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

Contents

Evaluation of Empirical Formulae for Determination of Hydraulic Conductivity based on Grain-Size Analysis

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Abstract: Several empirical equations to calculate hydraulic conductivity using grain size distribution of unconsolidated aquifer materials have been evaluated in this study. Grading analysis of soil samples extracted from test holes during groundwater investigation project was performed to determine their classification and particle size distribution characteristics; from which hydraulic conductivities were computed. Results showed that all the seven empirical formulae reliably estimated hydraulic conductivities of the various soil samples well within the known ranges. Kozeny-Carman formula proved to be the best estimator of most samples analyzed, and may be, even for a wide range of other soil types. However, some of the formulae underestimated or overestimated hydraulic conductivity; even of the same soils. Alyamani and Sen formula in particular is very sensitive to the shape of the grading curve, hence the need to be very careful when using. Most importantly, all these empirical formulae are to be used strictly within their domains of applicability. [The Journal of American Science. 2008;4(1):1-6]. (ISSN: 1545-1003).

Keywords: Hydraulic Conductivity, Empirical formula, Grain-size analysis.

Introduction

It has long been recognized that hydraulic conductivity is related to the grain-size distribution of granular porous media (Freeze and Cherry 1979). This interrelationship is very useful for the estimation of conductivity values where direct permeability data are sparse such as in the early stages of aquifer exploration .In groundwater hydrology, the knowledge of saturated hydraulic conductivity of soil is necessary for modeling the water flow in the soil, both in the saturated and unsaturated zone, and transportation of water-soluble pollutants in the soil. It also an important parameter for designing of the drainage of an area and in construction of earth dam and levee. Furthermore, it is of paramount importance in relation to some geotechnical problems, including the determination of seepage losses, settlement computations, and stability analyses (Boadu 2000). Above all, hydrogeologists always look for reliable techniques to determine the hydraulic conductivity of the aquifers with which they are concerned, for better groundwater development, management and conservation. Many different techniques have been proposed to determine its value, including field methods (pumping test of wells, auger hole test and tracer test), laboratory methods and calculations from empirical formulae(Todd and Mays 2005). However, accurate estimation of hydraulic conductivity in the field environment by the field methods is limited by the lack of precise knowledge of aquifer geometry and hydraulic boundaries (Uma et al. 1989). The cost of field operations and associated wells constructions can be prohibitive as well. Laboratory tests on the other hands, presents formidable problems in the sense of obtaining representative samples and, very often, long testing times. Alternatively, methods of estimating hydraulic conductivity from empirical formulae based on grain-size distribution characteristics have been developed and used to overcome these problems. Grain- size methods are comparably less expensive and do not depend on the geometry and hydraulic boundaries of the aquifer. Most importantly, since information about the textural properties of soils or rock is more easily obtained, a potential alternative for estimating hydraulic conductivity of soils is from grain-size distribution. Although in hydromechanics, it would be more useful to characterize the diameters of pores rather than those of the grains, the pore size distribution is very difficult to determine, so that approximation of hydraulic properties are mostly based on the easy-to-measure grain size distribution as a substitute(Cirpka 2003). Consequently, Groundwater professionals have tried for decades to relate hydraulic conductivity to grain size. The tasks appear rather straight forward but it found that this correlation is not easily established (Pinder and Celia 2006).

Numerous investigators have studied this relationship and several formulae have resulted based

on experimental work. Kozeny (1927) proposed a formula which was then modified by Carman (1937, 1956) to become the Kozeny-Carman equation. Other attempts were made by Hazen (1892), Shepherd (1989), Alyamani and Sen (1993), Terzaghi and Peck (1964). The applicability of these formulae depends on the type of soil for which hydraulic conductivity is to be estimated. Moreover, few formulas give reliable estimates of results because of the difficulty of including all possible variables in porous media. Vukovic and Soro (1992) noted that the applications of different empirical formulae to the same porous medium material can yield different values of hydraulic conductivity, which may differ by a factor of 10 or even 20. The objective of this paper therefore, is to evaluate the applicability and reliability of some of the commonly used empirical formulae for the determination of hydraulic conductivity of unconsolidated soil/rock materials.

Established Empirical Formulae

Hydraulic conductivity (K) can be estimated by particle size analysis of the sediment of interest, using empirical equations relating either K to some size property of the sediment. Vukovic and Soro (1992) summarized several empirical methods from former studies and presented a general formula:

$$K = \frac{g}{v} \cdot C \cdot f(n) \cdot d_e^2 \qquad (1)$$

where K = hydraulic conductivity; g = acceleration due to gravity; v = kinematic viscosity ; C = sorting coefficient; $f(n)$ = porosity function, and d_e = effective grain diameter. The kinematic viscosity (v) is related to dynamic viscosity (μ) and the fluid (water) density (ρ) as follows:

$$v = \frac{\mu}{\rho} \qquad (2)$$

The values of C, $f(n)$ and de are dependent on the different methods used in the grain-size analysis. According to Vukovic and Soro (1992), porosity (n) may be derived from the empirical relationship with the coefficient of grain uniformity (U) as follows:

$$n = 0.255\left(1 + 0.83^{U}\right) \qquad (3)$$

where U is the coefficient of grain uniformity and is given by:

$$U = \left(\frac{d_{60}}{d_{10}}\right) \qquad (4)$$

Here, d_{60} and d_{10} in the formula represent the grain diameter in (mm) for which, 60% and 10% of the sample respectively, are finer than.

Former studies have presented the following formulae which take the general form presented in equation (1) above but with varying ***C, f(n)*** and ***de*** values and their domains of applicability.

Hazen: $$K = \frac{g}{v} \times 6 \times 10^{-4}\left[1 + 10(n - 0.26)\right]d_{10}^2 \qquad (5)$$

Hazen formula was originally developed for determination of hydraulic conductivity of uniformly graded sand but is also useful for fine sand to gravel range, provided the sediment has a uniformity coefficient less than 5 and effective grain size between 0.1 and 3mm.

Kozeny-Carman: $$K = \frac{g}{v} \times 8.3 \times 10^{-3}\left[\frac{n^3}{(1-n)^2}\right]d_{10}^2 \qquad (6)$$

The Kozeny-Carman equation is one of the most widely accepted and used derivations of permeability as a function of the characteristics of the soil medium. This equation was originally proposed by Kozeny (1927) and was then modified by Carman (1937, 1956) to become the Kozeny-Carman equation .It is not appropriate for either soil with effective size above 3mm or for clayey soils (Carrier 2003)

Breyer: $$K = \frac{g}{v} \times 6 \times 10^{-4} \log\frac{500}{U} d_{10}^2 \qquad (7)$$

This method does not consider porosity and therefore, porosity function takes on value 1. Breyer formula is often considered most useful for materials with heterogeneous distributions and poorly sorted grains with uniformity coefficient between 1 and 20, and effective grain size between 0.06mm and 0.6mm.

Slitcher: $$K = \frac{g}{v} \times 1 \times 10^{-2} n^{3.287} d_{10}^2 \qquad (8)$$

This formula is most applicable for grain-size between 0.01mm and 5mm.

Terzaghi: $$K = \frac{g}{v} \cdot C_t \cdot \left(\frac{n - 0.13}{\sqrt[3]{1-n}}\right)^2 d_{10}^2 \qquad (9)$$

where the C_t = sorting coefficient and $6.1 \times 10^{-3} < C_t < 107 \times 10^{-3}$. In this study, an average value of C_t is used. Terzaghi formula is most applicable for large-grain sand (Cheng and Chen 2007.)

USBR: $$K = \frac{g}{v} \times 4.8 \times 10^{-4} d_{20}^{0.3} \times d_{20}^2 \qquad (10)$$

U.S. Bureau of Reclamation (USBR) formula calculates hydraulic conductivity from the effective grain size (d_{20}), and does not depend on porosity; hence porosity function is a unity. The formula is most suitable for medium-grain sand with uniformity coefficient less than 5 (Cheng and Chen 2007)

Alyamani & Sen:

$$K = 1300[I_o + 0.025(d_{50} - d_{10})]^2 \qquad (11)$$

where K is the hydraulic conductivity (m/day), I_o is the intercept (in mm) of the line formed by d_{50} and d_{10} with the grain-size axis, d_{10} is the effective grain diameter (mm), and d_{50} is the median grain diameter (mm). It should be noted that the terms in the formula above bear the stated units for consistency. This formula therefore, is exceptionally different from those that take the general form of equation (1) above. It is however, one of the well known equations that also depends on grain-size analysis. The method considers both sediment grain sizes $\mathbf{d_{10}}$ and $\mathbf{d_{50}}$ as well as the sorting characteristics

Materials and Methods

Samples Test: Four different soils samples were extracted from test holes during an ongoing borehole drilling project aimed at establishing the geological profile of an aquifer system. Samples from the cuttings were collected in containers and taken to the laboratory for further analysis. From the laboratory, the samples were treated and tested for grain size distribution according to the standard procedures of BS1377. The samples (1&3) with coarser particles were tested by the method of dry sieve analysis using a series of sorted BS sieves. The finer samples (2&4) on the other hand, were tested by Hydrometer method.

Grain-size Distribution Analysis: Table1 below shows the results of the particle size distribution analyses of the four soil samples studied. To further analyze the distribution of the particles and to help classify the samples, the test results were then plotted on a semi-logarithmic graph to obtain the grain-size distribution curves for each sample as shown in figure1 below. From the grain-size distribution curves, soil samples were classified according to particle size using a standard *British Soil Classification System*, detailed in *BS 5930: Site Investigation*. In this system, soils are classified into named basic soil-type groups according to size, and the groups further divided into coarse, medium and fine sub-groups. The classifications based on the grain-size distribution curves were as follows:

Sample1 - comprised 4% medium gravel, 19%fine gravel, 32% coarse sand, 28% medium sand, 17% fine sand and is therefore classified as *gravelly sand*.

Sample 2 - comprised 4% coarse sand, 82% medium sand, 14% fine sand; and classified as *medium sand.*

Sample 3 - comprised 13% fine gravel, , 37% coarse sand, 43% medium sand, 02% fine sand; with overall classification as *coarse sand*.

Sample 4 - comprised 02% coarse sand, 76% medium sand, 22% fine sand and is classified as *medium sand*.

Determination of K- values from Grain-Size Analysis: From the grain-size distribution curves in figure1 below, the samples were classified, diameters of soil particles at 10%, 20% and 50% cumulative weight determined, and the coefficients of uniformity, intercepts and porosity values were calculated. All these results, from which hydraulic conductivities were calculated using the seven empirical formulae discussed above, are presented in table2 below. Since the kinematic coefficient of viscosity is also necessary for the estimation of hydraulic conductivity, a value of $0.0874 m^2$/day derived for a water temperature of 20^oC is used in this study.

Results of Different Approaches

The hydraulic conductivity for gravelly sand is not available for Hazen and USBR methods since U > 5, a condition for which both methods are inapplicable. Hydraulic conductivities for medium sand samples are not available for Terzaghi method because the formula is only suitable for large-grain sand. On the other hand, conductivity value for coarse sand is not available for USBR since the method is only relevant for medium-grain sand.

Overall results showed that the hydraulic conductivities calculated by the USBR and Slitcher methods are in all cases lower than for the other methods, which is consistent with the conclusions by (Vukovic and Soro 1992) and (Cheng and Chen 2007). These two methods are always considered inaccurate. Likewise, Terzaghi method gave similar low values, may be due to the use of an average value (8.4×10^{-3}) of sorting coefficient(C) in the formula. Breyer method is most useful for analyzing heterogeneous sample with poorly sorted (well-graded) grains (Pinder and Celia 2006). It was therefore the best estimator for sample1 and a good one too, for sample3. However, for less heterogeneous (poorly graded) samples (2&4), the method underestimated the hydraulic conductivities values. Hazen formula which is based only on the d_{10} particle size is less accurate than the Kozeny- Carman formula which is based on the entire particle size distribution and particle shape (Carrier 2003). Therefore, the estimations by Kozeny-Carman for samples (2, 3, &4) were more accurate

than hazen, and possibly the best estimations in this study and others. Kozeny-Carman however, underestimated sample1 since the formula is not appropriate if the particle distribution has a long, flat tail in the fine fraction (Carrier 2003). Alyamani and Sen method is very sensitive to the shape of the grading curve and is more accurate for well-graded sample. Consequently, it was a fairly good estimator of samples (1&3) but underestimated samples (2&4) due to their poor grading.

Conclusion

Based on the aforementioned analysis and results, the following conclusions can be drawn:

a) Estimating the hydraulic conductivity of soils in terms of grading characteristics can relatively lead to underestimation or overestimation unless the appropriate method is used.

b) For the studied samples, and consequently may be for a wide range of soil type, the best overall estimation of permeability is reached based on Kozeny-Carman's formula followed by Hazen formula. However, Breyer formula is the best for estimation of highly heterogeneous soil sample.

c) Slitcher, USBR and Terzaghi formulae grossly underestimated the hydraulic conductivities in comparison to the other evaluated formulae.

d) Alyamani and Sen formula is very sensitive to shape of the grading curve and as such should be used with care.

e) Therefore, the most suitable formulae for the estimation of hydraulic conductivities in this study were as follows:

- Sample1(Breyer formula) = 114.009m/day,
- Sample2 (Kozeny-Carman) = 56.882m/day,
- Sample3(Kozeny-Carman) =112.495m/day; with Hazen and Breyer formulae acceptable
- Sample4(Kozeny-Carman) = 45.591m/day

Table1: summary results of soil particle size distribution tests

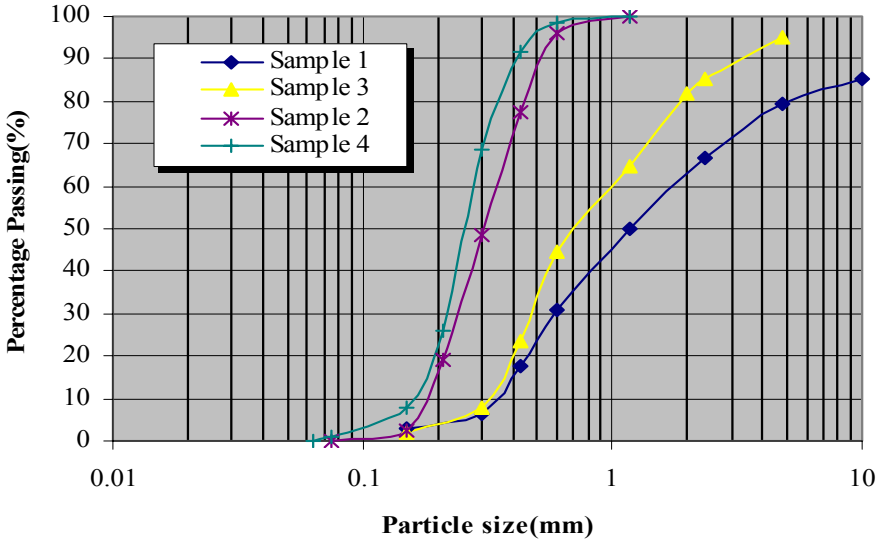
Sample-1								
Particle size (mm)	10	4.75	2.36	1.18	0.600	0.425	0.300	0.150
Percentage Passing (%)	85.23	79.57	66.73	49.99	30.75	17.55	6.28	2.84
Sample-2								
Particle size (mm)	1.18	0.600	0.425	0.300	0.212	0.150	0.075	-
Percentage Passing (%)	100	96.20	77.65	48.41	18.88	2.30	0.00	-
Sample-3								
Particle size (mm)	4.75	2.36	2.00	1.18	0.600	0.425	0.300	0.150
Percentage Passing (%)	95.05	85.08	82.02	64.66	44.76	23.42	7.96	2.11
Sample-4								
Particle size (mm)	1.18	0.600	0.425	0.300	0.212	0.150	0.075	0.063
Percentage Passing (%)	100	98.44	91.55	68.78	25.83	7.89	0.93	0.00

Table2: Hydraulic conductivities calculated from grain-size analysis using empirical formulae

Sample & its classification	d_{10} (mm)	d_{20} (mm)	d_{50} (mm)	(U)	(n)	(I_o) (mm)	Hazen (m/day)	K-C (m/day)	Breyer (m/day)	Slitcher (m/day)	Terzaghi (m/day)	USBR (m/day)	A/S (m/day)
1-Gravelly sand	0.339	0.468	1.180	5.309	0.349	0.249	NA	80.139	114.009	30.249	51.630	NA	94.788
2-Medium sand	0.180	0.220	0.330	1.917	0.433	0.157	44.454	56.882	39.347	17.327	NA	12.356	33.593
3-Coarse sand	0.310	0.400	0.720	3.226	0.395	0.254	113.500	112.495	105.787	38.001	66.381	NA	90.776
4-Medium sand	0.157	0.189	0.258	1.783	0.438	0.139	34.439	45.591	30.324	13.689	NA	8.713	26.038

Key: K-C = Kozeny-Carman; A/S = Alyamani & Sen

Figure1:Grain-size Distribution Curves for soil Samples



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Comparative study of a Fresh water Swamp of Doon valley

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Abstract: Fresh water swamps are the typical habitat where water oozes from the soil surface. They offer many important benefits including flood control, groundwater recharge, pollution cleanup, wildlife habitat, and recreation. In Doon valley fresh water swamp forest forms a distinct ecosystem. However increased population and developmental activities resulting in continuous encroachment upon forestland, many swamp areas have shrinked in around Doon valley. Today only a few small and scattered patches of swamps are left between the base of the outer Hills of Himalayas in the northern and the Shiwalik hills of the South. One of them is Mothronwala Fresh water swamp where the present work was carried out. It occurs as a compact area between 30^0 15' north latitude and 78^0 2' east longitude with an average altitude of 600m above mean sea level. In this paper we have attempted to assess the vegetation of Mothronwala Fresh water swamp and compare it with the vegetational data of Dakshini (1965). During the study a lot of changes have been recorded in the vegetation of Mothornwala Fresh water Swamp. The important characteristics tree, shrub, and herb species which was reported by Dakshini (1965) are missing now. The status of life form also declined from 224 to 194 in case of herbs, 52 to 34 in case of shrubs, and 38 to 25 in case of trees. 168 genera have emigrated as against to 116 immigrated. In case of species, the emigration was 212 species as against of immigration of 135 species. [The Journal of American Science. 2008;4(1):7-10]. (ISSN: 1545-1003).

Keywords: Floristic diversity, Swamp, Doon valley, Mothronwala,

Introduction

Swamps are typical habitat where water oozes out places known as "Oogals" which form perennial streams. At these places the subsoil water maintains constant level throughout the year above the surface of the soil. Swamps play a vital role in landscaping with a wide variety of flora and fauna. Due to variation in topography, a distinct floristic diversity is seen which is quite varied from the surrounding area. The Doon valley known for its swamps. There was a time when low lying areas of the valley were having a chain of swamps but human interference once started in the name of malarious climate (King, 1871) still persist. The trees were cut at that time and the openings created which resulted in the extinction of most of the swamps. Kanjilal (1901) drew attention to the botanical interest of the three detached bits of Swamp forest in the neighbourhood of DehraDun and he considered Mothronwala as the most important of them, where the present work was carried out.

Mothronwala fresh water swamp forest lies at the foot of the shiwaliks that forms the Southern flank of the Doon valley. The swampy zone consist of numerous pools with water oozing out in a series of deep but narrow ravines giving rise to number of streams which after union form a well defined channel. This channels runs out of the swamp forest and acts as a natural drainage. In present Mothronwala fresh water swamp forest is under great biotic stress and has been degraded to a great extent due to the clustering of villages around the periphery of the swamp. Resulting is that the vegetation structure has been changed in last few decades. The important characteristics species have been extinct from the study area and the place of these species various exotic hardy species are invading and replacing in indigenous flora.

A detailed study of Mothronwala Fresh water Swamp was studied by Dakshini (1960a, 1960b, 1965, 1968, 1970 &1974). The present work is an attempt to compare vegetational changes in last few decades of Mothronwala Fresh water swamp of Doon valley.

Material and Methods

Doon valley is situated on the North east corner of Uttaranchal between 78^0 5' east longitude and 30^0 24' north latitude. The present study was conducted during the year 2003 in Mothronwala Fresh water Swamp forest. The area was exhaustively surveyed for the study. Usual methods of collection, preservation and maintenance of specimen in herbarium were followed (Jain & Rao, 1977). During the field study the specimen of plants with flower and fruit were recorded. Collection of plant species were made throughout

the year. After collection, the specimen were processed, preserved and mounted on herbarium sheets. The herbarium sheets identified from the BSD herbarium and deposited in the Herbarium of Ecology Research laboratory, Botany Department D.A.V (PG) College, DehraDun.

Results

The study has been resulted that the vegetation of Mothronwala fresh water swamp has been depleted at much faster rate during last few decades. A considerable changes have been noticed in the vegetation of Mothronwala Fresh water swamp during the present study. Table 1 shows the comparison of floristic diversity of Mothronwala fresh water swamp after four decades. In present 278 plant species of angiosperms with 218 genera and 71 families were collected from the study area which is very less in the comparison of Dakshini (1965).

According to Dakshini (1965) the vegetational formula of study area was 38 Trees +52 Shrubs+235 Herbs. In present a major decline have been recorded in the vegetation structure of the swamp is 25 Trees +34Shrubs +25 Climbers +194 Herbs Table 2. The original forest vegetation of Mothronwala Fresh water swamps had dwindled to a large extent. A very common and characteristic species of Doon valley swamps *Bischofia Javanica, & Diospyros montana* reported by Kanjilal (1901) & Dakshini (1965) was not recorded from the study area in present. Besides these there are various characteristic tree species which was recorded by Dakshini (1965) are missing now. At the places of these tree species 11 new tree species was recorded form the study area Table 3. A major loss of characteristics species have been recorded from the study area. Table 4 shows the characteristics tree, shrubs and herbs species which have been loss their status from the study area. Table 5 shows the emigrated and immigrated species of the Mothronwala Fresh water swamp. During the study it is observed that, 168 genera have emigrated as against to 116 immigrated. In case of species, the emigration was of 212 species as against of immigration of 135 species.

