.65), communities. The diversity of shrubs was highest in *Quercus leucotrichophora* community (4.43), followed by *Rhododendron arboreum* (3.49) and *Pinus roxburghii* (3.37), communities. The diversity of saplings was highest in *Rhododendron arboreum* (2.78), followed by *Quercus floribunda* (2.74), *Rhododendron arboreum -Quercus floribunda* mixed (2.73), communities. Diversity of

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seedlings was highest is *Betula alnoides* (2.65) communities, followed by *Rhododendron arboreum* -*Quercus floribunda* mixed (2.33), and *Pinus roxburghii* (2.21). Herb diversity was highest in *Rhododendron arboreum* (4.66), followed by *Quercus leucotrichophora* (4.48), and *Pinus roxburghii* (4.08). These values were comparable to the previous records for various regions of west Himalaya [8, 54, 20, 39, 38].

Concentration of dominance (Cd)

Concentration of dominance of trees ranged from 0.06-0.49, seedlings from 0.08-0.45, saplings from 0.07-0.68, shrubs from 0.03-1.00 and herbs from 0.01-0.52. Concentration of dominance of trees was highest in *Myrica esculenta* community (0.49), followed by *Pinus roxburghii* (0.46), *Daphniphyllum himalayense* (0.44) and *Quercus leucotrichophora*-*Quercus floribunda* mixed *and Abies pindrow* (0.41), communities, it was lowest in *Quercus floribunda* community (0.06). For shrubs, it was highest for *Abies pindrow* community (1.00), followed by *Pinus roxburghii-Quercus leucotrichophora* mixed (0.15), *Persea duthiei-Rhododendron arboreum* mixed (0.11), communities, and lowest for *Rhododendron arboreum* community (0.03). For herbs, it was highest for *Rhododendron arboreum-Myrica esculenta* mixed community (0.52), followed by *Quercus floribunda* (0.31), *Persea duthiei-Rhododendron arboreum* mixed (0.12) communities. These values were comparable to the previous records [8, 9].

Socio economic and conservation values of the forest communities

Among all the communities, *Rhododendron arboreum* community (208 species; 127 economically important, 119 native, 2 endemic, 33 near endemic, and 6 rare-endangered species), followed by *Quercus floribunda*-*Rhododendron arboreum* mixed (98 species, 104 economically important species, 55 native, 1 endemic, 14 near endemic, and 5 rare endangered species); *Quercus leucotrichophora* (179 species, 98 economically important, 97 native, 2 endemic, 25 near endemic, and 6 rare endangered species); *Rhododendron arboreum*-*Quercus floribunda* mixed (127 species, 90 economically important, 70 native, 1 endemic, 18 near endemic, and 4 rare endangered species); *Quercus floribunda* (150 species, 85 economically important, 73 native, 1 endemic, 21 near endemic, and 5 rare endangered species); *Cupressus torulosa* (116 species, 69 economically important, 55 native, 1 endemic, 19 near endemic, and 2 rare endangered species), *Myrica esculenta*-*Rhododendron arboreum* mixed (97 species, 68 economically important, 45 native, 1 endemic, 18 near endemic, and 2 rare endangered species), *Betula alnoides* (93 species, 67 economically important, 51 native, 1 endemic, 20 near endemic, and 2 rare endangered species), *Cedrus deodara* (80 species, 59 economically important, 46 native, 1 endemic, 11 near endemic, and 1 rare endangered species), *Pinus roxburghii* (144 species, 59 economically important, 67 native, 1 endemic, 16 near endemic, and 3 rare-endangered species); *Alnus nepalensis* (77 species, 58 economically important, 43 native, 1 endemic, 10 near endemic, and 4 rare endangered species); and *Persea duthiei* (92 species, 52 economically important, 51 native, 1 endemic, 18 near endemic, and 4 rare endangered species), communities (*Table 3*). This clearly indicates the high socio-economic and conservation values of these communities, hence, need prioritization for conservation. The key species of the prioritized communities are *Rhododendron arboreum*, *Myrica esculenta*, *Selinium tenuifolium*, *Heracleum candicans, Buplerum longicaule, Berberis aristata, Sarcococa*