Table 1. Comparison of Taxonomic ranks of Mothronwala Fresh water Swmap

S.N	Taxon	1965	2003
1	Family	71	71
2	Genera	261	218
3	Species	365	278

Table 2. Change in life form of vegetation of Mothronwala Fresh water Swamp

S.N	Life form	1965	2003
1	Herbs	224	194
2	Shrubs	52	34
3	Climbers	42	25
4	Trees	38	25

Table 3. New Tree species recorded in Mothronwala Fresh water swamp after Dakshini (1965)

S.N	Tree spp	Family
1	*Bauhinia variegata* L	Leguminoseae
2	*Bombax cebia* L.	Bombacaceae
3	*Celtis australis* L.	Ulmaceae
4	*Cornus oblonga* Wall.	Cornaceae
5	*Grewia optiva* J. R. Dru.ex.Bur.	Tiliaceae
6	*Grevillea robusta* A.Cunn	Porteaceae
7	*Litsea monopetala* (Roxb)Pers.	Lauraceae
8	*Mangifera indica* L.	Anacardiaceae
9	*Melia azadirachta* L.	Meliaceae
10	*Pterospermum acerifolium* Willd.	Sterculiaceae
11	*Quercus leucotricophora* A.Camus	Fagaceae

Table 4. Emigrated and Immigrated taxa in last few decades

S.N	Taxon	Emigrated	Immigrated
1	Family	15	15
2	Genera	168	116
3	Species	212	135

Table 5. Loss of characteristic swampy species of Mothronwala fresh water swamp in last few decades

Life form	Species
Trees	*Bischofia javanica* Bl.
	Diospyrous montana Roxb.
	Shorea robusta Roxb.ex. Gaertner
Shrubs	*Elagenus conferta* Roxb.
	Trachelospermum lucidum (D.Don) K.Schumann
Herbs	*Adenostema lavenia* (L.) Kuntze
	Floscopa scandens Lour.
	Rotala mexicana Chamisso & Schlechtendal

Discussion

Doon valley has many fresh water swamps, due to its unique topography and peculiar situation in the foot hills of Himalaya. One of such swamp is Mothronwala Fresh water swamp, where the present study was carried out. The study concluded that in present, Mothronwala Fresh water swamp is under great anthropogenic pressure has been degraded to a great extent during the last few decades. The major portion of the swamp has been encroached upon by human settlement, agriculture, cultivation and related developmental activities by there nearby villagers. Villagers are depend on the swamp for fullfill their daily requirements such as fuel, food, fodder, timber and other domestic purposes. The villagers have occupied the peripheral area for cultivation of various fodder species. Lopping of trees by neighbouring villagers has resulted in the deformity of some of the trees with the consequent effect on the ground floor vegetation. Resulting is that exotic species like *Lantana camara, Eupatorium adenophorum, Parthenium hysterophorus, Ageratum conyzoides, Ipomea carnea, Malvastrum coromendelicum* invaded in a large number into the swamp area and have been changed the vegetation structure of the swamp.

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Plant Spacing, Dry Matter Accumulation and Yield of Local and Improved Maize Cultivars.

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Abstract: Field experiments were conducted at the Teaching and Research Farm, Federal University of Technology, Owerri, Nigeria to determine the influence of plant spacing on the yield and dry matter accumulation of local and improved maize varieties. The experiments were laid out as a split plot in a randomized complete block design (RCBD) with four replications consisting sixteen (16) treatments per replicate. The results obtained showed that maize growth and yield was significantly (P = 0.05) affected by the different plant spacing used. The highest dry maize grain yield was obtained in the hybrid varieties using plant spacing of 25 x 75cm while the lowest yield was obtained in the local maize type with plant spacing of 100 x 100cm. The trend observed in the other plant attributes measured such as the Mean Leaf Area (MLA)(cm^2), the plant height and the Dry Matter Accumulation (DMA) showed that the hybrid maize varieties performed significantly better than the local ones and had higher nutrient efficiency and conversion rate than the local cultivars although the yield was predicated on plant population. Based on the research findings, growing maize sole using plant spacing of 25 x 75cm remains the best recommendation for optimum maize grain yield in the field and an improvement of the local maize cultivars genetically for sustainability and food security purposes. [The Journal of American Science. 2008;4(1):11-19]. (ISSN: 1545-1003).

Keywords: *Hybrid maize, local maize types, Plant Spacing, leaf area Dry matter accumulation.*

Introduction

Maize (*Zea mays L.*) is a staple human food, a feed for livestock and raw material for many industrial products. It is an important food crop grown commercially in large scale and at subsistence level by many resource poor farmers. It matures earlier than most food crops and it is used in homes to prepare different dishes especially during the "hungry period" of June - July when most other crops had been planted by the farmers. Maize is a crop of world repute and has a remarkable adaptability in a wide range of climates, and it is more extensively distributed over the earth than any other local crops (Onwueme and Sinha, 1991). Great variations occur in the yields of maize and these large differences in yield cannot only be accounted for by climate or soil variability alone since areas with the same climate and rainfall pattern show markedly different average yields.

Ibeawuchi and Ofoh; (2000) reported that majority of our food is produced by the resource poor farmers, thus, among farmers in Southeastern Nigeria especially the resource poor ones there is an argument amongst this group of farmers on the yield, colour and taste of improved and local cultivars of maize. The resource poor farmers regard their local best (yellow or white) as being better than the improved ones. Most times differences may occur to a large extent due to cultivation techniques including use of fertilizers. However, our local maize is open pollinated while the improved ones are scientifically developed using several methods of guided cross-pollination. The most important thing about the improved maize variety is the development of improved inbreeds that would produce greater F_1 seed yield when hybridized.

Plant spacing is an important agronomic attribute since it is believed to have effects on light interception during which photosynthesis takes place which is the energy manufacturing medium using green parts of the plant. Also, it affects the photosphere and rhizosphere exploitation by the plants especially when spacing is inadequate and the plants suffers clustering together. Good plant spacing gives the right plant density, which is the number of plants, allowed on a given unit of land for optimum yield (Obi, 1991).

Despite the fact that a lot of research work had been carried out on maize by many agricultural scientists not much have been done in understanding the yield and dry matter accumulation of improved and local cultivars maize varieties with different endosperms in relation to different plant spacing. This gap in record and knowledge led to the initiation of this experiment on the effect of plant spacing on the yield and dry matter accumulation of local and improved maize cultivars with a view to proffering solution to the confused state of resource poor farmers as regards to local and improved maize cultivars cultivation.

Materials and methods

The experiment was conducted in 2003 and repeated in 2004 at the Teaching and Research Farm of Federal University of Technology, Owerri Nigeria (5^0 $27^/$ N and 7^0 $02^/$ E) on an elevation of 57.5m above sea level.

Meteorological data collected from Owerri Met. Centre showed that the environment had an annual rainfall of 2311.20mm and 2334.40mm in 2003 and 2004 cropping seasons respectively. It has a mean annual temperature of $31.^0$C and relative humidity of 89%.

Laboratory soil mechanical analysis showed that the soil had a sand value of 85%, silt 9.2% and clay fraction of 5.8% while the chemical soil analysis showed a pH of 4.92 (1 soil: 2.5 water), organic matter 1.98%, total nitrogen 0.08%, 9.88 ppm phosphorus Bray 2-P and exchangeable cations calcium, magnesium and potassium of 0.85, 0.55 and 0.51 Cmol $^{(+)}kg^{-1}$.

The experimental site was ploughed, harrowed and laid out as a split in a randomized complete block design with four treatments – (improved white and yellow maize and local white and yellow maize cultivars) using four spacing and were replicated 3 times giving a total of 48 plots.

The treatments include two local maize cultivars with yellow and white endosperms bought from Nkwo-ukwu Ihiagwa market and improved (hybrid) maize with yellow and white endosperm called Oba super obtained from the Imo State Agricultural Development Programme ADP, Okigwe Road Owerri. The maize cultivars were split into four spacing in a randomized complete block design.

The spacing used includes:		Plant population per hectare
25 x 75 cm	=	53,333 maize plants ha^{-1}
50 x 100cm	=	20,000 maize plants ha^{-1}
30 x 50cm	=	66,667 maize plants ha^{-1}
100 x 100cm	=	10,000 maize plants ha^{-1}

Each main plot measured 3 x 4m ($12m^2$) and this was split into 4 subplots measuring 1.25 x 1.75m with a 50cm gap between each subplot across the length and width of the sub plots. There was a 50cm gap between each block with a 1m-guard area round the experimental area, a total of $253.5m^2$.

The four maize cultivars were planted in the field according to the spacing with two seeds per hole and weak later thinned down to one plant per hole 2 weeks after. The experimental plots were weeded two times at 4 and 8 weeks after planting (WAP). N: P: K 15:15:15 fertilizer was applied to the plots 5 weeks after planting at $400kgha^{-1}$ or 87.5g per subplot. Data were collected from 2 to 12 WAP

Data collected were on plant height (cm), Leaf Area (MLA)(cm^2) dry matter accumulation; (DMA) Francis *et al* (1969) and grain yield ($t.ha^{-1}$). Mean leaf area was determined using the formula by Ogoke *et al* (2003). Post harvest operations were carried out on the maize cob to obtain the grain. The grains were weighed with a weighting scale and appropriate records were taken.

Treatment effects were determined using analysis of variance (ANOVA) as described by Wahua (1999).

Results and Discussion

Results

Main effects of plant spacing on selected growth parameters of the Hybrid and local maize cultivars at 2,4,6,8 and 10 WAP.

Plant Height

There were variations observed in plant height of the improved and local maize cultivars. The local maize also varied in heights between the local yellow and white cultivars being significantly different from the hybrid maize cultivars.

Within the hybrids, the heights were not significant except hybrid white at 6 WAP that showed significant different amongs the maize plants but this disappeared at 8 and 10 WAP showing that they are genetically improved. However, the local maize cultivars yellow and white, with spacing 30x50cm and 25x75cm had significantly taller maize plants than those spaced 50x100cm and 100x100cm. They were also significantly taller than the hybrids yellow or white.

Mean Leaf Area (MLA) (cm^2)

There was an increase in mean leaf area from 8 to 10 WAP for both the hybrid and the local maize types in all the different plant spacings used (Table 2). Plant spacing of 100 x 100 and 50 x 100cm largest mean leaf area had the for both the hybrid and were maize types land race while plant spacing had the least MLA. Significantly different from maize plants with 25 x 75cm and 30 x 50 cm respectively (Table 2)

Dry Matter Accumulation (DMA)

The dry matter accumulation of the maize cultivars is presented in Table 3 at 6, 9 and 12 WAP and the results indicate that the different plant spacing influenced biomass accumulation. Plant spacing 100 x 100cm, 50 x 100 and had the highest above ground biomass at taselling and silking for the hybrid and the local maize cultivars while plant spacing 30 x 50cm had the least biomass in both cultivars.

Maize Yields and Monetary Value

Results presented Table 4 are the grain yield and the corresponding monetary value of the different maize cultivars. The table shows that maize grain yield was greatly influenced by the different plant spacing used. In both the hybrid and the local types, plant spacing 25 x 75cm with plant density of 53,333 plants/ha had the highest grain yield closely followed by plant spacing 30 x 50cm that had 66,667 plants/ha while plant spacing 100 x 100cm with plant density of 10,000 plants/ha had the least grain yield. The same trend was also observed in terms of the monetary value. Plant spacing 25 x 75cm with grain yield of 3/ha had the highest monetary value of N63, 000.00 (white endosperm), while plant spacing 100 x 100cm and 50 x 100cm with grain yield of 1.1/ha and 1.20/ha respectively had least monetary value of N25, 000.00.

Discussion

The yield of a crop is a function of a number of factors and processes such as light intercepted by the canopy, metabolic efficiency of plants, translocation efficiency of photosynthates from leaves to economic parts and sink capacity or sink strength amongst others (Doku, 1977) and the genetic make up of the crops. Also, it is recognized that the photosynthetic capacity of the plant determines the overall productivity; the extent of development of each yield character is also dependent on the interrelationship between the various yield components. More so, consideration must be given to the microenvironment, which supports the growth and yield of the plant and translocation efficiency and conversion rate of the plant.

Different plant spacing with different plant densities generally influenced maize plant height. The 50 x 100cm and 100 x 100 plant spacing thus followed the observed trend in the plant heights in which the local cultivars with plant spacing of 25 x 75 cm and 30 x 50 cm had the tallest plant height at 10 WAP. The plant spacing, for hybrid maize plant, which had the least plant, height could be explained by the competition for scarce growth resources available, the genetic makeup and environmental factors of the plant. It means that these identified factor could be harnessed especially close spacing which cause competition and removal of nutrients for growth and genetic makeup either for tallest or shortness for the particular plant.

The increasing trend observed in leaf canopy cover and leaf area is evidence of good photosynthates assimilation, which resulted in higher vegetative growths. Plant spacing 25 x 75cm (hybrids) allows the maize leaves to capture enough sunlight and other resources, which subsequently was converted into more vegetative growth resulting in higher grain yield due to high metabolic efficiency and conversion rate of the hybrid plants. A plant forms adequate number of leaves and branches when it has adequate supplies of light, nutrients and water. Plant spacing 100 x 100cm had the highest mean leaf area at 8 and 10 WAP (Table 2), thus more solar radiation were absorbed and used for photosynthesis. Because of the improved nature of the hybrid cultivars, they were genetically more advanced and enhanced to take up plant nutrients from the soil faster than the local ones coupled with high densities, which always give room for competition for light energy. The reduction observed in maize dry matter accumulation in plant spacing 30 x 50cm at the 9 WAP in both the local and the improved cultivars may be as a result of

competition for nutrients and other growth factors. This points to the fact that closer spacing in a cropped field, may lead to greater reduction in dry matter accumulation as a result of competition. Earlier reports of Makinde and Alabi (2002), and Sterner (1984) support this observation. Therefore, it is not surprise as closely spaced plants compete for nutrient and other growth factors, they tend to grow taller than those with wider spacing. This fact is evident in this experiment.

A given plant population may be arranged in several ways, leading to variation in intensity of interaction between the cultivars concerned. The supply of growth factors such as light, water and nutrients to plants is affected by the interaction between the plants and thus, efficiency of the use of the limiting resources (Martin and Snaydon, 1982). This fact has to be further investigated for an intercropping system considering the number of crop combinations in an intercropped field.

Maize leaf area was not significantly affected by the plant spacing. Maize plants with wider spacing had higher leaf area than those with closer spacing 100 x 100cm spacing has significantly higher leaf areas and heavier seed weight per a thousand seed weight than the others with closer spacing in this experiment. This indicates photosynthetically higher production and may serve as prediction tool for crop growth and yield. This may be misleading since many factors govern yield of any crop. The primary production outlook may be determined if the leaf area is considered over the land area upon which the crop is produced. A higher lead area correspond to higher lead mass and these shows that there will be higher respiration rate of the maize crop (Table 2)

Maize grain yield was significantly affected by the different plant spacing with the hybrid varieties yielding more than the local maize variety. Evans (1993) had earlier stated that, although no single process holds the key to greater crop yield, physiological comparison between older and newer higher yielding varieties have often been used to identify characteristics that may have contributed to crop improvement. From the study conducted by Ding Kuhu *et al* (1991), the longer a crop is able to grow in the particular site in a season, the greater is its biomass production in that site. Thus, increase in biomass production with longer duration of growth reflects not only the opportunity for more prolonged interception of photo synthetically active radiation by the crop, but also the greater opportunity for uptake of nitrogen and other nutrients especially in low input condition (Yoshida, 1993). It is however, noted that for grain crops, grain yield will often increase with duration up to a certain point but what happens beyond that point depends on environmental and agronomic conditions (Evans, 1993). From this experiment, hybrid yellow and white with 25 x 75 cm spacing had higher yield than the other hybrids but were not statistically significant. However, there were statistically significant yields difference between the hybrid yellow and white and the local maize cultivars yellow and white. The hybrids yielded higher than the local cultivars in all spacing used. For the 1000 seed weight, the hybrid and local maize cultivars yellow and white with 100x100cm spacing had higher weight per 1000 seed than other spacing either of hybrid or local maize cultivars. According to Standhill (1981), crop physiologists have established that the increased solar interception achieved by the large and larger living crop canopies can largely explain the high yield levels in modern crop production by the adapted cultivars. From this experiment the genetic composition of the two maize cultivars came into play as the more genetically improved ones performed better in total yield than the yet to be improved local maize cultivars. It could be that the 25x75cm spacing had higher maize grain yield by number and not by weight while maize plant with 30c50cm spacing were higher in density but produced grains with light weight whereas the 100x100 cm spacing had higher 1000 maize grain weight than all of other treatments in the experiment.

Conclusion

Adequate plant spacing coupled with plant population per unit area gives a good yield. However, the experiment observed that high dry matter accumulation without improved genes to metabolize the products of photosynthesis as in the case of local maize cultivars leads to low crop yield. Further research is recommended for plant spacing and the numbers of maize seeds per hill say, 1,2,3, or4 maize. Seed per hill with a new to understanding their performance as sole since most resources poor farmers use this system in their multiple cropping systems. Farmers and maize growers should be encouraged not only to use proper plant spacing but also to grow hybrid maize varieties since these perform far better than the unimproved local land maize types.

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Table 1: Effects of plant spacing on plant height (cm) of the improved and local maize cultivars at 2,4,6,8 and 10 WAP

	Hybrid maize										Local maize									
	Yellow endosperm					White endosperm					Yellow endosperm					White endosperm				
	Sampling time (WAP)										Sampling time (WAP)									
Plant spacing (cm)	2	4	6	8	10	2	4	6	8	10	2	4	6	8	10	2	4	6	8	10
25 x 75 (Ps_1)	15	28	37	84	132	17	37	58	97	150	10	38	47	75	180	11	39	53	87	199
50 x 100 (Ps_2)	16	30	43	79	128	19	37	47	90	101	13	35	42	81	169	12	29	44	93	174
30 x 50 (Ps_3)	12	35	42	82	130	18	30	38	90	125	12	34	33	70	182	18	20	38	86	198
100 x 100 (Ps_4)	18	24	34	83	120	15	28	38	84	129	14	24	32	88	160	16	24	40	89	171
LSD(0.05)	NS	NS	NS	NS	NS	NS	NS	3.31	NS	NS	NS	NS	NS	NS	2.25	NS	NS	NS	NS	2.70

Table 2: Effects of plant spacing on mean leaf area (cm^2) of the improved and local maize cultivars at 2,4,6,8, and 10 WAP

	Hybrid										Local land race									
	Yellow endosperm					White endosperm					Yellow endosperm					White endosperm				
	Sampling time (WAP)										Sampling time (WAP)									
Plant spacing (cm)	2	4	6	8	10	2	4	6	8	10	2	4	6	8	10	2	4	6	8	10
25 x 75 (Ps_1)	5	7	10	26	40	9	10	12	18	40	2	8	20	28	49	4	8	16	20	36
50 x 100 (Ps_2)	4	8	11	19	36	7	10	21	33	55	3	10	17	20	63	3	5	16	28	36
30 x 50 (Ps_3)	6	7	12	17	25	5	9	11	16	34	2	10	16	23	47	2	6	18	29	39
100 x 100 (Ps_4)	4	6	17	36	59	4	17	20	42	60	2	10	18	19	101	3	5	18	22	87
LSD(0.05)	NS	NS	NS	NS	4.22	NS	NS	NS	3.57	5.88	NS	NS	NS	NS	2.94	NS	NS	NS	NS	4.6

Table 3: Effects of plant spacing on dry matter accumulation (Dm)(g) of the improved and local cultivars at 6,9 and 12 WAP

	Hybrid						Local land race					
	Yellow			White			Yellow			White		
	Sampling time (WAP)						Sampling time (WAP)					
Plant spacing (cm)	6	9	12	6	9	12	6	9	12	6	9	12
25 X 75	900	1500	1900	800	1790	1800	600	950	1909	670	980	1860
50 X 100	700	990	1890	680	890	1790	50	790	1829	600	960	859
30 X 50	690	65	1009	760	770	1060	700	780	1290	574	684	1090
100 X 100	690	900	1880	760	790	1790	500	540	1725	438	890	1894
LSD (0.05)	5.50	12.75	24.63	17.40	20.66	22.55	19.73	22.39	21.97	16.55	22.65	20.45

Table 4: Grain Yield (tons/ha) and monetary value (N) of the improved and local cultivars using different plant spacings

	Hybrid						Local land race					
Plant spacing (cm)	Yellow t/ha Endosperm		Monetary Value (N)	White t/ha endosperm		Monetary Value (N)	Yellow t/ha Endosperm		Monetary Value (N)	White t/ha endosperm		Monetary Value (N)
25 x 75	2.80	78	765352	3.00	68	742500	1.89	65	453600	1.90	61	414231.30
50 X 100	2.50	81	683350	2.10	70	519750	1.20	66	288000	1.20	60	263004.00
30 X 50	2.75	58	751685	2.60	54	643500	1.75	50	420000	1.30	48	284921.00
100 X 100	2.10	98	574014	2.00	95	495000	1.16	95	278400	1.08	90	236703.60
LSD (0.05)	NS	5.81		1.12	6.25		NS	7.22		NS	9.30	

Mean market surveys 2003	local: white:	yellow	Hybrid white	yellow
Ekeukwu Owerri (main market)	210	230	250	280
Afor Enyiogugu market	200	210	230	250
Relief market Owerri	220	250	230	250
	N210	N230	N236.67k	N260
Mean market surveys 2004				
Ekeukwu Owerri	230	260	265	310
Afor Enyiogwugwu market	220	240	250	260
Relief market Owerri	235	250	260	290
	N228.33	N250	N258.33	N286.67
Mean price per kg =	N219.17,	N240.00	N247.50,	N273.34

A Framework for Agile Methodologies for Development of Bioinformatics

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Abstract: The realm of Bioinformatics has entered into the post genomic era where the emphasis is not on collection of the data but it has shifted to analyzing the collected data meaningfully through computational and analysis tools specifically developed for Biologist and Scientists. Conventional software engineering frameworks and methodologies are unsuitable for the development of biological applications due to their peculiar characteristics. The agile methods/methodologies have been favored by the software engineering practitioners for developing applications of the evolutionary domain such as Bioinformatics. These methods suffer the shortcomings of non-iterative analysis process and also the temporal information of the development process is not recorded. In this paper, we identify a set of characteristics which are used in identifying the domains in which the agile practices should be adapted. We also propose a framework that can be used to develop software development methodologies for developing agile software systems/applications. [The Journal of American Science. 2008;4(1):20-32]. (ISSN: 1545-1003).

Keywords: Evolutionary systems, agile methodologies, bioinformatics, temporal history, Prototype based methodology, software engineering

1 Introduction

The explosion of bioinformatics as an emerging field during the last few years has brought both opportunities and challenges for the researchers in the areas such as computer science, biomedicine, agriculture, pharmaceutical industry etc. A huge volume of genomic data is being produced at the exponential rate by the corresponding accelerated experimentation and analysis. These novel types of data are originated from a variety of experimental techniques among which many are further capable of producing data at the different levels such as entire cells, organs, organisms, and even populations. The main driving force behind this expansion of the bioinformatics domain is emergence of new, efficient experimental techniques primarily deoxyribonucleic acid (DNA) sequencing that has led to an exponential growth of linear descriptions of protein, DNA and ribonucleic acid (RNA) molecules (Alberts, B., 2002). Other new data producing techniques work as massively parallel versions of the traditional experimental methodologies. Consequently, the computational support in the experiment design, processing of the results, and their analysis and interpretations has become important (Bornberg-Bauer, E. & Paton, N.W., 2002).

The developments and advancements in the bioinformatics domain have extended scope of the domain and the emphasis has progressively switched from the data acquisition and accumulation to its interpretation. The interpretation of this huge volume and diversified data depend on our ability to develop new methods/methodologies for the development sophisticated software that can handle and analyze this new type of data.

Currently available software tools and techniques are not meeting the requirements of the researchers and not providing much help in the research. The main reasons of their inability are: i) they were not develop using the principles of software engineering, ii) there is no formal framework and software development methodologies for this new domain of applications.

We identify those characteristics of the biological data and information which make it different and difficult to handle from the conventional (or non-biological) data. Consequently, these characteristics also makes different and difficult to develop software for the handling and maintaining of this type of data. In biology, the data and its inter-relationships have a profound effect on the system of which they are part of therefore it is important and useful to identify the characteristics of both data and the information of their

relationships in a biological system (Bornberg-Bauer, E. & Paton, N.W., 2002). The characteristics are listed and described as follows.

(i) Biological data and its information are highly evolutionary, uncertain and incomplete. The latest research invalidates the established facts as they are completely changed or modified (Ostell, JM., S.J. Wheelan & J.A. Kans. 2001).

(ii) This is unprecedented type of data (Marijke Keet., 2003). For example, a molecule, such as a bacteriocin, can be coded 'mostly' on plasmids and transposons, though 'rarely' on chromosomal DNA, plus a transposon can insert itself into a plasmid: should one classify the gene location as transposon or plasmid, or both.

(iii) Depending on the environment, the behavior of an object/entity of a biological system can vary (Alberts, B., 2002).

(iv) Both functional requirements and information about the data of bioinformatics need to be studied and analyzed simultaneously because they are closely related to each other and interdependent (Shah, A, 2000).

(v) Same data item (biological object) can have different structure in the different environments (Baldi, Brunak, 2004).

(vi) Data in bioinformatics is semi-structured (Alberts, B., 2002).

(vii) Even accurately studied and analyzed a biological system can become erroneous and liable to be discarded due to data curation, interpretations, tinkering and experimentation at some later stage (Baldi, Brunak, 2004).

(viii) A biological data object can increase its size in the incremental fashion with the availability of new information about the object (Marijke Keet., 2003).

(ix) The usage of bioinformatics is very broad, with different perceptions and objectives.

(x) Determining the functional specifications is a difficult task because they are always imprecise and incomplete. For example, some genes may survive in a particular environment and useless in a laboratory environment (Ostell, JM., S.J. Wheelan, & J.A. Kans. 2001).

(xi) Biological data is explorative and iterative in nature as it depends upon scientific inquiry.

The above mentioned characteristics of this domain especially the characteristics of bioinformatics make both the existing software development frameworks such as the Waterfall and Spiral life-cycle models, and the software development methodologies such as Structured Analysis and Design, Structured Analysis and Design Technique (SADT), Jackson Structured Design (JSD), Fusion, Object Modeling Technique (OMT), Object-Oriented Design Methodology (OODM) (Embely D., Jackson R., & Woodfield, S, 1995; Shah, A., 2001; Fatouhi, F., Shah, A., Ahmed, I., & Grosky,1994; Shah, A., 2001) unsuitable to use for the development of applications of this domain. Main reasons for their unsuitability are the unprecedented functional requirements and type of data of this application domain. Moreover, the existing agile software development methodologies are not supported by any framework which gives a basis to the methodologies. In this paper, we address this problem and propose a framework for a new class of software development methodologies. This proposed framework can be used a basis in the development of the new class of software development methodologies for the new domain applications including bioinformatics.

The remainder of the paper is organized as follows. In Section 2, we give the related work. In section 3, we discuss how the Prototype based framework as proposed by Shah(Shah, 2001) provides the necessary foundations for our proposed framework. The proposed framework is presented in Section 4. In Section 5, we give concluding remarks and future directions of this work.

2. Related Work

In this section, we present review of the bioinformatics discipline and describe the problems and challenges of this area. We also present an analytical review of software development life-cycle models.

2.1 Bioinformatics

Information science has been applied to biological sciences for producing the field called Bioinformatics. It is the application of computer technology to the management of biological information. Computers are used to gather, store, analyze and integrate biological and genetic information which can then be applied to gene-based drug discovery and development. The need for bioinformatics capabilities

has been precipitated by the explosion of publicly available genomic information resulting from the Human Genome Project (Altschul S.F., et al 1997).

The simplest tasks used in bioinformatics concern the creation and maintenance of databases of biological information. Nucleic acid sequences (and the protein sequences derived from them) comprise the majority of such databases. While the storage and organization of millions of nucleotides is far from trivial, designing a database and developing an interface where by researchers can both access existing information and submit new entries is only the beginning. The most pressing tasks in bioinformatics involve the analysis of sequence information. Computational Biology is the name given to this process.

The greatest achievement of bioinformatics methods, the Human Genome Project, is complete now. Because of this the nature and priorities of bioinformatics research and applications are changing (Ostell, JM., S.J. Wheelan, J.A. Kans., 2001). We are now well in to "post genomic era" era. This has affected bioinformatics in several ways:

i) Now that we possess multiple whole genomes we can look for differences and similarities between all the genes of multiple species. From such studies we can draw particular conclusions about species and general ones about evolution. This kind of science is often referred to as comparative genomics.

ii) There are now technologies designed to measure the relative number of copies of a genetic message (levels of gene expression) at different stages in development or disease or in different tissues. Such technologies, such as DNA micro arrays have grown in importance.

iii) Other, more direct, large-scale ways of identifying gene functions and associations (for example yeast two-hybrid methods) have grown in significance and with them the accompanying bioinformatics of functional genomics.

iv) There is a general shift in emphasis (of sequence analysis especially) from genes themselves to gene products. This has lead to:

a) Attempts to catalogue the activities and characterize interactions between all gene products (in humans): proteomics.

b) Attempts to crystallize and or predict the structures of all proteins (in humans): structural genomics.

v) What some people refer to as research or medical informatics, the management of all biomedical experimental data associated with particular molecules or patients---from mass spectroscopy, to in vitro assays to clinical side-effects---has moved from the concern of those working in drug company and hospital I.T. (information technology) into the mainstream of cell and molecular biology and migrate from the commercial and clinical to academic sectors (H. V. Jagadesh., 2004).

Hence, the importance of developing and devising new methods and approaches in Computer Science specially in the areas related to Application Development, such as Software Engineering and Systems Analysis and Design, specifically tailored for Bioinformatics in post genomic era has grown.

2.2 Class-based and Prototype-based techniques.

The two object modeling techniques which are called class-based and prototype-based techniques form the basis of two types of software development methodologies, which are referred to as the *class-based methodologies* and *prototype-based methodologies*. These two types of methodologies mainly differ from each other because they use the two different object-modeling techniques. We list the main features of the prototype-based technique and methodologies to magnify the differences between these two types of object modeling techniques and methodologies. Note that further details of these features can be seen in (Shah, A., 2001).

(i) The prototype-based methodologies are more implementation-oriented than the class-based, because their emphasis is more on design and run-time (or dynamic) aspects of objects of a system.

(ii) The prototype-based methodologies do not organize objects of an application into a hierarchical structure as a class-lattice; rather they place objects of a system at the same level.

(iii) The knowledge-sharing pattern among the objects of the system is fixed at the run-time.

(iv) Objects of a system which are developed using some prototype-based methodology, pass only once through the analysis process (or phase) in their life-span when the system is developed for the first time.

(v) After the system development, each update to the objects of the developed system is only processed by the design and implementation phases. It means that objects of a system are

processed only once by the analysis phase and many times by the design and implementation phases of a prototype-based methodology

These above listed features will provide a basis to our proposed framework for the agile software development.

2.3 Evolutionary Applications Domain

More recently, agile software development methodologies have generated a lot of interest in software engineering community owning to their purported suitability for evolutionary, iterative and volatile domains such as bioinformatics and web based applications.

However, it is no necessary that a certain agile method suits all settings or individuals. In their comprehensive comparison of agile methodologies (Abrahamssons, P., Salo, O., Ronkainen, J., Warsta., J., 2002), it is pointed out that little emphasis has been placed on analyzing for which situations agile methodologies are more suitable than others. Contrary to their findings, a new domain of applications is identified in (Shah, A. 2001) by specifying characteristics of the domain for which agile development methodologies are more suitable. The applications such as Computer-Aided Construction (CAC), and the Web-based applications belong to this class of applications or domain. One typical characteristic of the objects of this class of applications is that they frequently change their structure (instance-variables and methods), state (or data values), or both.

For modeling the objects of this domain that is identified above, it has been suggested that the prototype-based object modeling technique is more suitable than the class-based object modeling technique (for more details and justifications see (Shah, A., 2001; Fotouhi, F., Shah, A. et al, 1994; Borning, A.H., 1986; Lieberman, L. 1986). Here, we list the main characteristics of the application domain as identified by (Shah, A. 2001). More details can be seen in (Shah, A. 2001). We also add a few more characteristics which are endemic to Bioinformatics, thus broadening the scope of the applications conforming to these characteristics. .

i) Applications without hierarchical structure
ii) Rapid prototype development
iii) Incremental growth of objects
iv) Desirability to trace back changes to a specific object
v) Where the grouping of objects is not important
vi) Simultaneous capturing of changes to both parameters (i.e., structure and state) of objects
vii) Polymorphic behavior of objects.
viii) Evolutionary Behavior of the Objects.
ix) Vague Functional Characteristics(This results from imprecise and incomplete data and information about the application domain)
x) The requirements are emergent i.e., the activity of developing, delivering and using the software itself yields more requirements thorough better understanding of the problem.

These characteristics can form the basis of identifying the situations where Agile software development approaches are more suited as compared to traditional approaches.

It has been concluded in (Shah, A. 2001) that the traditional and class-based object-oriented methodologies are unsuitable to use for the development of this new application domain because they lack in the capturing and modeling the characteristics of the application domain. Most of the characteristics listed in Section 1.2.1 and those listed above are common. These common characteristics suggest that bioinformatics also belong to same class of applications for which prototype-based technique has been suggested by (Shah, A. 2001).

2.5 Limitations of Existing Agile Methodologies

In our opinion, existing agile software development methodologies, though used for the development of computer applications relating to evolutionary domain, have their limitations because as mentioned above, they are not supported by a suitable frame work and also owing to the following characteristics of the evolutionary domain.

i) Explorative and iterative nature as mentioned in section 1.1.
ii) Difficulty in specifying functional requirements (Refer section 1.1)

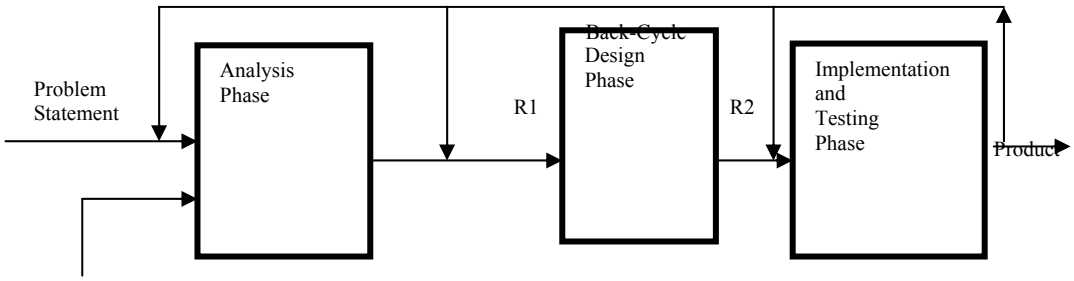
iii) Emergent requirements (Refer section 2.3).

The above characteristics necessitate an iterative analysis process .Moreover as mentioned in section 1.1 and section 2.3, the desirability to trace back changes and recording the historical information of a system adds a temporal aspect to software development. As information in evolutionary domains evolve overtime, it is not desirable to discard the old information. For example, in scientific experiments and research pertaining to some specific project, different alternative methodologies and experimental procedures may be tried to reach a specific goal and the best one selected later This may also be done to come up with some eclectic method by taking in to account many individual experiments and their mutual trade offs (Shah, A. 2001). For this reason it is important to associate temporal information with all the phases of an agile development methodology.

To the best of our knowledge, the existing Agile software development methodologies such as XP, Scrum, Crystal methodologies, Feature Driven Development and The Rational Unified Process do not have an iterative analysis process nor a mechanism to store temporal information of a system. Our proposed framework which we develop and present in next two sections have both an iterative analysis process and a mechanism for storing temporal information.

3. Towards Modification of Classical Water-Fall Model

The classical software development life-cycle Water-Fall model and its main development phases are shown in Figure 1. Note that in the figure, the maintenance phase is not shown. The function-oriented and class-based methodologies mainly follow the general guidelines of the classical water-Fall life-cycle model except for the following difference. The principle of *aggregation* in the *function-oriented methodologies* groups together functions of a system that are constituents of a higher level function implementation. In other words, the main emphasis of the function-oriented methodologies is on the system functionality, and the methodologies are referred to as the *function-oriented methodologies*. In the class-based (object-oriented) methodologies the principle of aggregation groups together functions (or methods) that operate on the same set of data. The main emphasis in the class-based methodologies is on designing object classes of a system and their organization (Wirfs-Brock, R., Wilkerson, B. & Wiener, L, 1991). Due to emphasis on different aspects of problem statement of a system in these two types of methodologies, the class-based methodologies spend more time and effort in the design phase than the analysis phase as compared to the function-oriented methodologies (for details see Wirfs-Brock, R., Wilkerson, B. and Wiener, L ,1991). In other words, the difference in the object-modeling techniques of the function-oriented methodologies and the class-based methodologies has affected the processing of the two phases (the analysis phase and design phase) of the classical life-cycle model. The difference is accommodated through a modification which recommends that for the class-based methodologies the design phase of the classical life-cycle model spend more time and effort than its analysis phase. We conclude that the difference between two classes of methodologies may pursue modifications to the classical life-cycle model to accommodate the difference.
Note that in Figure 1, an *additional knowledge* represents an update to an already developed system.



Additional Knowledge
(or New System Requirements and/or updates to the existing objects)

Legend:
R1: Analysis Report - output of Analysis Phase
R2: Design Report - output of Design Phase
Product: A Developed System

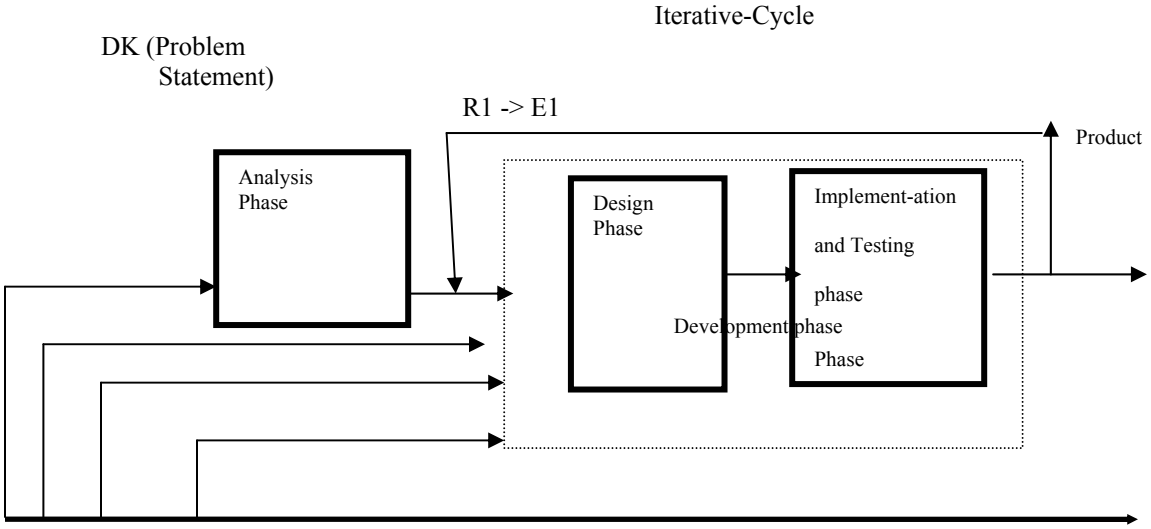
Figure 1: A common representation of classical Water-Fall life-cycle model

The weaknesses and deficiencies which further influence both the class-based and function-oriented methodologies as identified in (Shah, A., 2001) are as follows:

(i) The phases of the model work sequentially in both cases: the development of a new system, and to update an already developed system with additional knowledge. Referred to as *Static Order*.

(ii) During the development of a system, the only meta-data knowledge (i.e., functional requirements) of the system is considered and acquired. The data knowledge (data instances) of the system is neither considered nor acquired due to absence of guide-lines from the classical Water-Fall model.

(iii) The classical Water-Fall model does not provide explicit guidelines for the development of new and different types of application domains such as web-based applications and hypermedia systems (Nestorov, S., & Abiteroul, S., and Motwan, R., 1998). In these application domains, both data knowledge and meta-data knowledge are available before system development.



Legend:
DK: Default Knowledge (problem statement) of system at time t_0
AK_1: Additional Knowledge of system available at time t_1
AK_2: Additional Knowledge of system available at time t_2 **. . .**
AK_n: Additional Knowledge of system available at time t_n
R1, R2 and Product: hold the same meaning as they hold in Figure 1

Figure 2: The modified classical life-cycle model

These weaknesses and deficiencies in the classical Water-fall model and the characteristics of the new type of applications as mentioned in section 2.4 and identified by Shah (Shah,A.,2001) (i.e., web-based applications, hypermedia systems), led to the modifications in the classical Water-Fall model (Shah,A.,2001).

Figure 2 shows overall structure of the modified version of the classical Water Fall model, referred to as the *modified Water-Fall model* (Shah,A,.2001*)*. For the first case, when a new system is going to be developed from scratch both the modified Water-Fall model and the classical Water-Fall model function in the same fashion. But for the second case, when an additional knowledge is being incorporated into an already developed system, then both models differ in their working (compare Figure 1 and Figure2). These two cases are shown as two different types of events in Figure 2, which occur at different time instances on *Time-Line*. The time instance t_0 on Time-Line represents the occurrence of the first type of event, when a system is developed from scratch The set of time instances $\{t_1, t_2, \ldots, t_n\}$ represents the occurrences of the

second type of event on the Time-Line, when the instances of additional knowledge $AK_1, AK_2, \ldots AK_n$, respectively update an already developed system. At these time instances, the modified in Water-Fall model works differently from the classical Water-Fall model. After having the capability of incorporation the second type of event, the modified Water-Fall model becomes capable to provide a suitable framework for the application domains, which is described earlier.

It is already mentioned that both the design phase and implementation and testing phase of the modified Water-Fall model works iteratively and closely at occurrence of each time instance when an update is incorporated in an already developed system. An iteration means the processing of the two phases. Due to this reason both of these two phases are considered as a single phase, and it is refer it to as the *development phase* (see Figure 2).

The iterative property of the development phase is shown by the *Iterative-Cycles* in Figure 2. The Back-Cycles (in Figure 1) and the Iterative-Cycles (in Figure 2) differ in their objectives and functions. An Iterative-Cycle represents the incorporating process of additional knowledge into an already developed system, whereas, a Back-Cycle represents the incorporating Process of a revision to an under-development system. The first difference between them is that a Back-Cycle represents a revision to an under-development system and an Iterative-Cycle represents an update to an already developed system. The second difference between them is that a Back-Cycle can be initialed from any phase of the classical Water-Fall model, except for Analysis phase, whereas an Iterative-Cycle can only be initiated for the development phase (see Figure 2). Note that a *product* (in Figure 2) means an already developed system, and also after completion of each iteration of the development phase.

In the next two sections we propose modifications in the working guidelines of Analysis and Development phases of this modified model to provide us the basis for proposing a new framework suitable for the application domains such as Bioinformatics.

4. The Proposed Framework

Our proposed framework is further extension of the previous framework proposed by Shah that is described in Section 3 (Shah, A., 2001). This new framework takes an evolutionary, iterative and incremental approach both during the analysis and the development phases/process. This framework has been proposed keeping in view the characteristics of the domain applications given in Section 1. This proposed framework consists of three (3) phases, i.e., analysis phase design phase and implementation and testing phase. The design phase and implementation and testing phase are considered as a single phase which we have referred to as the development phase in section 3(Also see Fig. 2). The framework holds an *iterative* property which means that a system may be processed by the three phases more than once in its life-span.

Main feature of the agile systems is that they are inherently *adaptive*. Usually, functional requirements of an agile system is breakable into small *pieces/increments* that can be developed in pieces over periods of time, or iterations when each increment is available as proposed by Shah in (Shah A.,2001) and shown in Figure 2. Each iteration which is named as *Iterative Cycle*/*Back Cycle* in the figure can trigger analysis, design, or coding, and testing phase. The following types of changes can occur to a system.

Type I: If a change causes no loss or addition of information in an existing analysis or design artifact, then we can start the processing of the next phase.

Type II: This type of change causes no loss or addition of information in the analysis artifact but the design artifact changes due to this change. In this case, we can start the processing from Design Phase

Type III: If a change occurs in functional requirements of a system, then the next iteration starts from Analysis Phase.

Type I and Type II changes pertain to the development phase and will be discussed in Section 4.2. Type III change invokes the analysis phase and is discussed in detail in the next section.

We add the temporal component with all three (3) phases of the proposed framework (see Figure 2). By attaching time dimension with the analysis report, the design report and the resulting prototype system, we can maintain the history of changes in the development of the system. Each report and the resulting prototype system is identified by a tuple of the form $(T_s, Tc, A/D/P, T sc)$, where *Ts* and *Tc* represent start time and current time, respectively, and *A/D/P* represents analysis, design or prototype artifact. The time interval *(Ts, Tc) that is* denoted by *Tsc*, is referred to as the *time span* of the artifact. A pair of time-span and an artifact identity reduces the search space for a particular prototype associated with a particular design/analysis of a artifact. This might be used to establish the validity of an artifact during a

particular time span. By examining a sequence of tuples associated with each underdevelopment system, we can also measure the degree of evolution of the system.

The working of the proposed framework is described in the next sections.

4.1 Analysis Phase

The functional requirements of a system are gathered in the same manner as in the classical life-cycle model for the first iteration, or in the case the system is being developed from the scratch. As it has been mentioned in Section 3 and in Section 4 above, we modify Shah's framework (Shah, A., 2001) to provide a rationale for developing methodologies for the new application domain including bioinformatics. The main difference between Shah's framework and this framework is in the analysis phase. In this framework, the analysis phase is iterative.

There are some preliminary processing activities of the phase include comprehensive study of a problem statement, and identification and analysis of the system's functional requirements. The first iteration of this phase can be imitated after completing the preliminarily processing activities. This phase suggests to pick-up an initial list of prototypes referred to as candidate prototypes (or objects) of the system as mentioned in (Shah, A., 2001). This list of the candidate prototypes, can be prepared using similar criteria that are used by the class-based methodologies such as OMT (Pressman, R., 1992, Shah, A., Fotouhi, F., Grosky, W.,. Al-Dehlan, A., and Vashishta, A, 1993). The criterion that is commonly used by the class-based methodologies suggests for picking the *nouns* from a problem statement and considers them as candidate prototypes. After preparing this list, it is further processed, which includes prioritizing and discarding redundant and vague prototypes from the list of candidate prototypes, which might be included in the list during the subsequent iterations, and merging identical and closely related prototypes. This processing of the candidate list gives a *final list of prototypes/objects*. The output of the analysis phase is an Analysis Report/Requirement Document that is referred to as Requirements here, which is denoted as *R1* in Figure 4, and it mainly contains the final list of prototypes and other necessary information about the system such as relationships among the prototypes. This Analysis Report/Requirement Document is prioritized and added to a priority stack Figure 3).