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saligna, Viburnum cotinifolium, Quercus leucotrichophora, *Q. floribunda, Hypericum oblongifolium, Salvia lanata, Artemisia nilagarica*, *Acorus calamus, Origanum vulgare, Melothria heterophylla, Persea duthiei, P. odoratissima, Carpinus viminea, Pyrus pashia, Michelia kisopa, Zanthoxylum armatum, Cypripedium cordigerum, Cedrus deodara, Cupressus torulosa, Taxus baccata* subsp. *wallichiana, Habenaria edgeworthii, H. intermedia, Prinsepia utilis, Delphinium denudatum, Skimmia laureola, Bergenia ligulata, Ulmus wallichiana, Hedychium spicatum, Pimpinella acuminata, Goldfussia dalhoussiana, Onychium fragile,* and *Lepisorus excavatus*. The richness of economically important species in these communities indicates high anthropogenic pressure, which may lead to habitat degradation and extirpation of the species in near future. A significant positive relationship (r= 0.50, p< 0.01, n=31) has been found between the number of useful species and number of rare-endangered species, indicating that the use pattern of the species is directly proportional to the rarity of the species (*Fig. 2*.). If the rate of exploitation of the economically important species from these communities continues, there is much probability of extinction of these species from their natural habitats in near future and may lead to ecosystem imbalance. Therefore, there is an urgent need to initiate steps for the conservation of high value communities.

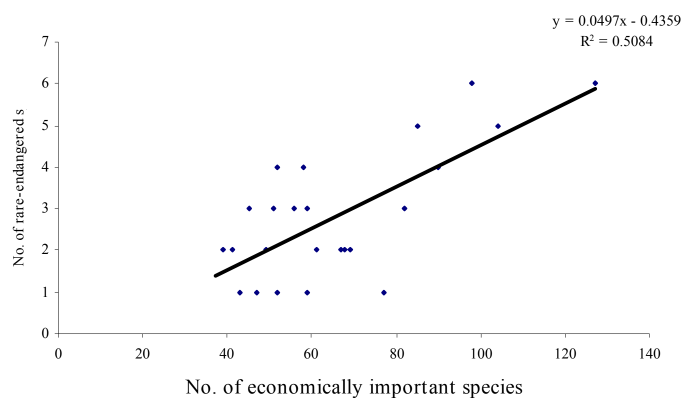

Figure 2. *Correlation between Economically Important and Rare-Endangered Species*

Prioritization of the forest communities for conservation and management

The conservation values of the communities are presented in (*Fig. 3*.) Amongst the communities, the *Rhododendron arboreum* community was ranked first, followed by *Quercus leucotrichophora, Quercus floribunda, Rhododendron arboreum-Quercus floribunda* mixed, *Quercus floribunda-Rhododendron arboreum* mixed, *Pinus roxburghii, Cupressus torulosa, Betula alnoides, Myrica esculenta-Rhododendron arboreum* mixed, *Persea duthiei, Cedrus deodara,* and *Alnus nepalensis*, communities. The prioritized communities represent the maximum numbers of economically important as well as native, endemic and rare-endangered species.