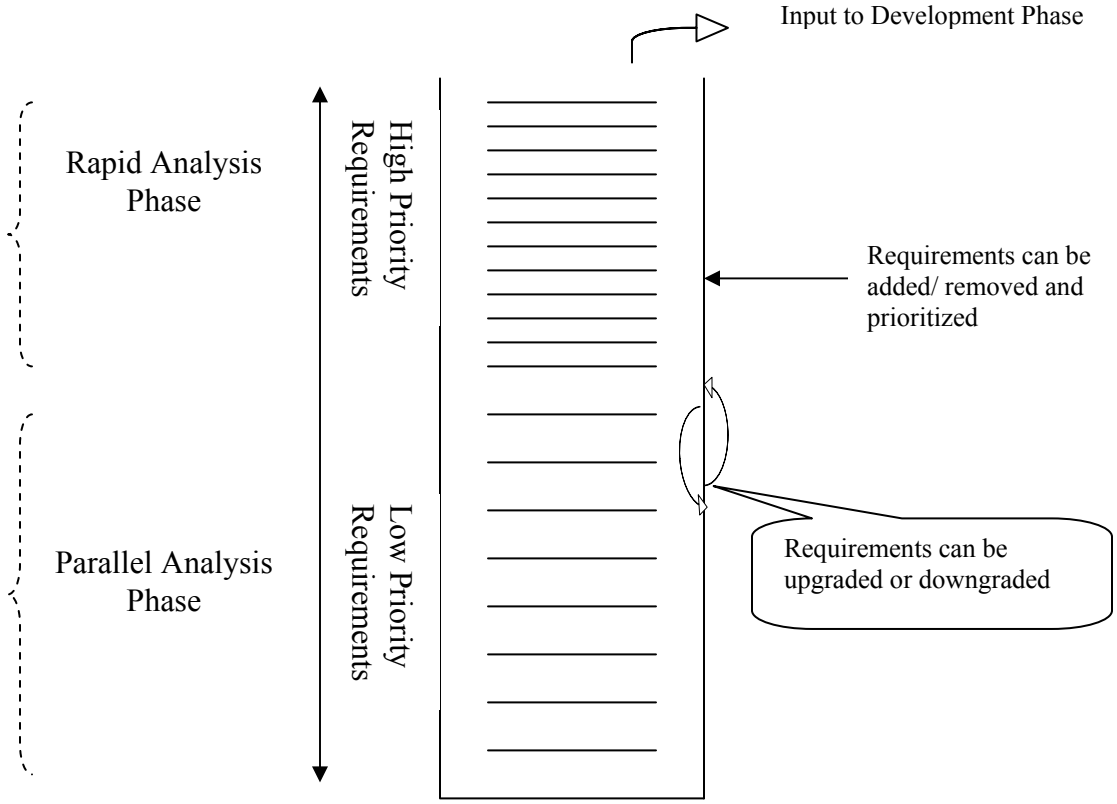
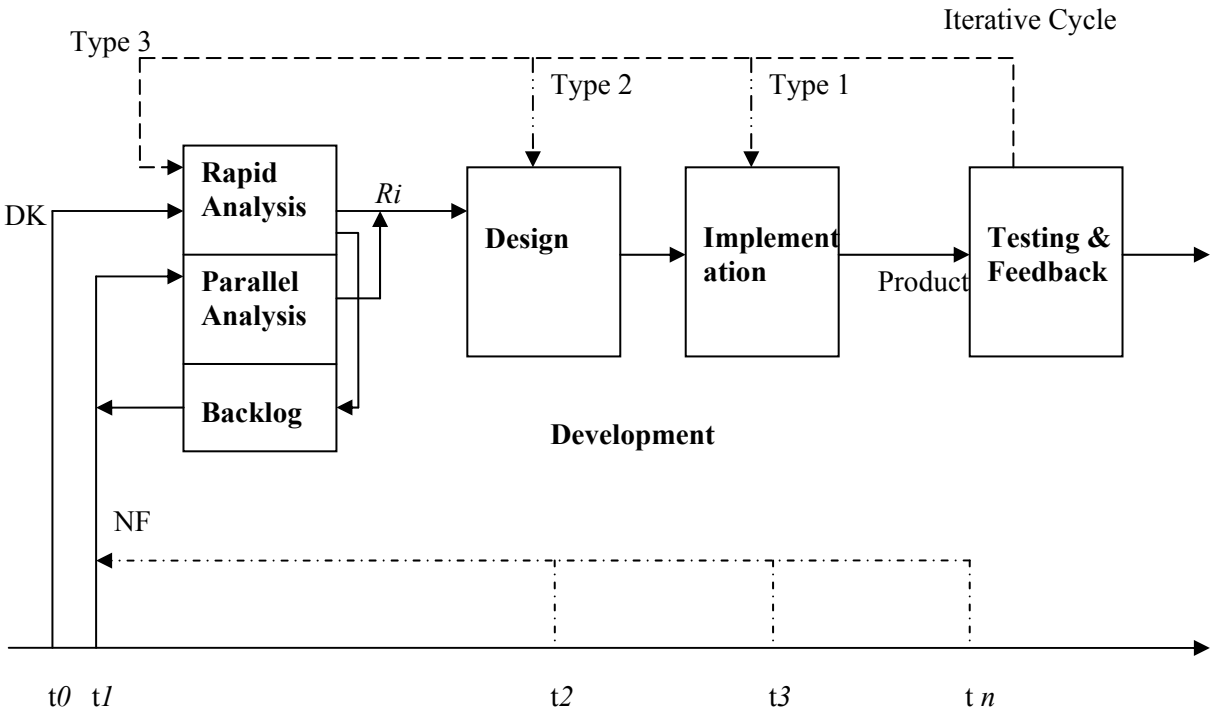


Fig 3: Priority Stack



Legend:

DK: Default Knowledge (problem statement) of system at time t_0
AK_1: Additional Knowledge of system available at time t_1
AK_2: Additional Knowledge of system available at time t_2 . . .
AK_n: Additional Knowledge of system available at time t_n
NF: New Facts about system discovered over time
R1 and Product: hold the same meaning as they hold in Figure 2
Ri: Analysis report(s) at later stages of development at any instant i

Figure 4: The modified Prototype based model for Bioinformatics domain

In the development of some systems like Bioinformatics may pass through this phase many times due to their typical characteristics which have been described earlier. Also, the feedback to this phase can come either from development phase through product/prototype testing or the feed back may be from Analysis Phase itself as new facts become known about the system. The working of Development Phase is described in the next section.

This proposed feedback mechanism makes Analysis Phase highly iterative, and iteration may be caused by the incorporation of some rapid changes occurred to a system. If the change affects the functional requirements, it is referred to as Type III change. In this case the next iteration starts from

Analysis Phase (see Figure 4). To incorporate this type of change/iterative, we suggest modification in the framework which has been proposed by Shah in (Shah A., 2001) and also given in Figure 2. The modified framework is shown in Figure 4. New information (or changes) occur to a system is incorporated in the system, and we refer it to as *New Facts* (NF) and is denoted by an arrow as shown in Figure 4.

The analysis phase in our proposed model is divided into two stages, i.e., Rapid Analysis Phase and Parallel Analysis Phase (see Figure 4). Rapid Analysis Phase processes those requirements which are high priority and temporally stable. Parallel Analysis Phase processes those requirements which are low priority and temporally unstable and they keep on evolving thought-out the life-span pf the system The requirements form Parallel Analysis Phase are passed to Rapid Analysis Phase as they are promoted to high priority and become temporally stable. It is the responsibility of the project/system stakeholders to provide, clarify, specify, and prioritize functional requirements. The priority can be either high or low depending on when they are input to Development Phase. Parallel Analysis Phase processes those functional requirements which evolve throughout life-span of a system until they can be upgraded to high priority as they become temporally stable. Functional requirements can appear throughout most of the project/system prioritized and added to the priority stack shown in Fig 3. In the figure, the requirements may be reprioritized at any time (upgraded/downgraded), or they may be removed from the priority stack (see Figure 3). The highest priority requirements from the top of the stack are input to Development Phase so that they can be implemented within the current iteration.

In the traditional Waterfall and Spiral lifecycle models the system stakeholders/customers specify their potential requirements early in the project, including ones they might need but really aren't sure about at that moment. They feel that it will be difficult to get them added later because of some change management/prevention process, which will be put in place once the requirements document is finalized. In our proposed framework, stakeholders elicit those requirements which they immediately need and are high on their priority list (It is unlikely that they will miss any of the high priority requirements). In conventional methods (Water Fall and Spiral) the majority of requirements efforts are performed at the beginning of the system development. For application domains such as Bioinformatics, it is unlikely that all of these requirements are still valid till the completion of the system. That is because application domains like Bioinformatics are evolutionary and dynamic as they are technology research driven. The iterative nature of analysis phase in our proposed framework suits the evolutionary domain such as bioinformatics because addition and modification of functional requirements is a continuing process.

4.2. Development Phase

Development Phase further consists of two phases, i.e., Design Phase and Implementation Phase. These two phases work closely to each other. Initially, Development Phase is triggered by the output (Analysis Report) from the Rapid Analysis Phase, R1 (see Figure 4). Once an executable prototype(product) is delivered , it goes through the iterative cycle and incrementally developed by using input both from Rapid and Parallel Analysis Phase (Rp). Development Phase can also be triggered by occurrence of the events *t1, t2, …tn*. These events are updates to an already developed system using additional recently acquired knowledge. In Figure 4, *n* number of triggers t are shown When a trigger *t* occurs, Development Phase takes both the product (an already developed system) and additional acquired knowledge as input, and processes them to incorporate the additional knowledge to the product. A continuous development or update is an essential characteristic of the agile applications and they need updates whenever requirements change or new information/knowledge about the application domain becomes known.

After the initial development of a system as we have discussed above, the system can experience different types of changes and the incorporation of these changes is a necessary and continuous task in its life-span. In Section 3.1, we have categorized three (3) changes. Now we propose their incorporation process to an agile system.

i) Type I: This type of change is the change that occurs only to the data parameter of the prototype. This type of change can easily be incorporated without affecting other parameters and to incorporate it, we do not need to do any intensive development effort. The existing implementation report can be used and the change can be incorporated directly in the implementation phase. Te existing analysis and design reports can also be consulted. In the figure, the two inputs to the implementation phase, the existing implementation report and the new information/data (change) are shown. After incorporating the change, it is necessary to update the implementation report for the future reference.

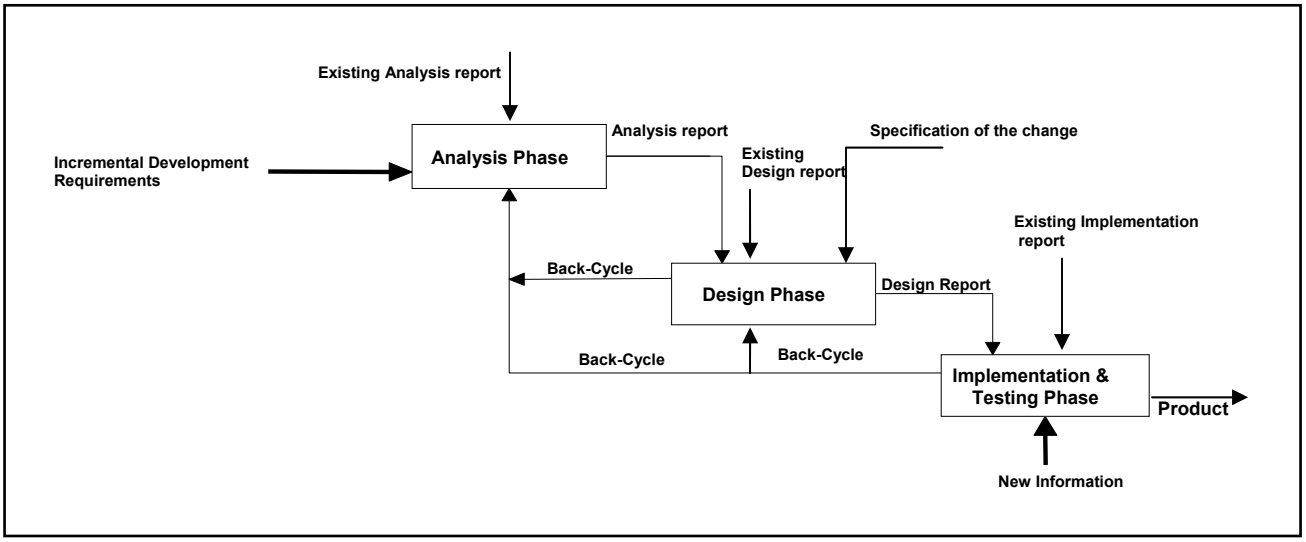


Figure 6: Development phase to incorporate Type I, Type II and Type III changes

ii) Type II change.

A change is a Type II change if it occurs only to the structure of the prototype. For incorporating this type of change, the inputs to the design phase are the existing design report and the specification of the change and the output is the new updated design report. This output is used as input to the implementation phase to physically incorporate the change in the application.

iii) Type III change.

If a change is both to the data and structure parameters, it is referred to as Type III change. A Type III change can significantly change the overall structure of an agile application. For incorporating such a change, we propose efforts in all the three phases as shown in Figure 6.In the figure the change is shown as incremental requirement development that are input to the analysis phase which results in a new analysis report for design phase and a subsequent new design report to be implemented in the implementation phase.

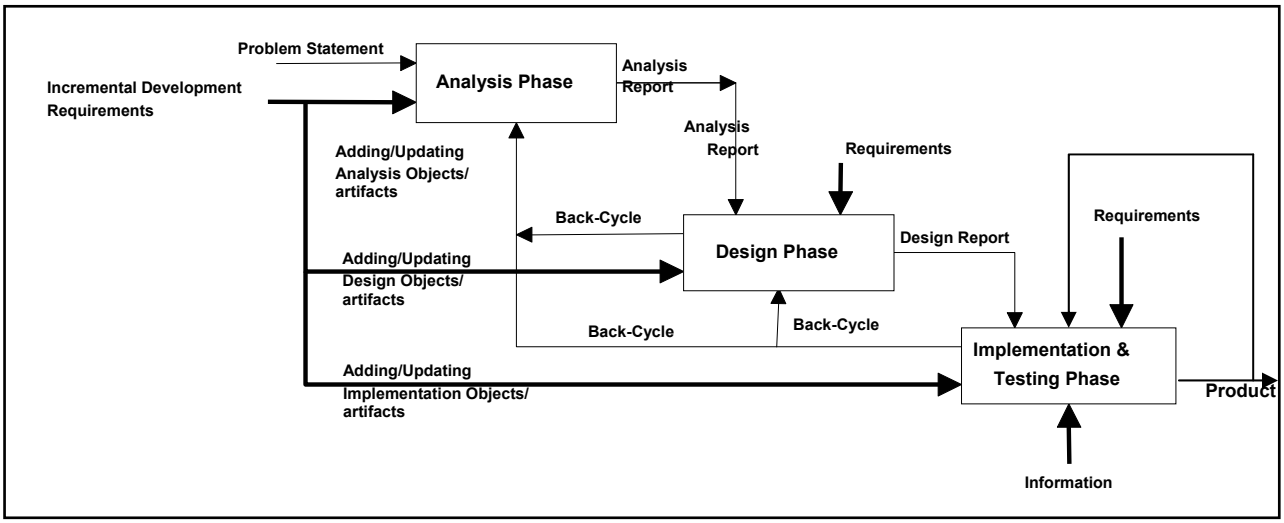


Figure 7: Detailed and Complete development phase for agile application development

Figure 7 illustrates that type 3 changes are input to the analysis phase where it results in a new analysis report which is subsequently input to the design phase to result either in the new design report or a back cycle if further evolutionary changes are reported. The new design report results in the executable prototype which is then tested. The testing phase may also output type 2 or type 3 changes which are then in out to the design or analysis phase through back cycles. The implementation and testing phase can also accommodate type 1 changes which may be reported after the testing of executable prototype or when new information about the system is known.

5 Conclusion and Future Works

The importance of computational methods for analyzing biological data has grown in the post genomic era. In this paper, we concluded that bioinformatics belong to a class of applications for which conventional models of software development are not suitable. This is due to peculiar characteristics of biological data and also because of wide breadth of biological data user base, in which there is no agreement, either vertically (within an organization) or horizontally(across various organization), on what is expected out of a biological data computational tool. This increases the complexity for a computer scientist who must come up with better tools and software engineering models to cater for this increasingly important and complex domain. Agile methods are well suited to the exploratory and iterative nature of Bioinformatics. But most of the agile methods do not prescribe a specific framework, but rater a philosophy for approaching software development. In this work we developed a framework which can form a basis of evolving a methodology for development of Bioinformatics. The frame work can provide the building blocks for a methodology to develop which can include the best practices being used in various stages of most used Agile practices such as XP, SCRUM or Crystal Methodologies.

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Germination and Seedling growth of Field Pea *Pisum sativum* Malviya Matar-15(HUDP-15) and Pusa Prabhat (DDR-23) under varying level of Copper and Chromium

Sandeep K. Pandey *

Abstratct: The heavy metal gradual impact on field pea (Pisum sativum) Var. Malviya Matar-15 (HUDP-15) and Pusa Prabhat (DDR-23) was assessed at germination, seedling growth and pigments concentration after having subjected it to different concentration of $CuSO_4$ and K_2CrO_4 .The germination percentage seedling growth and pigment concentrations were affected by elevating concentration, where Pusa Prabhat (DDR-23) is more sensitive to the elevated concentration of $CuSO_4$ and K_2CrO_4 solutions concentrations then Malviya Matar-15 . But the negative stress of the non essential Cr is more vigorous than essential Cu.This justifies that the field pea variety Pusa Prabhat is less suitable for the cultivation under situation where water and soil suffer from intermittent and momentary metal pollution like Copper and Chromium. [The Journal of American Science. 2008;4(1):33-47]. (ISSN: 1545-1003).

Keywords: Heavy Metals, Seed Germination, Chemical Treatments, Field Pea.

1. Introduction:

Heavy metals are the intrinsic component of the environment with essential and non essential both types. It is the unplanned municipal waste disposal, mining and use of extensive pesticides, other agro-chemicals uses are the significant cause of elevation in environment, its persistence is the cause of most concern. Copper is essential element required in trace amount to plants. Copper concentration increasing in the environment is the cause of disposal of copper containing waste water, sludge and combustion of fossil fuel. Its source may be mining, metal production, phosphate fertilizer production. It has been also incorporating by natural agencies like wind blown dust, decaying vegetation forest fire and sea spray. A very common practice sewage sludge amendment to agricultural soil about 1:20 ratio can expose >100 ppm of Cu to the plants, Singh, R.P.et.al. (2007). Since Cu does not break down in the environment and is continually accumulated by plants and animals thus Cu can influence plant diversity depending on acidity of soil and presence of organic matter, Anonymous b (2004). Cu is essential plant nutrients in trace in excess it is causing stunted growth, cholorsis and root malformation. Cook et.al. (1997) Foy et.al. (1978), Imaculada, Y. (2005).

Chromium is a non-essential and toxic element to plants; Chromium is found in all part of the environment, including air, water and soil naturally occurring in soil, normal range of Cr is from 10 to 50 mg/ kg depending on the parental material. In ultramafic soils (serpentine), it can reach up to 125 mg/ kg,

Adriano (1986). The leather industry is the major cause for the high invasion of Cr to the biosphere, accounting for 40% of the total industrial use Barnhart (1997). In India, about 2000–32,000 tons of elemental Cr yearly escapes into the environment from tanning industries. Average 1:20 ratio of soil and sewage sludge blending can expose >20 ppm of cr to the plants Singh,R.P et.al. (2007). Since seed germination is the first physiological process affected by Cr, the capability of a seed to germinate in a medium containing Cr would be indicative of its level of tolerance to this metal Peralta et al., (2001). High levels (500ppm) of hexavalent Cr in soil reduced germination up to 48% in the bush bean Phaseolus vulgaris Parr and Taylor,(1982). Peralta et al. (2001). The maximum quantity of element contaminant was always contained in roots and a minimum in the vegetative and reproductive organs. In bean, only 0.1% of the Cr accumulated was found in the seeds as against 98% in the roots Huffman and Allaway (1973). Decrease in root growth is a well-documented effect due to heavy metals in trees and crops Breckle, (1991); Goldboldand Kettner, (1991); Tang et al., (2001) Prasad et al.(2001) reported that the order of metal toxicity to new root primordia in Salix viminalis is Cd>Cr>Pb, whereas root length was more affected by Cr than by other heavy metals studied. Adverse effects of Cr on plant height and shoot growth have been reported (Rout et al., 1997), Shankar Arun,et. al. (2005).Barcelo,et.al.(1986) studied the chlorophyll-a,chlorophyll-b,total carotenoids and trace elements of initial and first trifoliate leaves of Phoseolus vulgaris. Plants grown with or without Cr(IV) in the nutrient solutions showed negative linear relation between chlorophyll and carotinoid contents due to chromium. High correlation coefficient was also obtained between pigment contents and Iron and Zinc contents. Chromium induced inhibition of Fe and Zn transport was considered to cause chlorosis in plants. Rai et.al.(1990) studied the effect of Cr,Pb,Ni, & Ag on growth, pigments DNA,RNA,heterocysts,frequency uptake of NH_4^+ and NO_3^- nitrate reductase

and glutamine synthetase activities of Nostic muscorum and revealed a direct positive correlation between molal concentration and inhibition of different processes. The inhibition of pigments was recorded in the order as Chll.<Phycocyanin<Carotinoid. No generalized trend for inhibition of macromolecules was observed. Field peas are the most consumable pulses crops, Malviya Matar-15 and Pusa Prabhat DDR-23 are the recently developed high yielding varieties. The required attribute for plants are metal uptake avoidance and accumulation without toxic symptoms development, these are the symptoms of tolerant plants, meanwhile the sensitive plant shows more stress symptoms, To determine tolerance capabilities, the plants were tested by germination test and seedling growth, chlorophyll content these are the key issues for sustenance of plants in any pre-existing conditions. The present work is to assess the effect of increasing concentration of heavy metals like Cu and Cr their possible gradual impact on physiology, biochemistry and heavy metal tolerance of the plants and also to find out the correlation between elevated concentration of heavy metals to pigments concentration and intra pigments dependencies on different concentration of heavy metals.

2. Materials and Methods:

2.1 Study area

The experiment was conducted at Deptt. of Evironmental Science, P.G.College, Ghazipur a suburban area of district head quarter, located in the eastern Gangetic plain of the Indian sub continent at $25^0$19`and $25^0$54`N latitude, $83^0$4`and $83^0$58`E longitude and 67.50 m above the sea level. The experiment was carried out between Sept.-Oct 2005. This period of the year is characterized by mean monthly maximum temperatures between 34.8 and 36.1 ^{0}C and mean monthly minimum temperatures between 16 and 23.5 ^{0}C. Maximum relative humidity varied from 95% to 100% and minimum from 69% to 71%.

2.2 Selection of Seed

Seed of Field Pea Malviya Matar-15 (HUDP-15) and Pusa Prabhat (DDR-23) was chosen for the experimental work has developed by B.H.U. Varanasi and Indian Agricultural Research Institute (IARI); New Delhi, both are resistant to PM, adoptive to the site of the study (Eastern U.P.) India. And a recently developed and exceedingly espouse high yield variety respectively.