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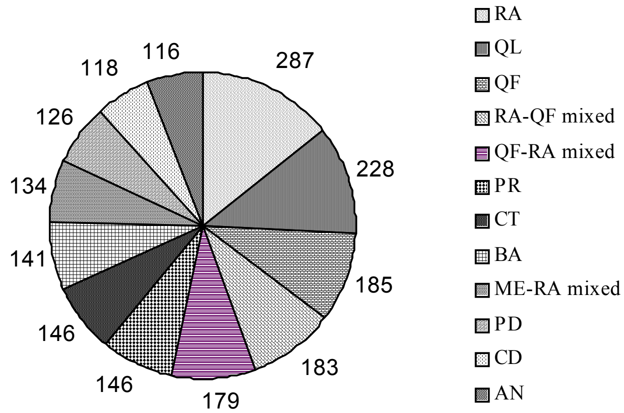

Figure 3. *Prioritization of Forest communities with their Conservation Values*
RA= *Rhododendron arboreum*; QL=*Quercus leucotrichophora*; QF=Quercus floribunda; RA-QF= *Rhododendron arboreum-Quercus floribunda* mixed; QF-RA= *Quercus floribunda-Rhododendron arboreum* mixed; PR=*Pinus roxburghii*; CT=*Cupressus torulosa*; BA=*Betula alnoides*; ME-RA mixed= *Myrica esculenta-Rhododendron arboreum* mixed; PD=*Persea duthiei*; CD=*Cedrus deodara*; and AN=*Alnus nepalensis*

Conclusions

The present study provides comprehensive information on site characteristic, habitats, community diversity, vegetation distribution pattern and forest composition of the species including richness of economically important, native, endemic and rare-endangered species, prioritization of communities for conservation. Based on the results, it can be concluded that the area has high potential in terms of number of species and communities. The occurrence of high number of native, endemic, economically important and rare-endangered species enhance the conservation as well as socio-economic values of the MRF. The day to day need of forest resources particularly fuel and fodder species has increased the pressure on forest trees and shrubs to a great extent. Furthermore, the over-exploitation of species for fuel, fodder, medicine, food (wild edibles), and house building may lead to the extirpation of these species from the area. Therefore, there is a need to develop adequate strategy and action plan for the conservation and management of habitats, species, and communities, so that sustainable utilization of the species could be ensured.

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EDITORIAL COMMUNICATION

In the previous volume of AEER 2007 from the article "Nowinszky-Mészáros-Puskás: The hourly distribution of moth species caught by a light trap" AEER 5(1): 103-107 Table 2 was missing.
We apologize for any inconvenience this may have caused.

Editorial Board

Table 2 *Light-trap catch (in %) of the examined species during night (UT)*

Species	*16-17*	*17-18*	*18-19*	*19-20*	*20-21*	*21-22*	*22-23*	*23-24*	*0-1*	*1-2*	*2-3*	*3-4*	*4-5*	*Individuals*	*Nights*
Plutellidae															
Plutella maculipennis Curt. (4)				15,0	18,0	19,0	25,0	5,0	5,0	6,0	1,0			262	24
Gelechiidae															
Recurvaria nanella Hbn.						17,0	34,0		17,0	34,0				6	
Tortricidae															2
Tortrix viridana L. (4)			0,4	4,5	25,6	23,7	21,1	11,7	7,1	4,5	0,8	0,8		266	10
Pandemis heparana Schiff. (4)			2,2	8,9	28,9	33,3	15,6	6,7	2,2	2,2				45	8
Pandemis ribeana Hbn. (3)			14,3	9,5	14,3	14,3	9,5	14,3	9,5	9,5	4,8			21	7
Hedia nubiferana Haw. (4)			0,6	2,5	15,4	14,8	22,2	11,7	14,2	11,7	4,3	1,2	1,2	162	14
Spilonota ocellana F. (4)				14,3	35,7		28,6	7,1	7,1				7,1	14	2
Laspeyresia pomonella L.			16,7	16,7		16,7	16,7	16,7				16,7		6	4
Pyralidae															
Oncocera semirubella Scop.						16,7	33,3	16,7	16,7	16,7				6	5
Sitochroa verticalis L. (4)				6,7	13,3	6,7	40,0			20,0	6,7	6,7		15	7
Microlepidoptera spec. indet.		0,2	1,3	5,3	12,0	14,0	22,1	12,0	13,0	11,6	6,8	1,7	0,2	2802	39
Drepanidae															
Polyploca ridens Hbn. (2)			7,9	26,3	34,2	0,0	7,9	2,6	7,9	5,3	7,9			38	5
Asphalia ruficollis Schiff. (2)		7,1	50,0	19,0	14,3	2,4	4,8				2,4			42	4
Drepana binaria Hfn. (4)				4,8	14,3	42,9	23,8		9,5		4,8			21	8
Geometridae															
Chiasmia clathrata L. (4)					9,1	22,7	31,8	9,1	9,1	9,1	9,1			22	13
Biston stratarius Hfn. (2)		2,4	18,1	32,1	22,1	5,6	8,0	4,8	0,8	4,0	0,8	1,2		249	21
Apocheima hispidaria Schiff. (2)		4,4	40,0	24,4	22,2	4,4	2,2				2,2			45	8
Lycia hirtaria Cl. (4)		8,9	6,7	4,4		2,2	24,4	11,1	8,9	15,6	13,3	4,4		45	10
Lycia zonaria Schiff.		12,5					62,5		12,5	12,5				8	3
Biston betularia L.						14,3	14,3	28,6	14,3	28,6				7	6
Erannis marginaria Bkh.					33,3		33,3	11,1	11,1		11,1			9	5
Bapta temerata Schiff.					42,9	14,3	14,3	14,3		14,3				7	1