2.3 Experimental Design and Set up

Forty seeds of uniform size for each variety were selected, the seeds were surface sterilized in 5% sodium hypochlorite solution to remove the microbial contamination then seed were thoroughly washed with di-ionized water. Water soluble salts of copper ($CuSo_4$) and Chromium were (K_2CrO_4) were taken for making the solutions by the A.R. Grade reagents first stock solution for 1000 ppm were made and desirable 25,50 and 100 ppm obtained by diluting them by distilled water. Seeds were spread on sterlised petri dishes lined with filter paper whatman No.-1 . The seed were irrigated with equal volume (20ml.) of different concentration of solutions for each treatment , the petri dishes were arranged in a completely randomized block design with three replicates and in each replicates 40 seeds .The experiment was carried out in a growth chamber at 25^0C , 12 hours dark and 12 hours light period with illumination of 2500 lux. Their germination were recorded on each day at fixed time and fixed intervals. The germination percentage was recorded in tabular form. After 15 days only 12 germinated seedlings of similar morphology and age groups of each variety were transferred in plastic trays with size 24``×10`` size with soil and irrigated on alternate days with 250 ml of respective concentration of 25, 50 and 100 % of Cu and Cr solutions. Each treatment having three replicates and each replicates contains 12 seedlings. The seedlings were harvested after 15 days from the date of seedling transferred in Trays.

2.4 Estimation of Germination %, Root length, Shoot length and No. of lateral roots

Germination percentage was calculated by dividing the seed germinated on each day by total no. of seed taken × 100. And finally adding the total percentage. Root, and shoot length measured by normal scale.

2.5 Estimation of Chlorophyll and Carotinoids

The chlorophyll and carotinoids contents of primary leaves were estimated with 80% acetone with help of spectrophotometer (Hitachi, Ltd. Tokyo) Jayaraman,J.(1981).

Chlorophyll a = (.0127) (OD663)-(.00269) (OD 645) gm/lit.

Chlorophyll b = (.0229) (OD 645) – (.00488) (OD 638)gm/lit.

Carotenoids = (OD 490)-(.114) (OD663) – (.638) (OD 645) gm/lit.

3. Results:

Table 1 (a): Effect of Copper and Chromium on percentage germination of Malviya Matar-15. (Mean±Stand.Error)

S.No.	Treatment	Concentration	% Germination	% Reduction in germination
1.	Control	------	100±.00[a]	------
2.	$CuSO_4$	25 50 100	88±3.0[b] 80±1.1[c] 70±1.5[d]	12 20 30
3.	K_2CrO_4	25 50 100	83±1.5[b] 75±1.5[c] 66±1.8[d]	17 25 44

Different letters in each group shows significant difference at P<0.05 levels.

Table 1 (b): Effect of Copper and Chromium on percentage germination of Pusa Prabhat (DDR-23). (Mean±Stand.Error).

S.No.	Treatment	Concentration	% Germination	% Reduction in germination
1.	Control	------	100±.00[a]	------
2.	$CuSO_4$	25 50 100	85±2.5[b] 78±2.5[b] 67±2.5[c]	15 22 33
3.	K_2CrO_4	25 50 100	83±3.6[b] 72±1.5[c] 63±3.0[d]	17 28 37

Different letters in each group shows significant difference at P<0.05 levels.

Table-2(a): Effect of Copper and Chromium on Root length, Shoot length and Number of lateral roots of Malviya Matar-15.(Mean±Stand.Error)

S.No.	Treatment	Concentration%	Root length(Cm.)	Shoot length(Cm.)	No. of lateral roots
1.	Control	------	6.5±.40[a]	9.9±.26[a]	9.2±.10[a]
2.	$CuSO_4$	25 50 100	5.4±.01[b] 4.6±.20[bc] 3.2±.15[d]	6.2±.20[b] 5.6±.35[b] 3.8±.11[c]	7.3±.40[b] 6.2±.15[c] 4.2±.15[d]
3.	K_2CrO_4	25 50 100	2.9±.45[b] 2.2±.15[bc] 1.3±.15[c]	3.6±.26[b] 2.9±.26[bc] 1.6±.30[c]	7±.40[b] 5±.20[c] 3.2±.20[d]

Different letters in each group shows significant difference at P<0.05 levels.

Table-2(b): Effect of Copper and Chromium on Root length, Shoot length and Number of lateral roots of Pusa Prabha(DDR-23).(Mean±Stand.Error).

S.No.	Treatment	Concentration	Root length(Cm.)	Shoot length(Cm.)	No. of lateral roots
1.	Control	------	6.8±.20[a]	9.1±005[a]	8.7±.11[a]
2.	$CuSO_4$	25	5.0±.75[b]	6.0±.30[b]	7.0±.30[b]
		50	4.2±.15[bc]	5.2±.15[c]	5.9±.005[c]
		100	2.9±.23[c]	3.7±.15[d]	3.9±.008[d]
3.	K_2CrO_4	25	2.5±.005[b]	3.3±.01[b]	6.6±.20[b]
		50	1.8.±.15[c]	2.0±.25[c]	4.7±.11[c]
		100	1.1±.1[d]	1.4±.26[c]	2.9±.26[d]

Different letters in each group shows significant difference at P<0.05 levels.

Table-3(a): Effect of Copper and Chromium on pigment content of Malviya Mater-15. (Mean±Stand.Error)

S.No.	Treatment	Concentration	Cholophyll'a'(g/lit.)	Chlorophyll 'b'(g/lit.)	Carotenoids (g/lit.)
1.	Control	------	.0042±.00002[a]	.0056±.00003[a]	.345±.00005[a]
2.	$CuSO_4$	25	.0036±.000005[a]	.0052±.000015[a]	.221±.00026[b]
		50	.0029±.00002[b]	.0049±.00002[b]	.168±.00015[c]
		100	.0021±.00002[c]	.0038±.000015[c]	.0978±.000046[d]
3.	K_2CrO_4	25	.0028±.000015[b]	.0050±.000050[a]	.208±.000025[b]
		50	.0019±.000057[c]	.0037±.000020[b]	.144±.00040[c]
		100	.0012±.000015[d]	.0025±.000015[c]	.0975±.000028[d]

Different letters in each group shows significant difference at P<0.05 levels.

Table-3(b): Effect of Copper and Chromium on pigment content of Pusa Prabhat (DDR-23). (Mean±Stand.Error).

S.No.	Treatment	Concentration	Cholophyll'a'(g/lit.)	Chlorophyll 'b'(g/lit.)	Carotenoids (g/lit.)
1.	Control	------	.0040±.000025[a]	.0054±.000016[a]	.339±.00011[a]
2.	$CuSO_4$	25	.0031±.000025[b]	.0047±.000015[b]	.218±.00011[b]
		50	.0024±.000026[bc]	.0040±.00002[b]	.162±.00011[c]
		100	.0019±.000015[c]	.0036±.000013[c]	.0973±.000017[d]
3.	K_2CrO_4	25	.0022±.000030[b]	.0050±.000026[a]	.200±.0000[c]
		50	.0020±.000015[b]	.0031±.00001[b]	.140±.00028[c]
		100	.0011±.0000[c]	.0022±.000015[c]	.0965±.00010[d]

Different letters in each group shows significant difference at P<0.05 levels.

Percentage germination of field pea (Malviya Matar-15) and Pusa Prabhat (DDR-23) has affected by the copper and chromium solutions. The rate of germination have highly retrograde in presence of higher concentration of solution than preceding lower concentration and control as mentioned in Table No. 1(a)and (b).

Root and Shoot length , No. of lateral roots and pigments of 15 days old seedlings at different concentrations of Cu and Cr are presented in Table No. 2(a),(b) and 3 (a),(b) respectively. The highest value of Root length, Shoot length and No. of lateral roots and amount of pigments reported in control condition for both species and treatments and least values were reported in case of 100 PPM concentration. There was considerable difference in root length shoot length, no. of lateral roots for both species treatments with Copper sulphate and Potassium chromate , the extent of decline was significantly greater in case of Pusa Prabhat than Malviya Matar-15. The root length was significantly influenced by varying concentration of copper sulphate and vigorously affected by potassium chromate solution. The comparative negative effect was higher in case of Pusa Prabhat than Malviya Matar-15. The two different metal treatments had a significant effect on shoot development. The shortest shoot length was observed at highest concentration 100 PPM of potassium chromate in Pussa Prabhat. However comparatively longer shoot length in case of Malviya Matar-15 reported in both chemical treatments as mentioned in Table No.-2. Negative effect of chromate solution is more on both crops then copper sulphtae solution. Percentage germination and number of lateral roots have reduced with each elevation in concentration in all the treatments. Effect of metal treatment on pigments had been significantly seen as the copper sulphate solutions decreases in the pigment formation like Chlorophyll a, Chlorophyll b and formation of carotenoids. While increase the concentration potassium chromate causes a more adverse effect on the vegetations. As lesser formation of chlorophyll a, chlorophyll b and carotinoids. ANOVA analysis indicates the P<.05 level of significance in all the treatment cases compare to control.

Table:4a. Co-relationship between varying level of copper concentration and pigments concentration in leaf of Malviya Matar-15.

Correlations

		CHLOROPH	CHLB	CARATINO
CHLOROPH	Pearson Correlation	1.000	.780**	.921**
	Sig. (2-tailed)	.	.003	.000
	N	12	12	12
CHLB	Pearson Correlation	.780**	1.000	.848**
	Sig. (2-tailed)	.003	.	.000
	N	12	12	12
CARATINO	Pearson Correlation	.921**	.848**	1.000
	Sig. (2-tailed)	.000	.000	.
	N	12	12	12

**. Correlation is significant at the 0.01 level (2-tailed).

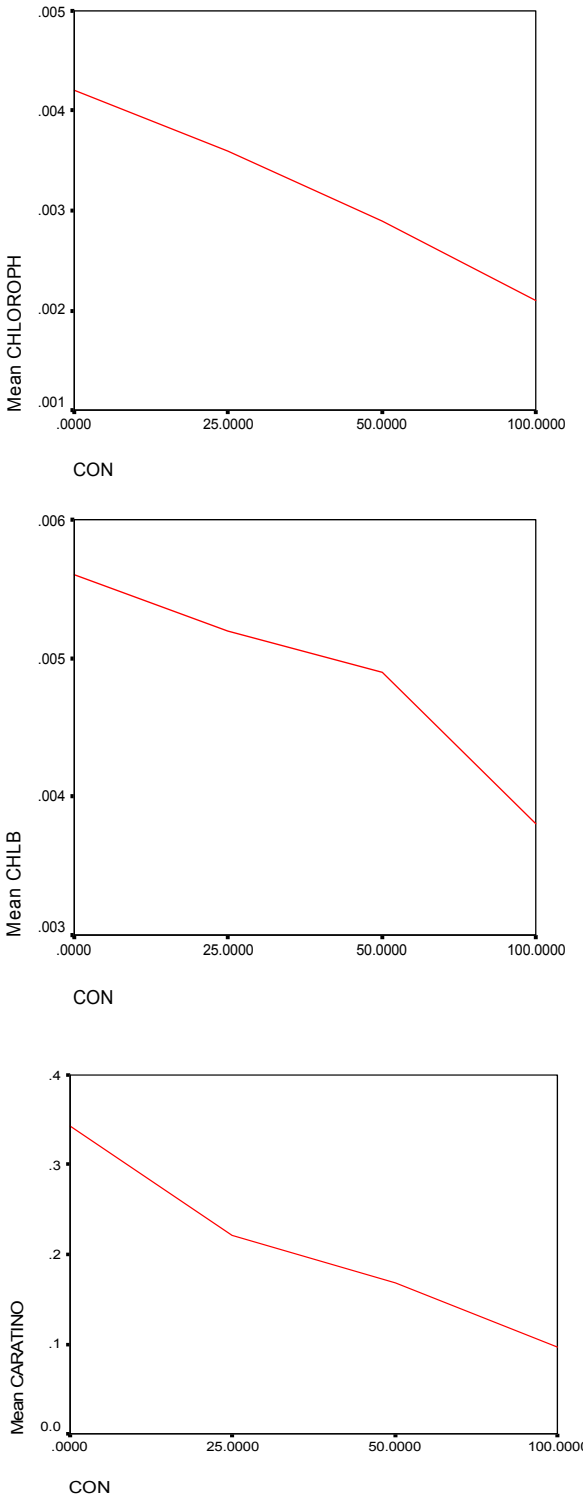
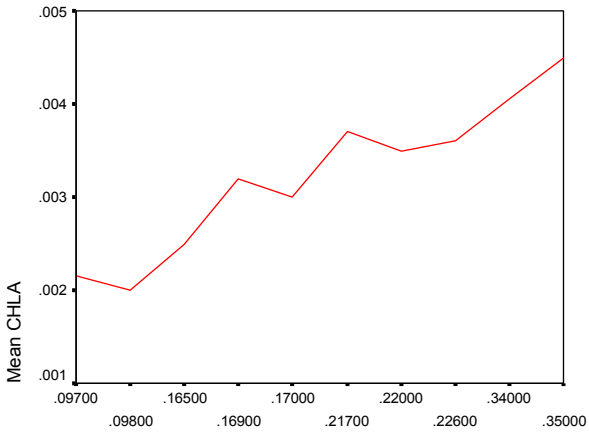
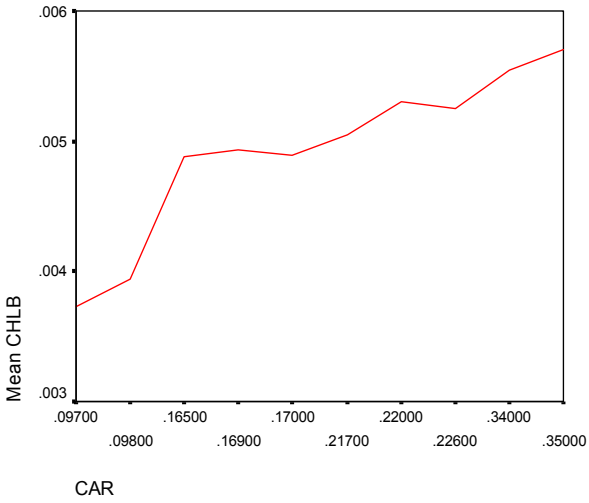


Fig.:1a. Relationship between varying level of copper concentration and pigments concentration in leaf of Malviya Matar-15.





.Fig.:1b. Rerelationship between varying level of carotinoids concentration with chlorophyll 'a' and 'b' concentration in leaf of Malviya Matar-15.

Table: 4b.Co-relationship between varying level of copper concentration and pigmentsconcentration in leaf of Pusa Prabhat.

Correlations

		CHLA	CHLB	CAR
CHLA	Pearson Correlation	1.000	.913**	.918**
	Sig. (2-tailed)	.	.000	.000
	N	12	12	12
CHLB	Pearson Correlation	.913**	1.000	.929**
	Sig. (2-tailed)	.000	.	.000
	N	12	12	12
CAR	Pearson Correlation	.918**	.929**	1.000
	Sig. (2-tailed)	.000	.000	.
	N	12	12	12

**. Correlation is significant at the 0.01 level (2-tailed).

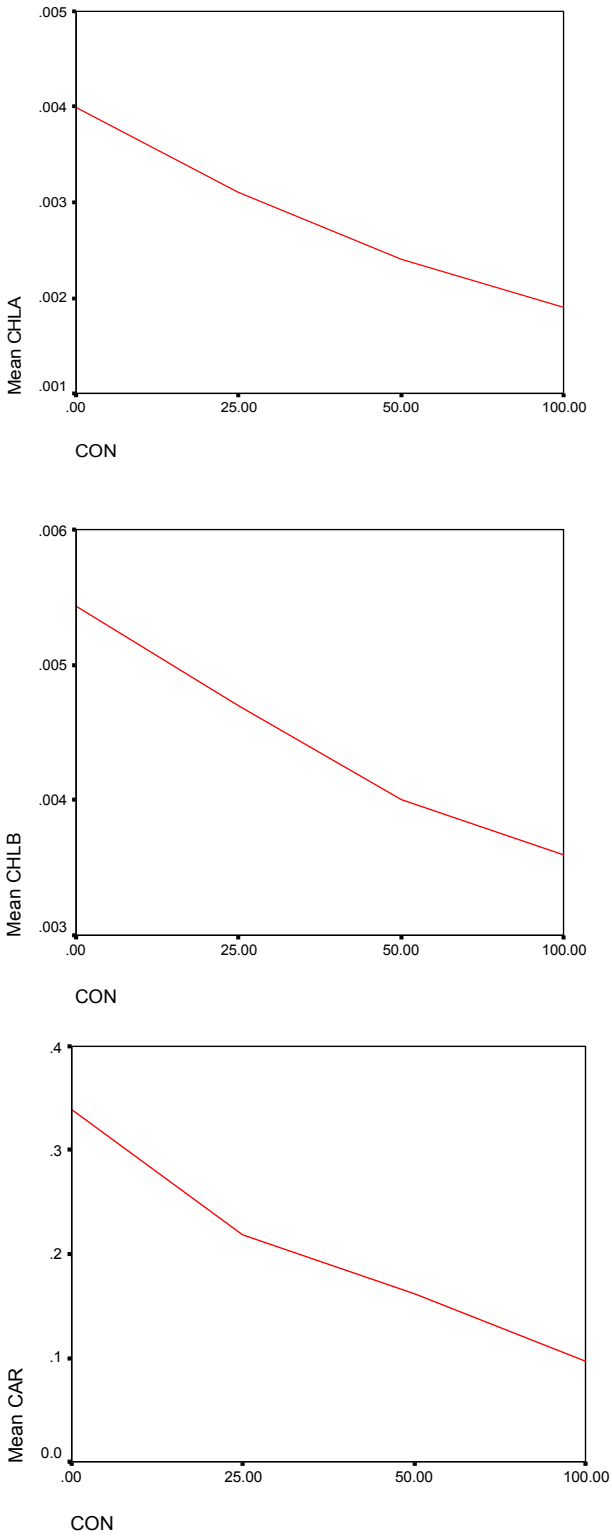


Fig.:2a. Relationship between varying level of copper concentration and pigments concentration in leaf of Pusa Prabhat.

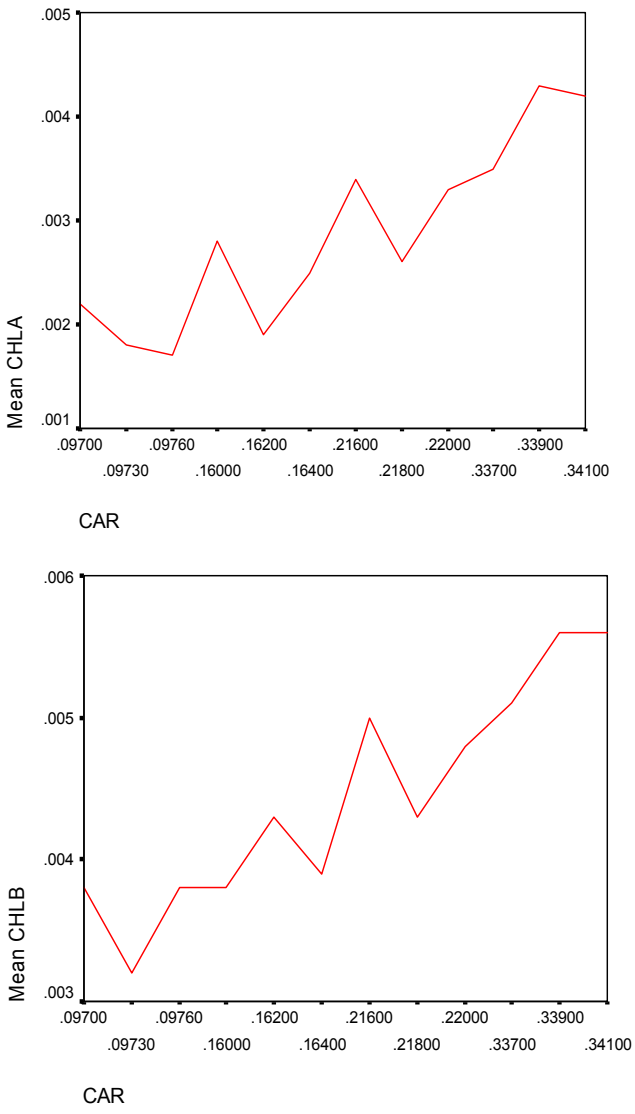


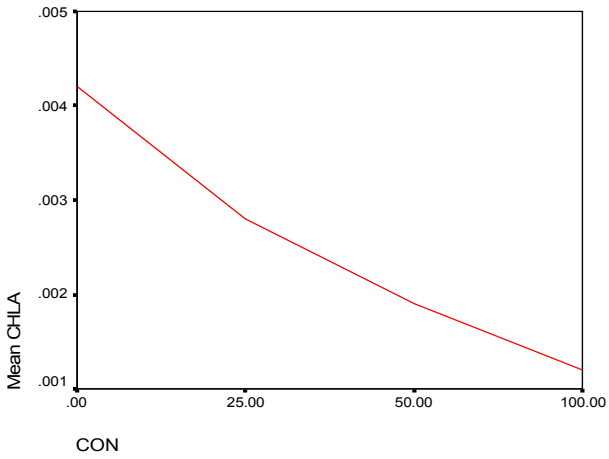
Fig.:2b. Rerelationship between varying level of carotinoids concentration with chlorophyll ‘a’ and ‘b’ concentration in leaf of Pusa Prabhat.

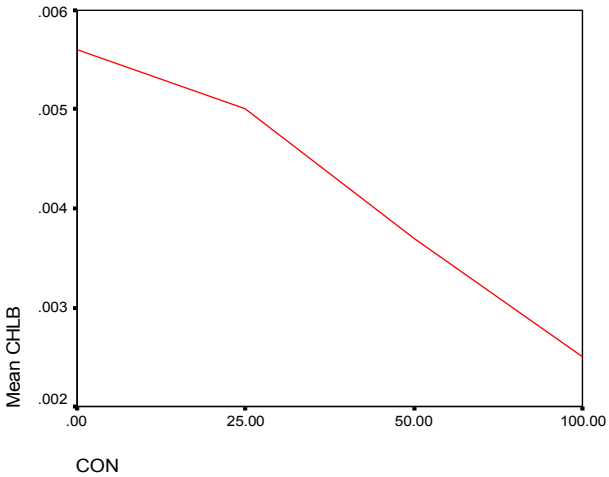
Table:4c. Co-relationship between varying level of Chromium concentration and pigments concentration in leaf of Malviya Matar-15.

Correlations

		CHLA	CHLB	CAR
CHLA	Pearson Correlation	1.000	.875**	.977**
	Sig. (2-tailed)	.	.000	.000
	N	12	12	12
CHLB	Pearson Correlation	.875**	1.000	.889**
	Sig. (2-tailed)	.000	.	.000
	N	12	12	12
CAR	Pearson Correlation	.977**	.889**	1.000
	Sig. (2-tailed)	.000	.000	.
	N	12	12	12

**. Correlation is significant at the 0.01 level (2-tailed).





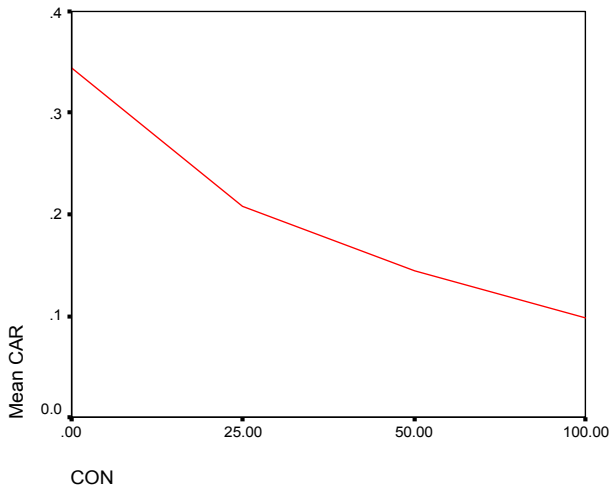


Fig.:3a. Relationship between varying level of Chromium concentration and pigments concentration in leaf of Malviya Matar-15.