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Table 2 (continuing) *Light-trap catch (in %) of the examined species during night (UT)*

Species	16-17	17-18	18-19	19-20	20-21	21-22	22-23	23-24	0-1	1-2	2-3	3-4	4-5	Individuals	Nights
Campaea margaritata L.			11,1	11,1	11,1	11,1	44,4	11,1						9	3
Siona lineata L.					14,3	14,3	42,9	14,3	14,3					7	4
Alsophila aescularia Schiff. (2)		12,5	22,5	27,5	12,5	5,0	7,5	12,5						40	3
Lygris pyraliata Schiff. (4)					7,1	10,7	32,1	21,4	14,3	7,1		7,1		28	2
Geometridae sp. indet.			0,9	3,5	10,6	15,0	15,9	14,2	13,3	15,9	4,4	5,3	0,9	113	15
Notodontidae															
Dicranura ulmi Schiff.				14,3		14,3		14,3	14,3	28,6	14,3			7	4
Notodonta tritophus Schiff.						30,0		10,0	50,0	10,0				10	1
Drymonia ruficornis Hfn. (4)			4,8	14,3	9,5	23,8	28,6	14,3	4,8					21	8
Noctuidae															
Apatele rumicis L. (4)			7,7	7,7	15,4	23,1	15,4	7,7	7,7	7,7	7,7			13	5
Minucia lunaris Schiff. (4)				7,1	14,2	28,6	35,7	7,1		7,1				14	8
Autographa gamma L. (4)				9,1		36,4	18,2	9,1		27,3				11	9
Brachinochia sphinx Hfn. (4)	3,1	6,3	3,1	0,0	6,3	9,4	15,6	28,1	18,8	3,1	3,1	3,1		32	4
Episema coeruleocephala L. (2)	5,0	30,0	25,0	5,0	5,0	15,0		5,0		5,0	5,0			20	1
Charanyca trigrammica Hfn.							50,0	16,7	16,7	16,7				6	4
Dicycla oo L. (4)						11,1	14,8	40,7	25,9	7,4				27	2
Cosmia trapezina L.							83,3	16,7						6	2
Agrochola lychnidis Schiff.			16,7	16,7	33,3	16,7	16,7							6	2
Amathes c-nigrum L. (4)				6,2	16,9	23,1	26,2	15,4	6,2	3,1	3,1			65	15
Agrochola litura L.		11,1		11,1	33,3	11,1	22,2		11,1					9	4
Eupsilia transversa Hfn. (3a)		5,9	17,6	5,9	5,9	11,8	11,8	5,9	17,6	5,9		11,8		17	3
Conistra vaccinii L. (3a)	2,3	25,6	9,3	11,6	7,0	16,3	20,9	4,7			2,3			43	13
Conistra vaupunctatum Esp.						12,5	25,0	25,0	12,5	25,0				8	5
Conistra erythrocephala Schiff. (3a)		7,1	7,1	28,4	7,1		28,4	14,2	7,1					14	4
Lithophane ornitopus Hfn. (2)		5,0	25,0	20,0	15,0	5,0	10,0	5,0		15,0				20	10
Valeria oleagina Schiff. (2)		5,6	11,2	16,7	28,0	16,7	5,6			5,6		11,2		18	5
Ammoconia caecimacula Schiff. (2)	3,0	36,4	21,2	12,1	6,1	9,1	3,0	3,0	6,1					33	11

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Species	16-17	17-18	18-19	19-20	20-21	21-22	22-23	23-24	0-1	1-2	2-3	3-4	4-5	Individuals	Nights
Apamea anceps Schiff. (3)			10,0	13,3	16,7	13,3	10,0	13,3	13,3	6,7	3,3			30	6
Procus (Oligia) Hbn. (4a)					31,8	18,2	18,2	9,1	9,1	4,5	4,5		4,5	22	4
Discestra trifolii Hfn.					33,3		33,3	16,7				16,7		6	4
Harmodia luteago Schiff.					28,4	14,2	28,4	14,2	14,2					7	3
Mamestra brassicae Hfn.						33,3	33,3	16,7	16,7					6	3
Orthosia incerta Hfn. (4)		5,4		10,8	32,4	16,2	18,9	5,4		8,1		2,7		37	14
Orthosia gothica L. (4)		2,9	5,9	5,9	11,8	14,7	14,7	17,6	14,7	11,8				34	17
Orthosia cruda Schiff. (2)		5,7	15,6	18,0	10,7	5,7	10,7	9,8	7,4	11,5	3,3	1,6		122	12
Orthosia stabilis Schiff. (2)		6,9	4,2	18,1	20,8	9,7	12,5	9,7	1,4	8,3	5,6	2,8		72	15
Orthosia munda Schiff.		11,1	11,1	11,1		11,1	22,2			11,1	11,1	11,1		9	4
Xylomania conspicillaris L.				33,3			33,3		16,7	16,7				6	5
Perigrapha i-cinctum Schiff. (2)		7,1	7,1	21,3	21,3	21,3	7,1	7,1				7,1		14	5
Eugnorisma depuncta L. (4a)			13,8	10,3	17,2	3,4	10,3	13,8	17,2	6,9	6,9			29	2
Agrochola humilis Schiff. (4)		5,7	9,4	7,5	17,0	32,1	22,6		5,7					53	4
Cerastis rubricosa Schiff.			15,4	15,4	23,0	15,4		15,4				15,4		13	1
Scotia exclamationis L. (4)				1,9	14,8	5,6	29,6	20,4	14,8	11,1	1,9			54	12
Scotia segetum Schiff. (4)		6,7		13,3		13,3	13,3	26,7	20,0	6,7				15	10
Noctuidae spec. indet.			2,9	7,8	26,2	19,4	12,6	17,5	5,8	2,9	3,9		1,0	103	11
Lymantriidae															
Lymantria dispar L. (2)			12,5	25,0	12,5	18,8		18,8	6,3	6,3				16	3
Arctiidae															
Ocnogyna parasita Hbn.						12,5	50,0	12,5	25,0					8	2
Phragmatobia fuliginosa L.			10,0	10,0	50,0		10,0	10,0	10,0					10	5

Notes: The Tchernishev's activity type number can be seen after the species name in brackets. The 3a and 4a sample differs, there are two activity peaks in the first part of night, or rather during whole night.

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