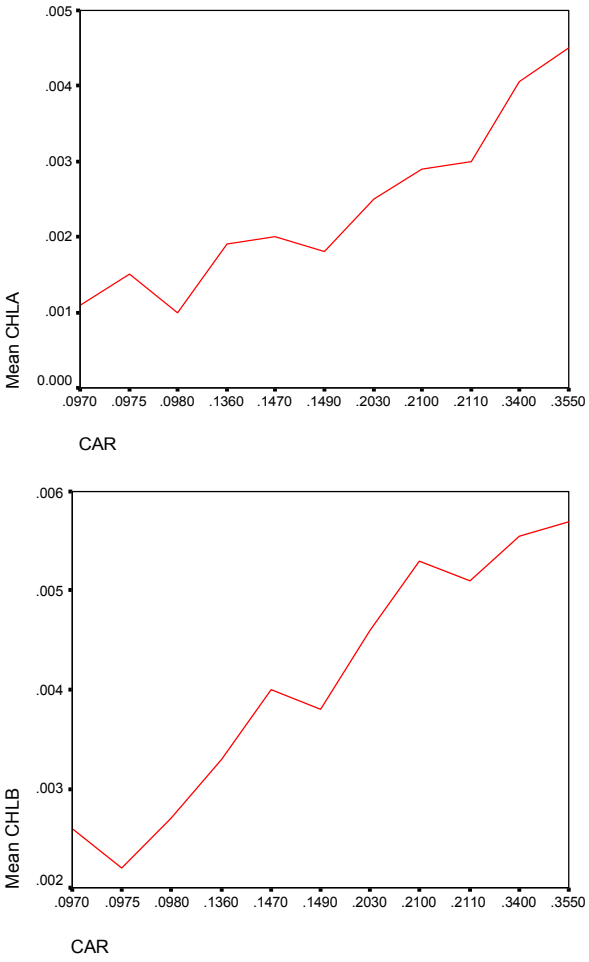


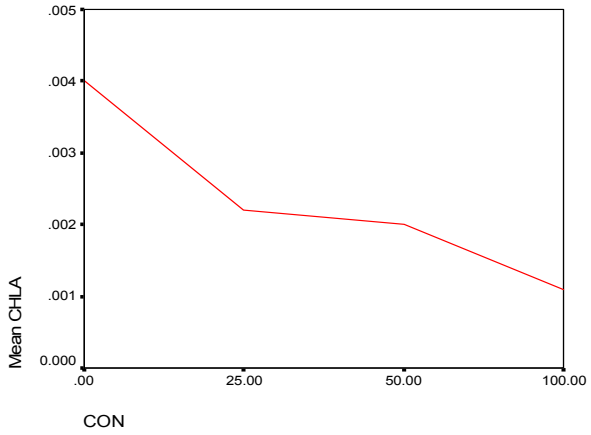
Fig.:3b. Rerelationship between varying level of carotinoids concentration with chlorophyll ‘a’ and ‘b’ concentration in leaf of Malviya Matar-15.

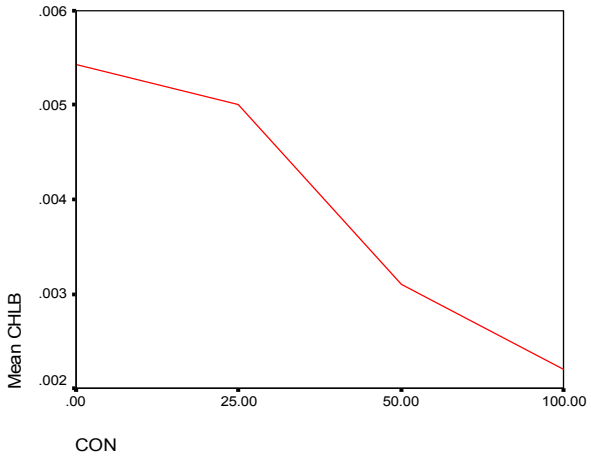
Table:4d. Co-relationship between varying level of Chromium concentration and pigments concentration in leaf of Pusa Prabhat.

Correlations

		CHLA	CHLB	CAR
CHLA	Pearson Correlation	1.000	.787**	.948**
	Sig. (2-tailed)	.	.002	.000
	N	12	12	12
CHLB	Pearson Correlation	.787**	1.000	.882**
	Sig. (2-tailed)	.002	.	.000
	N	12	12	12
CAR	Pearson Correlation	.948**	.882**	1.000
	Sig. (2-tailed)	.000	.000	.
	N	12	12	12

**. Correlation is significant at the 0.01 level (2-tailed).





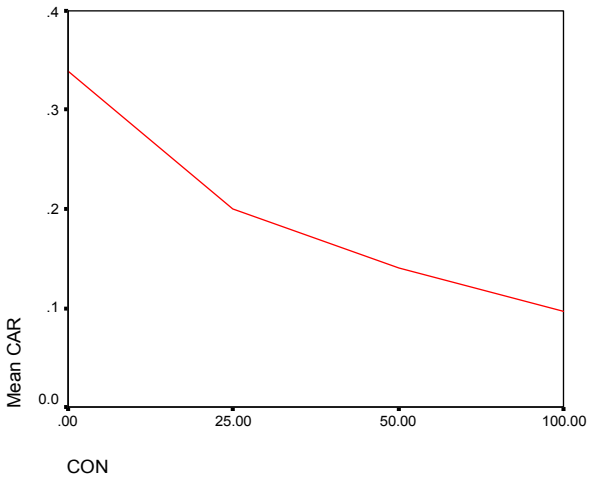


Fig.:4a. Relationship between varying level of Chromium concentration and pigments concentration in leaf of Pusa Prabhat.

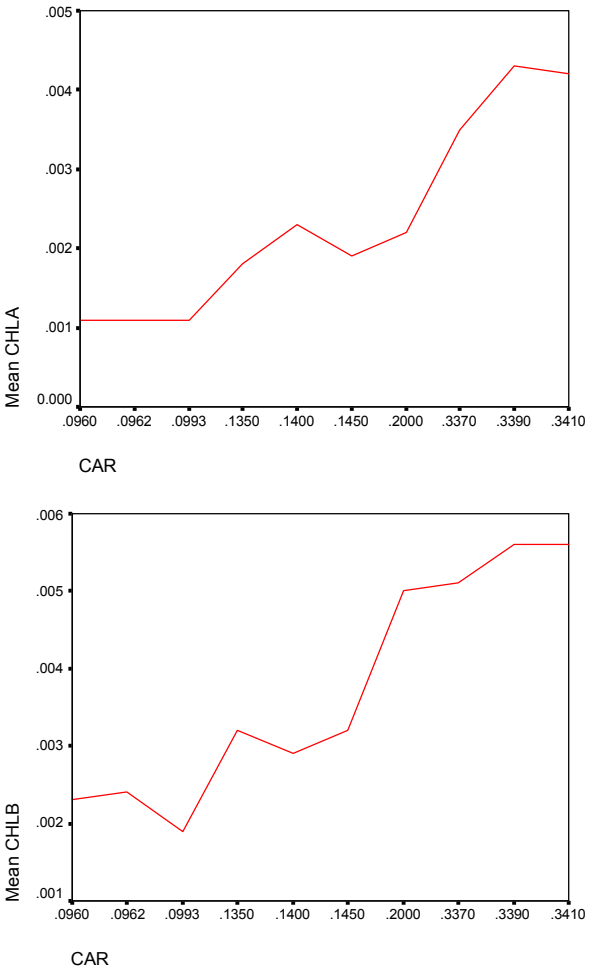


Fig.:4b. Relationship between varying level of carotinoids concentration with chlorophyll 'a' and 'b' concentration in leaf of Pusa Prabhat.

Correlation studies shows that the increase in concentration of solution is inversely related to the concentration of the all pigments while the correlations between carotinoids and chlorophyll 'a' and 'b' is positive as increase in concentration of chlorophyll increase in concentration of carotinoids.

4. Discussion and Conclusions:

The present result showed that the germination of both varieties was significantly influenced by both chemical treatments. The results clearly indicates that increasing concentration of these two metals retrogrades the germination with its percentage increase. The result was consistent with Rout et. al. (2000). Jain et. al.(2000) Peralta (2001). The reduced germination of seeds under Cr stress could be a depressive effect of Cr on the activity of amylases and on subsequent transport of sugars to the embryo axes Zeid (2001).Heavy metal causes a significant adverse effect on seedling growth and pigment formation. The study showed reduction in root length, shoot length, no. of lateral roots and pigment formation. The effect of negative stress is max.in case of Cr then Cu. This could be due to the inhibition of the root cell division/root elongation or to extension of the cell cycle in roots Barcelo et. al. (1986).Result similar to Cu toxicity on root growth has been reported by Sheldon,A. and Neal W. Menzies (2004). Shoot growth reduction through the toxicity of Cr has been reported by Rout et. al. (1997). Barton (2000). The reduction in shoot growth is due to stunted root growth results lesser nutrients and water supplied to aerial parts, where Cr and Cu causes direct impact on the cellular metabolism causes shortning of shoot height. Hanus and Tomas(1993).At elevated level of Cu treatment of seedlings the Chl a, Chl b and carotinoids content decreases supported by Miller (1938), Patsikka (2002). Where author suggested that reduced chlorophyll contents observed in the plants grown in presence of very high copper content due to iron deficiency. Meanwhile the elevated level of chromium shows decreased level of Chl a,Chl b and carotinoids as supported by Ganesh Shankar,K. et. al. (2006).Where author have suggested that morphological germination study parameters were decreased with respect to increase of chromium concentrations. In concluding remark we can say that both metal treatments are harmful for the plant germination and seedling growth behaviors at elevated concentration level from its optimum. Where Cr is comparatively more toxic than Cu. The Pusa Prabhat is more sensitive to the metal pollution like Cr and Cu pollution than Malviya Matar-15.

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Poly(amidoamine)(PAMAM) /CMS Dendritic nanocomposite for controlled drug delivery

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ABSTRACT: Dendrimers are one of the emerging delivery systems with the capability to present such hydrophobic agents in a formulation with better prospective[1] .These dendritic macromolecules with a large number of suface terminal groups and interior cavities offer a better opportunity for delivery by becoming charged and acting as static covalent micelles.These are biocompatible, nonimmunogetic , and water- soluble and possess terminal functional groups for binding various targeted or guest molecules[2]. The host – guest properties of dendrimers based on hydrophobic and ionic interactions apart from physical entrapment have been thoroughly studied .In the present study , the conjugate carboxymethyl starch (CMS) /PAMAM dendrimer were exhaustivly studied as controlled – release systems for parenteral administration of a model drug 5- aminosalicyclic acid (mesalamine) and analyzed using various release kinetic studies. The synthesized nanocomposite have been characterized by Fourier transfer infrared(FTIR) spectrophotometer. The morphology of these composites was studied by scanning electron microscopy. [The Journal of American Science. 2008;4(1):48-52]. (ISSN: 1545-1003).

KEYWORDS: PAMAM dendrimer , CMS, 5-amino salicyclic acid(5-ASA), nanocomposite.

INTRODUCTION

The invention of the dendrimer is attributed to Donald Tomalia, who first published his report of poly(amidoamine) dendrimer synthesis in 1979 from his laboratory at the Michigan Molecular Institute in Midland, Michigan (Tomalia 1995)[3].His first poly(amidoamine) dendrimer was the result of reacting three methylacrylate molecules to an ammonia core, followed by the addition of three ethylenediamine molecules to form the G0 amidoamine. By continuing this two-step process of methylacrylate/ethylenediamine addition, successive amidoamine generations are produced, doubling the number of terminal amine groups each time[4] .

Concurrently, Fritz Vögtle of the University of Bonn published his own dendrimer synthesis consisting of ammonia derivatives with acrylonitrile. Later, George Newkome would publish an alternative synthesis of similar molecules that he called “arborols,” after the tree-like symmetry, but his 1985 discovery would be largely overshadowed four years later when a collaboration between Cornell University and AT&T Laboratories would revolutionize dendrimer synthesis, setting off an explosion of dendrimer research around the globe[5] .

In 1989, Jean Fréchet of Cornell University and Timothy Miller of AT&T Laboratories jointly developed a convergent synthesis for producing dendrimers. Rather than иeginning with a core molecule and building each generation onto the core outwardly, Fréchet and Miller were able to begin with the dendrimer periphery and inserted the molecular core as the last step[6]. In this manner, high-purity dendrons of the desired generation could be synthesized, and then by reacting these dendrons with the core molecule, dendrimers could be produced with the same high purity[7]. Prior to the development of convergent dendrimer synthesis, only a handful of scientific papers had been published on dendrimer research; in the five years that followed, dendrimer research literally exploded within the scientific community (Tomalia 1995)[8].

Because of their early discovery and thus the amount of research that has been conducted with them (Bosman, Janssen et al. 1999), PAMAM dendrimers are among the very few commercially available dendrimers, available in generations 0 to 10 from Aldrich, Inc. The PAMAM dendrimers available from Aldrich, Inc., are prepared by a divergent synthesis[9].

Dendrimers are one of the emerging delivery systems with the capability to present such hydrophobic agents in a formulation with better prospective .These dendritic macromolecules with a large number of suface terminal groups and interior cavities offer a better opportunity for delivery by becoming charged and acting as static covalent micelles.These are biocompatible, nonimmunogetic , and water-soluble and possess terminal functional groups for binding various targeted or guest molecules[10]. The host – guest properties of dendrimers based on hydrophobic and ionic interactions apart from physical entrapment have been thoroughly studied[11,12] .

In the present study , the PAMAM dendrimers were exhaustivly studied as controlled – release systems for parenteral administration of a model drug 5- aminosalicyclic acid (mesalamine) and analyzed using various release kinetic studies[13,14].

Experimental Methods

Materials

All reactions were performed under an atmosphere pressure . All reagents and solvents , unless otherwise specified,were obtained from Merck Chemical Company.

Poly(amidoamine)(PAMAM) generation 4 contains 64 surface primary amino groups was obtained from Dendritech ,Inc. , Aldrich company.Melting points were obtained on a Mel-Temp melting point apparatus . Analytical TLCs were run on commericial Merck plates coated with silica gel GF250(0.25mm thick). Fourier transfer infrared(FTIR , Bruker)spectroscopy was used to identified the polymer surface.Specta were obtained in the wave number range of 400-4000cm^{-1} Spectra of samples were recorded from KBr in 1:10 (wt/wt) ratio.

Firstly ,the 0.5 g cornstarch and 120 ml 2-propanol were placed in a 500 ml vessel and stirred for 2 h . The 5 g sodium hydroxide was added and reacted for 1 h at 78-80 ˚C .After thet , the 10 g chloroacetic acid was added to the vessel and stirred for another 2 h at 50 ˚C .The product was filtered and washed several times with ethanol , then dried under vacuum . The resulting carboxymethyl starch (CMS) was crused in a mortar[degree of substitution (DS) = 0.49].

The condensation reaction between poly(AmidoAmnie)(PAMAM) and the activated carboxymethyl starch (CMS) was carried out in a borate buffer (PH=8.5). The solvent(methanol) was vacuum – evaporated from PAMAM prior to reaction.Then, 1.62mM, 23 mg PAMAM dendrimer 11.6 mM , 38.9 mg CMS dissolved in 50 cc borate buffer (PH= 8.5) and incubated at the room temperature for 24 h with shaking .Crude reaction mixtures of conjugates were dialyzed against distilled water using dialysis tubing for 24h.

Then, 5-aminosalicylic acid(5-ASA) was dissolved in methanol following which the dendrimer was added .The reaction mixture was stirred for 24 h in the dark ,then evaporated using rotaevaporator to remove methanol . The traces were dried under vacuum in order to remove methanol completely .To these traces , deionized water was added . This solution was stirred in the dark for 24 h. This was extract the drug – dendrimer complex ,as dendrimer is soluble in water while 5-ASA is not. The solution was then filtered through PTFE membrane(Millix Millipore) of pore size 200nm ,and then lypophilized to remove water.After approximately 180 min, the sample were sprayed into a liquid nitrogen bath cooled down to 77° K, resulting in frozen droplets. These frozen droplets were then put into the chamber of the freeze-dryer. In the freeze-drying process, the products are dried by a sublimation of the water component in an iced solution.The drug –CMS/dendrimer complex obtained was in the form of a brown powder.

RESULTS AND DISCUSSION

Figure 1 shows the SEM of PAMAM(generation 4)dendrimer/CMS / Mesalamine(5-ASA) nanocomposite that synthesized by chemical reaction. This nanocomposite is very sensitive to the temperature that due to the intraction electron and sample . Scanning electron micrography images were

obtains from a diluted solution of the nanocomposite particle . The white spots are drug nano particles .The SEM image shows the presence of 5-ASA spherical particles in polyfunctional dendrimeric matrix, which are homogenenously distributed throughout the composites ,which is also confirmed from ^{1}H-NMR studies. The ability of the dendrimer to form a complex with drugs depends on the core- suface groups of dendrimer ,electrostatic interactions between the dendrimer and the drug , and the ability of the drug to form a conjugate with the dendrimer through chemical bonding . Therefore , it is possible to manipulate the incorporation process for a given drug by appropriate selection of the dendrimer and the surface functionality.One might expect that the mesalamine with the carboxylic group may form a complex with surface NH_2 groups of PAMAM dendrimer.

Fig. 2a shows the FT-IR spectrum of pure carboxymethyl starch (CMS) , where the % of transmittance is plotted as a function of wave number (cm^{-1}) . The wide peak around 3411 cm^{-1} is attributing to the O-H stretching vibrations of CMS.The peaks at 1597 and 1417 cm^{-1} attribute to the COO^- unsymmetrical and symmetrical stretching vibration respectively .The peaks from the FTIR spectrum of CMS-PAMAM 4.0G complex at 3250 cm^{-1} showed the presence of terminal primary amino groups (figure 2b).In case of drug – dendrimer complex , the broad peak at 3200 cm^{-1} is the strong evidence of the presence of NH_3^+ showing the electrostatic association of drug with dendrimer (figure 2c).For learn of effect of the nature and size of the drug in drug delivery, we study drug release of the polymers containing 5-ASA as a pharmaceutically active compound as a function of time is shown in figures 3. The concentration of 5-ASA released at selected time intervals was determined by UV spectrophotometry at 205 and 235 nm, respectively. In order to study potential application of nanocomposite containing 5-aminosalicylic acid as pharmaceutically active compounds, we have studied the drug release behavior of the polymers under physiological conditions. The concentration of drugs released at selected time intervals was determined by UV spectrophotometry. Important parameter for increasing of diffusion coefficient is decreased of particle size. It appears that the degree of drug release polymers depends on their particle size. In odder hand, the chemical structure of the drug too is an important factor in hydrolytic behavior of polymeric prodrugs. 5-ASA contains both amine (basic) and carboxylic acid (acidic) functional groups. This factor ultimately result in an increase hydrophilicity of 5-ASA in acidic media.

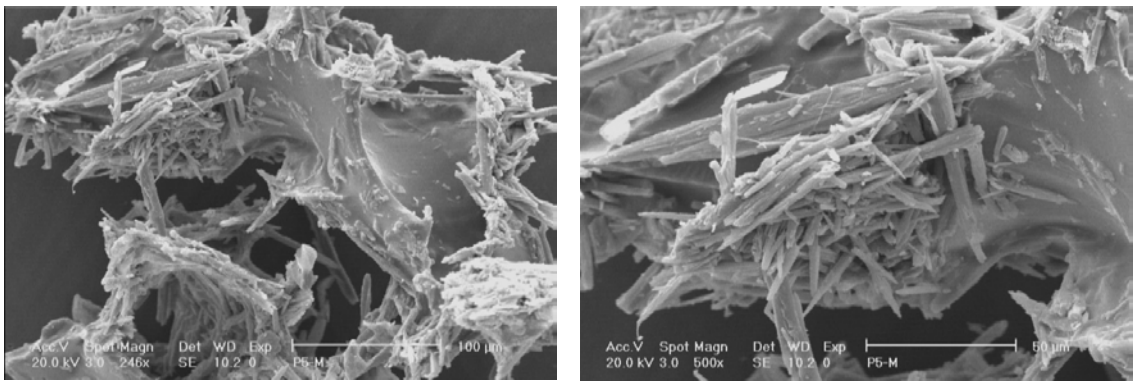

Fig.1 – SEM of CMS-PAMAM(G4)–(5-ASA) nanocomposite

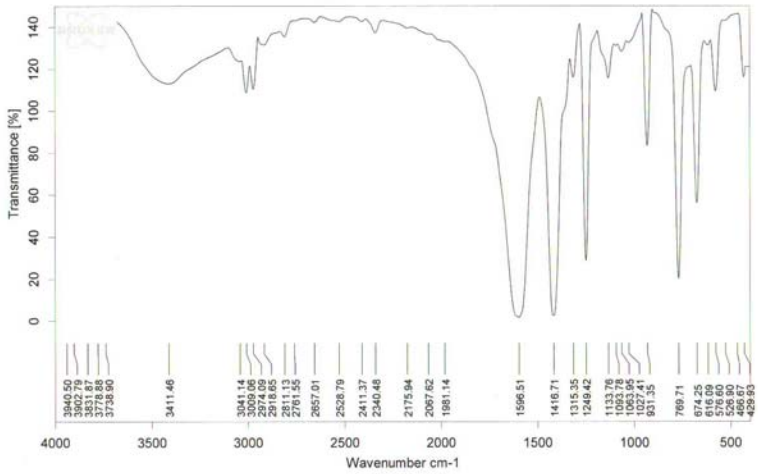

Fig.2a – FT- IR spectrum of pure CMS

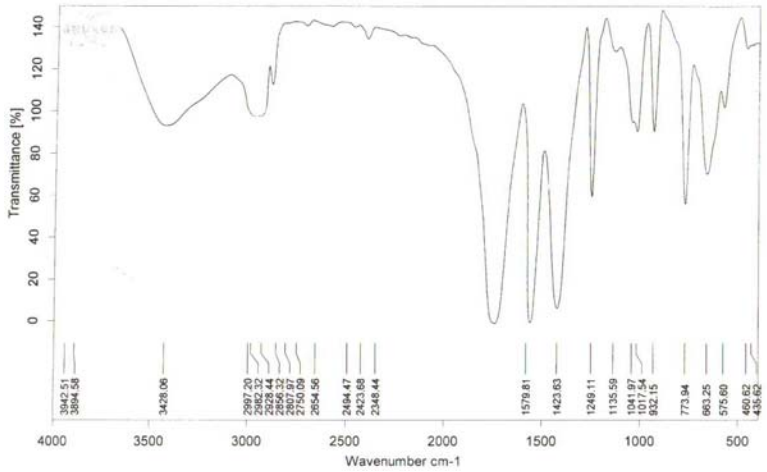

Fig.2b – FT- IR spectrum of CMS-PAMAM complex

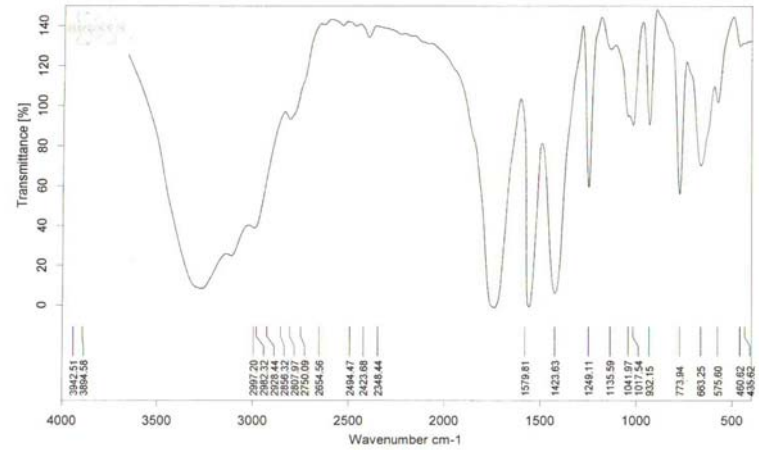

Fig.2c – FT- IR spectrum of CMS-PAMAM-Drug complex

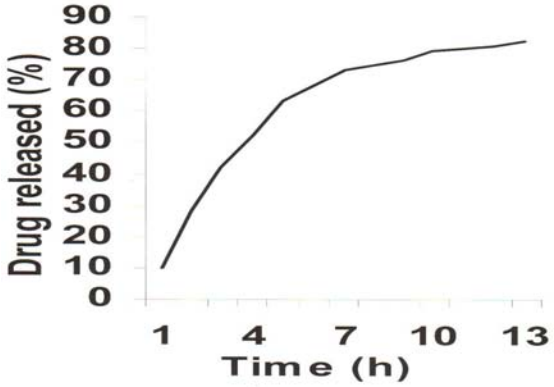

Fig. 3 – Drug release percentage from CMS-PAMAM- (5-ASA) nanocomposite

CONCLUSION

The present study reveals that the conjugate CMS-PAMAM dendrimer interact with hydrophobic 5-ASA molecules to bring it in its ionized state and hence enhace solubility.At the same time dendrimers can localize the drug at the site of inflammation and the drug can provide effective pharmacological action .However , the potential role of our system in various other categories of the drugs for drug delivery is still under investigation.

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Movements of Radio Collared Wolves and Their Significance on Pack Assembly

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Abstract: Little is known about wolf pack assembly throughout the year, such as whether or not pack members do most everything together as a group. This study, however, presents the first quantified evidence of how the movements of individual pack members can indicate the degree of pack assembly over an annual period. The movements of three radio collared wolves (*Canis lupus)* in the Fishtrap pack of northwest Montana, were monitored over a 603-day study period and 532 daily telemetry surveys. Each survey presented a variety of possible pairing results, from finding a single collared wolf to finding all three together at the same location. All possible combinations were observed, and the three collared wolves were found together in only 31.0% of the surveys. For the pack to have been fully assembled, the three collared wolves would have had to be present. Therefore, this pairing combination represented when the pack potentially could have been fully assembled, although no assumption was made that they were. Nevertheless, these preliminary results indicated that pack members were together in 31% or less of the surveys. Although the percentage of surveys did not correlate directly with total time spent together, it did suggest the Fishtrap pack spent the minority of time fully assembled throughout the year. [The Journal of American Science. 2008;4(1):53-58]. (ISSN: 1545-1003).

Introduction

Wolves are known to live in social groups called packs in which interactions among individuals are guided by a hierarchy system (Mech and Boitani, 2003; Packard, 2003). Little is known, however, about the extent of pack assembly throughout the year, and how often all members are actually present at the same time, either at a static location or during movement through their territory. Most studies of wild wolves have focused on the movements of individuals rather than the pack as a whole, although Murie (1944) noted that the wolves he studied usually traveled in packs and occasionally traveled alone or in various combinations.

There seems little doubt wolf packs break into smaller groups for at least the purposes of hunting (Haber, 1977; Mech and Boitani, 2003), and perhaps because of social factors (Haber, 1977) and to find potential mates (Allen, 1979). However, this seems to happen more for some packs than others (Allen, 1979). During the spring and summer, when a pack's focus is on rearing the pups, pack members periodically leave the den and rendezvous sites, and then return to help care for the pups (Murie, 1944; Chapman, 1977; Mech, 1988a). By fall, the pups have matured enough to participate in hunts. The pack then becomes nomadic and moves as a group throughout their territory during the winter (Burkholder, 1959; Mech, 1966b; Peterson, 1977; Jedrzejewski et al, 2001). Some researchers have suggested that a basic wolf pack consists of a breeding pair and its offspring which function as a tight-knit unit year-round (Mech and Boitani, 2003), although it was not specified if tight-knit meant the pack was physically together or not.

Other studies have focused on wolf movements with regard to predation (Mech and Peterson, 2003) or dispersing individuals (Mech and Seal, 1987; Mech, 1987a; Boyd and Pletcher, 1999) rather than on the degree of pack assembly during these times. Radio telemetry studies have tracked individual wolves in which daily movements, territory use, and activity periods were monitored (Jedrzejewski et al, 2001; Theuerkauf et al, 2003), as well as the speed and distance traveled by individual wolves (Musiani et al, 1998; Merrill and Mech, 2000). Again, however, this information was not related to the movements of other pack members, nor was it conducted over several seasons or years. Consequently, to what extent wolf packs are assembled throughout the year has remained unknown. Descriptive phrases such as "moves as a group" or "tight-knit year-round," therefore, have been open to interpretation. A group of wolves could consist of the majority of pack members or all of them. Tight-knit could mean the pack was fully assembled or the wolves acted as a cohesive group even though they spent time apart, like a human family. The definitions of these statements were either nonspecific and unquantified, or just assumed and not defined at all.

Unless all members of a pack were radio-collared, or located by some other method, it would be impossible to know how often they were physically together throughout the year. Instead, this study used the locations found for three radio collared wolves, as they moved through their territory over a two year period, as a general measure of pack assembly throughout the year. Clearly, packs break-up periodically for a variety of reasons, but to what degree was the question posed in this study. The hypothesis, therefore, states that the Fishtrap pack spent the minority of time together as a pack throughout the year. Because one pack was involved in this study rather than several, the results are only a preliminary test of the hypothesis and may not apply to other wolf packs.

Methods

Radio monitoring of the Fishtrap pack began in June 2003, under the guidance of U. S. Fish and Wildlife Service. At that time, an adult male and female wolf had been collared. In January 2004, a female pup from the 2003 litter was also collared. The presence of three collared wolves allowed for the collection of pairing data that could be used to document pack assembly throughout the year. The study period reported here began 8 January 2004 and ended 1 September 2005, and involved radio telemetry surveys conducted mostly on a daily basis. Although the young female was collared two months after the nomadic season began, this season didn't end until 20 April 2004, over three and a half months later. Data from this season was truncated to included all three collared wolves and used in the data analysis. The estimated size of the Fishtrap pack during the study period was determined by telemetry surveys, howling surveys, and snow tracking. In 2004, the pack consisted of at least 12 individuals, and in 2005 there were at least 9 pack members.

Telemetry surveys varied in length from one to five hours, during which I drove through the pack's territory on logging roads. Occasionally, surveys were conducted by walking or on an All Terrain Vehicle. Surveys occurred in both daylight and nighttime hours that varied throughout the seasons, and throughout most of the 24-hour cycle. The exception was between the hours of 0300 and 0800 in which only several surveys were conducted.

I relocated collared wolves by triangulation of radio signals using a handheld yagi-type directional antennae. Triangulations usually involved three azimuths, but occasionally weather conditions and topographical features limited this process to two azimuths. In either case, the bearings were plotted onto 7.5 minute topography maps (scale 1:24,000) in the computer application Topo!. When three azimuths were used, the wolf location was plotted inside of a small triangle, most of which were ≤ 1.3 km^2. GPS locations were obtained by estimating the center of the polygon and using the coordinates generated by Topo!. Using this method, the wolves were found either kilometers apart or within ≤ 0.6 km of one another. This natural break in the data helped determine pairing classifications. In some instances, such as at the den site, individual collared wolves could occasionally be found up to 0.6 km away from the den entrance where other collared wolves were located. The same occurred at rendezvous sites, in which some wolves congregated while others were a short distance away. This information was found using radio telemetry as well as howling surveys. So I kept the distance of ≤ 0.6 km to define when collared wolves were paired to accommodate the movement of the wolves during telemetry surveys, such as walking around the immediate area. Therefore, pairing did not necessarily mean the collared wolves were standing next to each other, but rather they were in the same immediate vicinity. This was analogous to human family members at home, but in their rooms, or visiting together in the living room, etc. The same logic applied to when the wolves were traveling. If they were less than ≤ 0.6 km apart and moving in the same direction, they were considered paired. Pairing combinations were documented throughout all seasons of the year and defined as adult female-adult male (AF-AM), adult female-young female (AF-YF), adult male-young female (AM-YF), and all three collared wolves found together at the same location (AM-AF-YF).

For data analysis, an annual cycle was divided into three "wolf seasons" in which the collared wolves' movements were based on several contexts: denning, rendezvous sites, and nomadic (when the wolves roamed their territory throughout the winter with that year's litter). During the study period, each of these seasons was documented twice and defined as Den 04 and Den 05 (denning), Ren 04 and Ren 05 (rendezvous sites), Nom 04 and Nom 05 (nomadic). Because the surveys were conducted on almost a daily basis, the data showed a distinctively different pattern of wolf locations within a day or two after the end of one season and the beginning of another, therefore, seasons were easily distinguishable. Because the collared wolves monitored in this study were all from the same pack, they did not represent a sample of the greater wolf population in northwest Montana. Consequently, data analysis was limited to mostly descriptive statistics, along with a non-parametric chi square test.

Results and Discussion

Over the 603-day study period, 532 telemetry surveys were conducted throughout the Fishtrap pack's known territory. One or more collared wolves were found in 437 (82%) of these surveys, which were used in the analysis. Significant differences in pairing occurred throughout the seasons (χ^2=79.95, *df*=30, $P \leq 0.001$), and when viewed as a percent of observed pairings (Figure 1), the most common occurrence was when all collared wolves were found together (AM-AF-YF). Overall, this category averaged 31.0% of possible pairings throughout the study period (Figure 2). The other pairing categories were missing at least one collared wolf, which raised the possibility that perhaps the missing wolves were actually present and the radio telemetry equipment did not pick up their signals because of local topography, the wolves were behind a knoll, or other environmental factors. Although this scenario was possible, it seemed unlikely. At least five to six telemetry stations were conducted in the immediate area where collared wolves was found, and spaced over two to three miles. This would have eliminated most topographical features that would have blocked a collared wolf's signals. In addition, if I continued to search for the other collared wolves, I found them at other locations 40% of the time. If I didn't find them, they were either in areas I didn't search, or the telemetry equipment missed their signals. In the AM-AF-YF category, all three collared wolves were accounted for and found together in the same vicinity, so there was no problem with the telemetry equipment potentially missing their signals, unless all three collared wolves were missed at the same time. Again, this seemed unlikely given the previous explanation.

At the beginning of the study period, I assessed the accuracy of the telemetry azimuths to determine if they could be used reliably to classify the collared wolves as paired. I did this by comparing them to azimuths obtained from howling surveys which were known to be accurate. For example, in June 2003, I found the Fishtrap pack's den site by using howling surveys. I accrued numerous triangulations of the wolves' responses, including those of the pups. When the wolves vacated the den area to begin their rendezvous site season, I used the handheld GPS unit at the den site to determine the den's precise location. The majority of howling survey azimuths were within the uncertainty range provided by the GPS unit, which was ±7.6 to 45.7 m. Therefore, I considered the azimuths from howling surveys to be accurate. I then compared the azimuths obtained from telemetry surveys at the den area with those from the howling surveys. The majority of telemetry azimuths were within ±1 to 2 degrees of the howling azimuths, with occasional extremes of ±3 to 4 degrees, or an exact match. Both the telemetry and howling surveys were conducted at a distance of 0.5 to 0.8 km from the den site, and the uncertainty of the telemetry azimuths fit well within the definition of when the collared wolves were considered paired. So the telemetry azimuths were found to be accurate enough to at least determine pairing.

Of all the possible pairing combinations, the AM-AF-YF pairing was the most significant, because it was the category on which the study's hypothesis was based. It was the only category in which all collared wolves were found together. Had the pack been fully assembled at that time - and no assumption was made that they were - then a fully assembled pack could have only occurred in 31% of the surveys or less, on average (Figure 2). Although the percent of surveys did not necessarily equate to the exact amount of time the collared wolves spent together, it did sample the degree in which the entire pack was potentially assembled at particular moments in time, and over almost a 2-yr period. This result suggests the pack spent the minority of time together throughout the year, although the exact amount of time remains unknown.

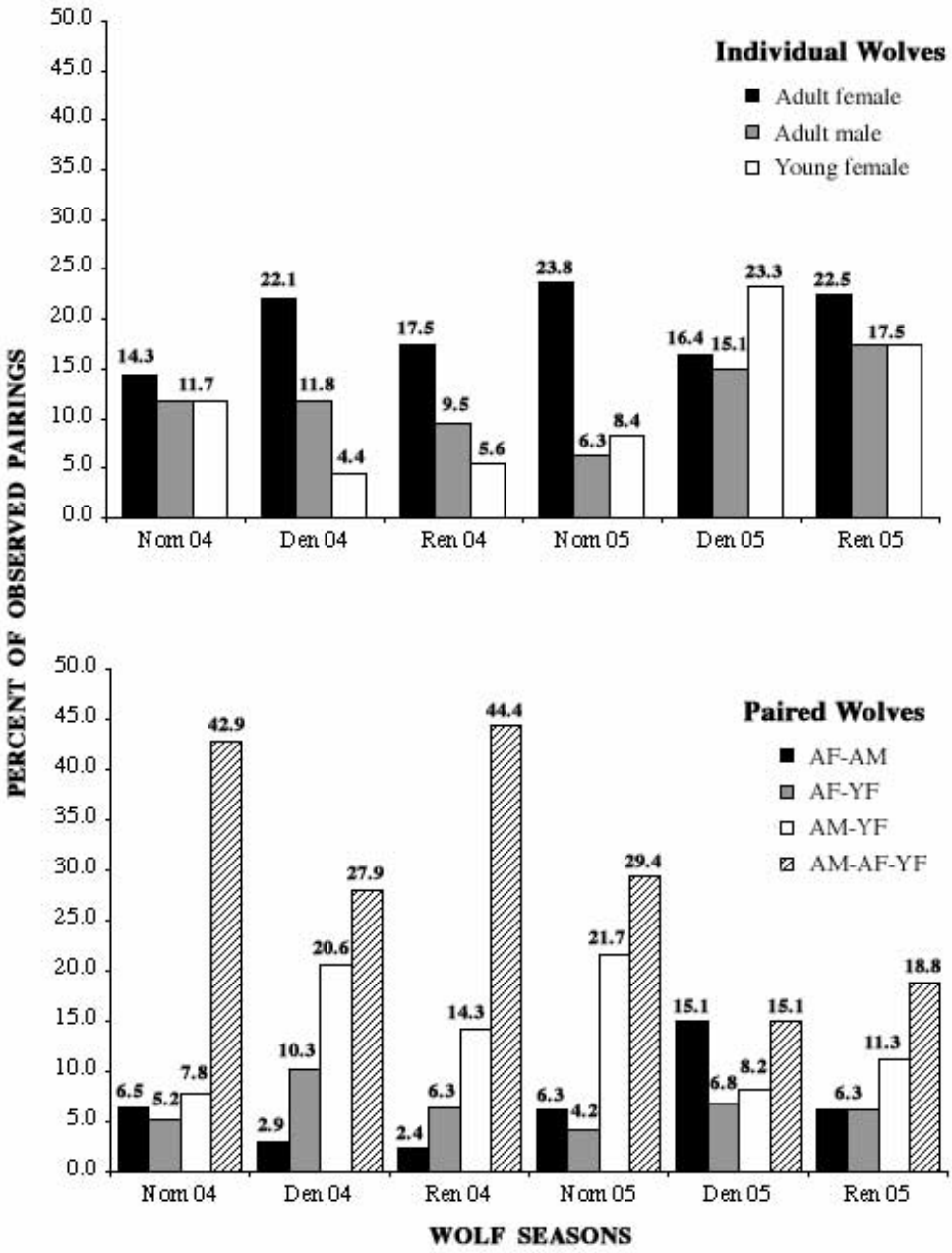

Figure 1. Percentage of observed pairings found over wolf seasons. The number above each column is the percentage for that pairing. From the 357 surveys represented in the graph, there were 567 pairings, ranging from individual collared wolves found in separate locations to all possible pairing combinations: adult female-adult male (AF-AM), adult female-young female (AF-YF), adult male-young female (AM-YF), and all three collared wolves found together at the same location (AM-AF-YF).

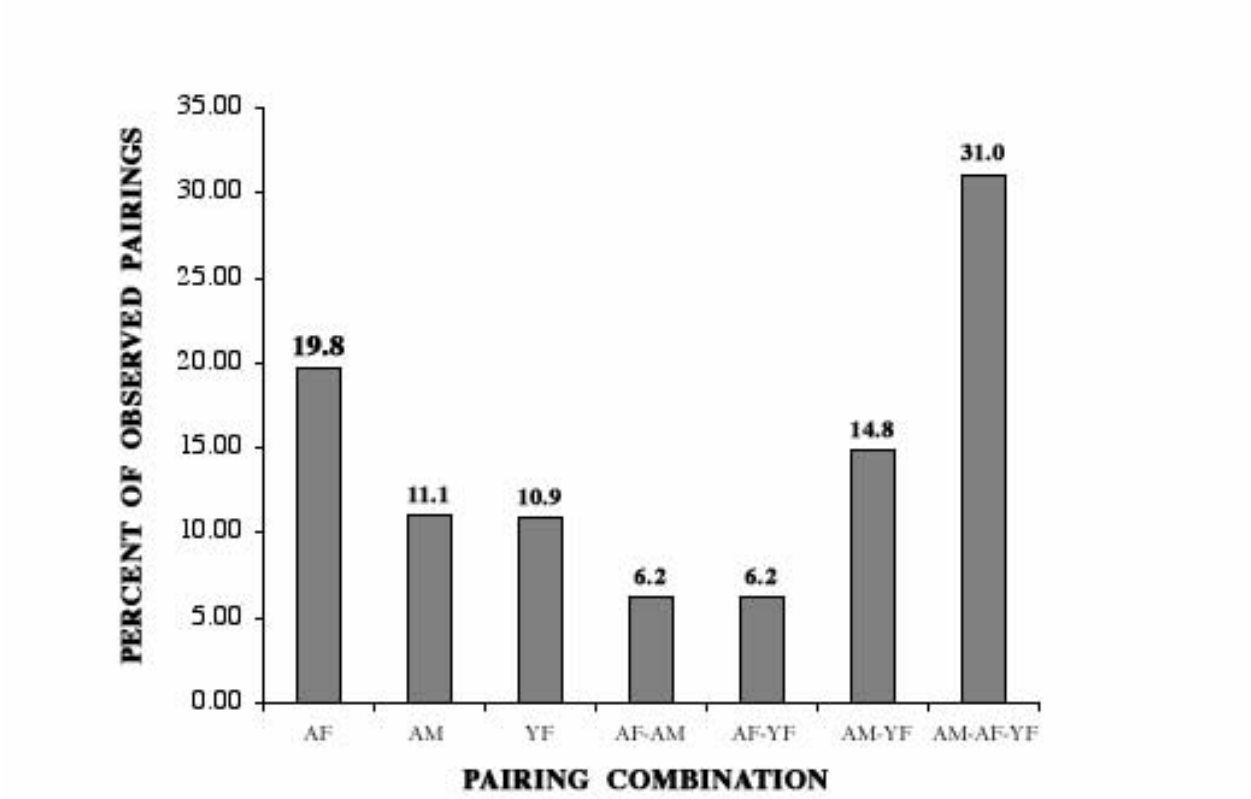

Figure 2. Average percentage of observed pairings found throughout all seasons of the study period.

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Finite and Infinite Populations in Biological Statistics: Should We Distinguish Them?

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Abstract: The paper discusses necessity of distinguishing finite and infinite populations in statistical research, with a focus on biological applications. It is shown that statistics for these two types of populations is different and that lack of proper understanding of this issue may do much harm. [The Journal of American Science. 2008;4(1):59-62]. (ISSN: 1545-1003).

Key words: statistics, survey sampling, inference, interpretation.

1. Introduction

There are finite and infinite populations. Both are studied in biological sciences. Surprisingly, there are people, both statisticians and those who apply statistics to interpret results of their research, who are not aware of it. This is a worrying situation because finite and infinite populations are, basically, different and usually should be approached in completely different manners. It is, therefore, important to be aware of it and to be able to distinguish the two statistical philosophies: that related to a finite and to an infinite population.

Whether or not infinite populations exist is a topic for a broad discussion. The main argument of those who do not accept infinite populations is that the universe is finite, so there is no possibility to consider an infinite number of anything. However, for simplicity, in this paper we assume that an "infinite" population approximates the imaginary infinite population when a number of elements of this population is unimaginable. This approximation is necessary to apply classical statistics and interpret properly the results obtained. Hereafter, we take no notice of this approximation and consider finite and infinite populations, keeping, however, this approximation in mind.

There have been not many people who worked on both types of populations. One of them was Jerzy Neyman, a distinguished Polish statistician, whose works on infinite (e.g., related to confidence intervals and hypothesis testing; see, e.g., Neyman and Pearson 1928a, b and 1933a, b, Neyman, 1942) and finite (e.g., related to stratified sampling; Neyman 1934) populations are known among statisticians throughout the world, and whose contribution to statistics of both finite and infinite populations is indisputable. However, one can feel alarmed by the fact that many researchers are not aware of the existence of these two types of populations.

At the very beginning of an investigation, one has to decide whether one considers a finite or infinite population. This decision may sometimes be difficult. It may, and likely will, affect a way of collecting data (the experimental design); data handling at the estimation stage of the study, including a choice of models and statistical methods to be used; and, last but not least, interpretation of the results obtained. Applying classical statistics for a finite population may do more harm than good. Sometimes it may, however, occur to be a reasonable solution, but it must not be a matter of luck, but the reasonable decision of a statistician. Therefore, it is important for a statistician to be aware of which type of population he or she is dealing with.

The aim of this paper is to discuss the importance of distinguishing finite and infinite populations. For the sake of simplicity, let us base on biological studies, and let us keep in mind that the discussion we provide relates also to other studies. Sometimes a decision on the type of population to study may be tricky and have quite an impact on the results and interpretation. I will not discuss statistics for both cases—there are plenty of textbooks and papers on both of them. I will just show that both philosophies are distinct, and why it is so. I hope to convince the reader that this knowledge is important to apply statistics correctly. The paper is directed to those who study, learn or teach statistics of either infinite or finite populations, and to those who apply statistics. Whichever population type a reader deals with, some parts of the paper will provide him/her just basic information, and the remaining should be interesting for him/her. Nevertheless, because most of the classical statistics deals with infinite populations, I will slightly focus on finite populations and show that finite-population studies are quite often in various fields of biology.

2. When do we consider finite and infinite populations?

Whether one should consider a finite or infinite population depends on a study's aims. Based on them, it normally should be obvious which type of population should be defined and which population the interpretation should be linked to. Below, both types of populations are briefly described in relation to biology.

Infinite populations. Classical biological, economic, engineering and other experiments, in which one aims to study a particular process or processes for a specific population or populations, deal with infinite populations. In such studies, inferences and interpretation have a general meaning that helps understand the processes in the

population. A classical agricultural example is a fertilization experiment, whereby one studies whether fertilizer combinations provide similar yield of a crop species. Here, a number of populations considered is equal to a number of fertilizer combinations, and each such a population is infinite in nature. The elements of the populations may be plants of the cultivar or genotype of a species (e.g., rice, wheat, etc.) grown under the specified conditions, fertilizer combinations in our case. However, the populations should be defined more precisely, because the experiments almost always refer to some (broader or narrower) environmental conditions and agrotechnical practices.

There may be a lot of various infinite-population studies in biology, such as studies on resistance of a plant to a pathogen; occurrence of a species in a high-moisture soil environment; or co-existence of two eriophyoid mite species on *Pinus sylvestris* in a National Park. All of such studies have this similarity that they aim at *general* interpretation of the biological processes of study, irrespective of a time point.

Finite populations. Each population the elements of which exist in a particular time is finite. However, some populations are so large that one is unable to imagine all its elements. For example, a number of plants of rice in the world harvested in 2005 is impossible to be counted so may be thought of as an infinite population. On the other hand, a population of farms in India on 1 November 2006 (or November 2006) is imaginable, countable, and as such, should be thought of as a finite population. Note that a finite population may be considered in several occasions; it is still, however, the finite population the elements of which may be changed, removed, or added.

The most common finite-population studies are those related to official statistics. It is enough to mention here agricultural censuses (Wanke 2003) and sample surveys (Kursa and Lednicki 2006) conducted by statistical offices. The censuses/surveys aim to provide information on agricultural production and market in the country and its provinces.

Questionnaire surveys are another example of a finite-population approach. Taking into account only chosen biosciences, they may be useful in surveys on various food science and epidemiology problems (e.g., Willett et al. 1985, Khokhar and Fenwick 1994, Richardson-Harma et al. 1998, Sandström and Faergemann 2004), agricultural practices and production (e.g., Jackson-Smith 1997, Nazarko et al. 2003, Herzog et al. 2006), ecology and wildlife biology (Seiler et al. 2004, White et al. 2005, and the citations therein), not to mention health sciences (e.g., Sammarco et al. 2001). One must be aware, however, that questionnaire samples may also relate to infinite populations (which depends on how a researcher defines a sample and a population). There are many methodological problems connected with questionnaire surveys, like difficulties in obtaining a random sample, high non-response rates, and the like (Särndal et al. 1992).

The following are two finite-population examples from botany/phytosociology. Studying a particular site for occurrence of a particular plant species, a researcher takes samples from this site (Elzinga et al. 1998). The population studied is the site, that is, the geographical area, and the variable of interest—occurrence of the species. A parameter to be estimated may be, for example, a total number of occurrences of the species in the site. If, for any reason, the whole site area may not be surveyed, the samples are taken subject to a chosen sampling design, and the parameter is estimated based on formulas appropriate for the design.

Consider another survey in which one aims to study occurrence of a plant species in National Parks of a country. From each National Park, samples from several randomly chosen sites (the National Park is/may be divided to) are taken to determine whether this species occurs there. This is a stratified sample where a National Park constitutes a stratum from which a sample is taken. A sample from each National Park (stratum) may be taken using simple random sampling without replacement (e.g., quadrat samples from the whole stratum [National Park]—see Elzinga et al. 1998) or more complex designs (especially when the National Park is geographically large) such as a stratified or multistage sampling design. If the complex designs are used within National Parks, the whole design (i.e., for the whole population) becomes complex since it is stratified sampling with different designs within the strata.

Producer groups (such as a group of organic or ornamental farms) may be surveyed to examine their adaptation to the group's requirements or to determine the group's characteristics (e.g., productivity, agrotechnical practices, environmental conditions, etc.). An animal species in a zoological garden(s) may be studied for infection with a pathogen. Apples in an orchard may be studied for maggoty. Deer in a district may be studied for disease infection. Animals in a country may be studied for disease symptoms after a national epidemic. A much longer list of examples of finite-population biology research might be provided here, but the above-given show the importance of ability to distinguish finite and infinite populations in biology.

3. Why is it important to distinguish finite and infinite populations?

In previous sections, differences between finite and infinite populations have been pointed out and the examples of both types of populations have been given. Now it is time to show why a researcher should be able to distinguish finite and infinite populations.

Classical, infinite-population statistics assumes that a sample be simple, which, basically, means that all the sample observations should be stochastically independent and follow the same theoretical distribution. In

case of sampling from a finite population, a sample is simple only for simple random sampling with replacement, which, practically, is not the case in any survey and has only theoretical importance. Note that limiting distributions in a classical sense are concerned with a simple random sample. These limiting distributions, proper for infinite populations, had to be adapted to work for finite populations (see, e.g., Hájek 1960). This is mainly because samples from finite populations are taken using complex sampling designs and schemes, which makes the samples non-simple. Not to mention more complex sampling designs, such as stratified or multistage sampling, even a simple random sample taken without replacement is non-simple because observations for the population units sampled are dependent. For most finite-population sampling schemes, estimators for simple population parameters, like mean or proportion, are different from those for simple random sample (which work for infinite populations). In addition, formulas for variance of the estimators differ for the two cases, which, in turn, makes the confidence intervals for the estimators also differ. Testing hypothesis for finite populations under non-simple sample is also different from that for infinite populations, and even classical tests need to be modified. Therefore, any classical statistical methodology that is correct for infinite populations is usually not correct for finite populations.

What has already been said relates to statistical theory and application. However, statistics aims to provide tools for researchers to interpret the results of their studies. And here mixing up finite and infinite populations may cause most harm. This is because interpretation about finite populations is based on completely different philosophy and has completely different meaning than that about infinite populations. For finite populations, one usually attempts to picture processes one wants to study in this population, usually in relation to a particular time. This makes the interpretation has no general meaning (as it usually is for infinite populations), but simply refers to this particular set of elements in this particular time. For infinite populations, one may provide interpretation having a general meaning, which is not generally linked to particular elements of the population and to a particular time point. Knowledge of processes in an infinite population may, for example, help the researcher understand some general processes in a population or several populations (e.g., yielding of a crop species in particular environmental conditions; resistance of a cultivar to a pathogen; influence of fertilization on a yielding level; occurrence of a species in a high-moisture soil environment; co-existence of two eriophyoid mite species on *Pinus sylvestris* in a National Park in general; etc.). Inferences based on finite populations are of different character, and usually describe the population in a particular time (e.g., average yielding of a crop species in a country in 2006; resistance of a cultivar to a pathogen in the particular region of a country; influence of fertilization on a yielding level in a population of farms in a region in 2006; occurrence of a species in a National Park in 2006; co-existence of two eriophyoid mite species on *Pinus sylvestris* in a National Park in 2006; etc.).

4. Conclusion

Distinguishing finite and infinite populations is of importance for biostatisticians because they have to choose appropriate methods to apply, and for researchers applying statistics because they have to know how to draw inferences about their populations. Without this basic knowledge, results and interpretation of a research may occur to be false and unreliable, and convincing the researcher that one should consider finite-population or infinite-population approach might be difficult. It is also possible to find a statistician who makes light of an approach he or she does not work on. This is alarming because none of the approaches is better than another: they both are important in various sciences, including biology, and they should not be mixed up.

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Asian Elephant (*Elephas maximus*) and Riparian Wildlife Corridors: A Case Study from Lesser-Himalayan Zone of Uttarakhand

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Abstract: Developmental activities are increasingly recognized as the cause of habitat fragmentation and shrinking of wildlife corridors. This study assessed the status and importance of the Chilla – Motichur and Khara – Anjani riparian wildlife corridor in northern India. We review the potential involvement of developmental activities in the recent past, which has affected frequent movement of Asian elephant (*Elephas maximus*) within their home range. These corridors linking the Chilla forest with Motichur forest and Shyampur forest are subjected to severe anthropogenic pressures. Livestock grazing, fuel wood collection and movement of people are the major activities observed in both corridors. These anthropogenic activities have substantially affected the movement of elephant within their home range and have led to the loss of forests connectivity. The long-term effects will include genetic isolation, habitat fragmentation within the same forest and enhancement in the human-elephant conflict in adjoining areas. Genetic isolation of elephant populations may also increase the chances of replacement of inter-breeding to intra-breeding, and thereby reduce the population persistence even for wide ranging wildlife species. Additionally, minimization of all kinds of biotic pressures in the corridor areas and providing higher protection to riparian wildlife corridors are highly recommended. [THE JOURNAL OF AMERICAN SCIENCE. 2008;4(1):63-75]. (ISSN: 1545-1003)

Key Words: Asian elephant, *Elephas maximus*, riparian wildlife corridors, conservation, developmental activities, Rajaji National Park.

Introduction

The Shivalik foothills are one of the world's most spectacular landscapes, encompassing the tall grasslands and the *Shorea robusta* (Sal) forests. This entire belt is natural home of Asian elephants (*Elephas maximus*) besides many other wild animals like *Panthera tigris* (tiger), *Panthera pardus* (leopard), *Melursus ursinus* (Sloth bear), *Hyaena hyaena* (Hyaena), *Muntiacus muntjak* (Barking deer), *Axis axis* (Spotted deer), *Cervous unicolor* (Sambhar), *Sus scrofa* (Wild boar), *Ophiophagus hannah* (King cobra) etc. It is interesting to mention here that this protected area is western-most limit of Asian elephant, tiger and king cobra.

The Shivalik landscape is one of the last few places in the world where elephants exist and offers urgent need for conservation. This protected area in India's lesser Himalayan region falls under sub tropical moist deciduous forest type with extensive stands of *Shorea robusta* (Sal), *Mallotus phillipinensis* (Rohini), *Acacia catechu* (Khair), *Adina cordifolia* (Haldu), *Terminalia bellirica* (Bahera), *Terminalia* tomentosa (Sain), *Ficus bengalensis* (Bar), *Dalbergia sissoo* (Shisham) etc. in its premise besides many other important fodder plant species. From conservation point of view it appears to be India's one of the most successful national park and its development has helped to boost the population of Asian elephant in their natural habitat.

The natural continuous forest ranges of India has been broken up into many parts due to agriculture, urbanization, increasing road traffic and development related activities as well as other anthropogenic activities. This situation creates many problems for various organisms living in forests. Genetic isolation, limitation of dispersal and migration and the decline of populations of animals requiring large territories are the most common problems connected with fragmentation of forests and other components of the environment. A serious threat was also recorded to European wildlife resulting from the dynamic development of a transportation infrastructure network within the Trans-European Transportation Network (TEN-T) programme. This transportation network disrupts migration corridors of large terrestrial mammals

and causes a fragmentation of their environment on a scale not previously recorded (Nowak and Myslajek, 2005).

Elephants have suffered most grievously as compared to other wild herbivores on account of loss of their natural habitats and corridors as they require larger space for their various activities. Increasing human pressure inside the deeper forest regime and developmental projects has given rise to management and conservation problems. These include crop raiding by elephants outside the park area and even some human fatalities. The human population around the Rajaji National Park alone has doubled during past one decade and rapid urbanization and industrialization has resulted in the loss of many forestlands to townships and thereby increasing the major problem during the recent past. The present note is a part of our long term study on the behavioural biology of Asian elephant in sub tropical moist deciduous forests of India.

Methods

Study area

Rajaji National Park [29^0 15' to 30^0 31' North Latitude, 77^0 52' to 78^0 22' East Longitude] is spread over an area of 820.42 Km^2 in and around the Shivalik foothills, which lies in the lesser Himalayas and the upper Gangetic plains (Figure 1). Spread across Hardwar, Dehradun and Pauri districts of Uttarakhand state, Rajaji National Park (RNP) has been designated as a reserved area for the "Project Elephant" by the Ministry of Environment and Forests, Government of India with the sole aim of maintaining the viable population of Asian elephants in their natural habitat. The Shivalik foothills offer the most prominent geomorphic features of this tract. The river Ganges has cut across these hills at Hardwar. The Chilla forest area of the RNP lies in the east of the river Ganges and is attached by the Garhwal Forest Division. The study is ongoing in Hardwar (District-Hardwar), Chilla (District-Pauri) and Motichur (District-Dehradun) forest ranges of the RNP. Besides, few of the adjoining forest areas (Shyampur forest range of the Hardwar forest division) were also incorporated in this study. The altitude lies between 302-1000 m asl. The study site falls in sub-tropical moist deciduous forest type and landscape comprises of undulating hills with plain patches.

Data collection

For studying the movement pattern of elephants and analyzing the impact of developmental activities on riparian wildlife corridors and on elephant's movement, the Chilla – Motichur and Khara – Anjani corridors were surveyed in depthly from 2000 to 2007. The traditional movement tracks along with feeding grounds of elephants were searched and observed directly. Different forest blocks of concerned forest ranges were chosen one after another sequentially and searched for elephants for about 10 – 12 hours (depending upon weather conditions) in a single day search. The observations started at early hours in the morning being the best time to search and observe the elephant in open areas and four hours in the afternoon i.e. before the sunset. The data collected is as part of the animal monitoring activities. The daily record is based on direct sighting of animals, indirect evidences like feeding sign, footprints impression time and fresh dung piles. The direct sighting were noted in duly prepared proformas, recording the group composition, age and sex, if observed in groups and also the place of sighting, time and vegetation type. Besides, villagers of adjoining areas, Gujjars (where available), staff of forest department, the researchers from various scientific institutions and non-government organizations and other individuals working on this problem, were also interviewed. Field binocular was also used for observing their movement behaviour without disturbing the animal from an adequate and safe distance.

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

Results

Riparian wildlife corridors

Riparian zones are important migration corridors for migratory animals and due to annual flowing of water in various streams, these corridors also tend to be rich in nutrients and very diverse in both structural and vegetation characteristics, thus creating and sustaining a diversity of micro-habitats capable of

supporting many different wildlife species. Riparian corridors may provide safe travel routes between habitats reducing the negative impacts of forest fragmentation on wildlife populations. Riparian zones support high soil moisture and associated moisture-loving vegetation. These areas of high biological diversity often provide the necessary elements for survival. Because riparian corridors represent the area where upland and aquatic habitats merge, well-developed riparian corridors tend to contain a relatively high degree of wildlife diversity, having both upland-dependent and aquatic-dependent wildlife species present.

RNP comprises of several annual and perennial water streams and few of them are traditionally working as natural corridor for elephant's long-term migration. These riparian wildlife corridors generally comprise of mixed vegetation type and several important grass species were also present in between its edges. Ghasiram, Mundal, Soni, Dogadda, Siddh and Pili water streams and Binj and Rawasan rivers are few of the important riparian wildlife corridors, through which elephants interchange the forest zones. During hot period elephants use these corridors for resting purpose and for performing their movement towards river Ganges.

The corridor suitability was compared and analysed with different variables as settlements, road access, continuous forest patches, water bodies, religious places inside the protected area, fodder species and elephant movement over the corridor. It was observed during the course of study that presently only two major corridors, Chilla – Motichur and Khara – Anjani corridor are being utilized by elephants for their long-term migration. Both of these corridors are having rich fodder species in their premise and blessed with several annual rivers. Additionally Motichur, Song and Suswa rivers might act as alternative migratory route for elephants.

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

Chilla forest range of the RNP and Shyampur forest range of the Hardwar forest division are well connected forest zone therefore; elephant utilizes both of these forest ranges round the year. These forests also provide connectivity to the Lansdowne forest division for elephant movement towards Corbett National Park. But during the recent past number of construction work along with huge amount of anthropogenic activities has restricted the frequent movement of elephants within their home range. It was observed during the present investigation that most of the elephants in these forest pockets do not represent their long-term migration mainly because of anthropogenic barriers present in between the forest areas. In the course of this study we have encountered many elephant groups and solo bulls and on the basis of their physical identity it was inferred that they are only restricted to move to some extent. In this area we have reported two major corridors, which are currently in use by the elephants.

The motor roads, which are adjacent to the forests like Hardwar-Dehradun National Highway, Hardwar – Bijnor National Highway, BHEL roads etc. have heavy traffic pressure. As per a preliminary study, the average number of vehicles passing on Dehradun-Hardwar road per day is 7,929 and all the wild animals, including elephants, are not in a position to cross this track at any time due to the presence of heavy traffic (Singh and Sharma, 2001). Same situation is with other corridors present adjacent to the RNP area. Kotdwar – Lansdowne road runs parallel to the river Kho and crosses the Rajaji-Corbett corridor, the major movement track of northwestern elephant population between the Yamuna and river Sharda. This road serves as the major transport link between Pauri town and Kotdwar area. The presence of traffic on the road, construction of steep retaining walls and the presence of human population along the entire corridor area have almost restricted the migration of elephants (Johnsingh and Williams, 1999).

Chilla – Motichur corridor

This corridor is about 7 kilometers long beginning at the tail end of Mundal valley and links the Chilla forest range on the eastern portion to the Motichur forest range on the west. Elephants used this corridor traditionally but currently elephants are not utilizing this corridor regularly. Sometimes solo bulls were

reported to follow this route and very occasionally group movements was also observed but only up to the island area, which is situated in between river Ganges. This corridor area comprises of many fodder plant species like *Mallotus phillipinensis* (Rohini), *Acacia catechu* (Khair), *Dalbergia sissoo* (Shisham), *Tectona grandis* (Teak), *Zizyphus mauritiana* (Ber), *Aegle marmelos* (Bel), *Ficus bengalensis* (Bar), *Ficus glomerata* (Gular), *Grewia oppositifolia* (Bhimal), *Bombax ceiba* (Semal), *Lannea grandis* (Jhingan), *Bauhinia variegata* (Kachnar), *Lagerstroemia parviflora* (Dhauri), *Kydia calycina* (Pula), *Syzygium cumini* (Jamun) and *Ehretia laevis* (Chamror). Besides, elephants also use various grasses and shrubs as their food resources, which include *Dendrocalamus strictus* (Bamboo), *Helicteres isora* (Kapasi), *Saccharum munja* (Pula), *Saccharum spontaneum* (Kans), *Cynodon dactylon* (Doob Grass), *Eulaliopsis binata* (Bhabhar Grass) etc. Presently Gujjars (tribal people) are completely relocated from Chilla and Motichur forest ranges of the RNP but the programme for resettling them to rehabilitation site from Gohri forest range is still ongoing.

Because, few of the forest pockets of the Gohri forest range also falls under this corridor area, therefore, it will be needed to resettle the Gujjars from this forest range. Elephant's movement was restricted in this area mainly due to biotic pressure and cattle grazing inside the forest area. Before 2002, we have observed large herds of elephants (maximum 38 elephants) in Kunao forest beat and in Binj river but presently their large groups have been subjected to dispersed in small ones.

There are four islands within the river in this region, which form part of the park. However, in the 1950's and 60's a number of developments, having drastic effect of land use came up on the western bank. The BHEL set up a major plant to the west of Ganges in the southern part of this trans-Ganga corridor for wildlife. Later the IDPL set up a large factory in the northern part of the corridor, also to the west of Ganga. The Army for a large ammunition dump has utilized the area in between and subsequently some remaining land was given away for the rehabilitation of Tehri Dam oustees. Thus, on the west bank most of the corridor stands diverted and rendered unusable.

On the east bank yet another major development activity has all, but destroyed the ecological corridor. A hydro-electric power project was set up in the 1970's. A barrage was constructed across the Ganga at Kunao just outside the park in the middle of the northern boundary. From here a deep power channel runs parallel to the east of Ganga for about 14 kilometers up to Chilla where the powerhouse is located. Although there are a couple of narrow bridges over the channel, these are not generally used by the animals. There have been cases of wild animals and even elephant mortalities, in attempts to cross these bridges.

Rarely elephant bulls and the group are known to cross, but otherwise there is complete isolation between western and eastern components of an internal ecological unit. The presence of army camp in the elephant corridor has also adversely affected the movement of wild animals. Besides, Khand village (48.5 hectares) is also located in the elephant migration corridor and is an obstacle to their movement. Ganga Bhogpur and Kaudia villages are also situated in eastern side of river Ganges and peripheral to Ganga canal. This area also lies under corridor area and elephants sometimes used to move in these villages in search of cultivated crops. Dudhia forest beat (island) due to its proximity to the Haripur Kala village is one of the most sensitive area as far as elephant casualties are concerned. During the study period occasionally, the movement of only solo bulls was observed in this part of the park. Group movement is almost restricted in this forest pocket mainly due to anthropogenic activities. Despite the fact that Dudhia area is rich in *Dalbergia sissoo* (Shisham) and *Acacia catechu* (Khair) forest, the preferred food item of the elephants. Besides, few of the fodder grass species like *Saccharum munja* and *Desmostachya bipinnata* are also grow in profusion in this area.

A major developmental project, which has divided the Rajaji – Corbett elephant habitat into two regimes is the 14 kilometers long Kunao – Chilla power channel, which was constructed on the east bank of river Ganges. In the early 1970s, this canal is 22 meter wide, nine meter deep and with full flow of water. The side of the canal is at an angle of 45^0 and cemented except for 500 meter; therefore, do not offer foot-hold to the elephants (Kumar, 1995). There are three places at which bull elephants and groups cross the power channel and go to Ganges.

1) Binj water stream – in Gohri forest range.
2) 60 meters long aqueduct connecting Dogadda with Ganges – in the edge of Gohri and Chilla forest ranges.
3) Bridge across the power channel, 2 kilometers from Chilla – in Chilla forest range (Soni Shroth).

In summer, bulls were observed more to use these tracks for their movements towards river Ganges but occasionally groups also follow this route, when their movement is towards western direction. Elephants generally use the Ghasiram water streams and Soni shroth bridge for interchanging these forest zones.

Khara – Anjani corridor

This corridor is about 5 kilometers long and connects the Khara forest beat of the RNP with Chandi, Siddh and Anjani forest beat (Shyampur forest range) of the Hardwar forest division. Presently this is one of the major corridor, which is regularly in use by elephants. The internal corridor area consists of the fodder species that Chilla forest comprises. The Anjani forest beat is attached with river Ganges and the forest comprises of *Acacia catechu, Dalbergia sissoo, Bombax ceiba, Helictres isora, Tectona grandis* and *Ficus bengalensis* trees. Besides, few of the important grass species are also present in the island area. During the last three years, state Government has constructed about four flyovers in Hardwar – Bijnor National Highway and due to this about 18 kilometers forest stretch along both the sides of the highway has got destructed mainly due to huge amount of anthropogenic activities. Besides, agricultural expansion adjoining to river Ganges has lead to the loss of forest wealth, which is also hindering the traditional movement of elephants.

It was observed during the present study that mostly adult bull elephants are utilizing this route. Sometimes few of the male elephants through associating, follow this route to enter the Anjani forest, which is peripheral to river Ganges. Elephants cross the national highway (Figure 2) and river Ganges in evening hours and re-enters to forest area in early morning hours. During this long journey elephants sometimes spent more time to feed on the plant species those are present in the island situated in between river Ganges. This forest stretch is one of the major corridor for elephant movement and presently has got disturbed mainly due to habitat loss around the national highway. Besides, elephants also utilize Gaziwali bridge, Shyampur bridge and Pili bridge those are situated over east Ganga canal for their outside movement and to feed on the cultivated crops in nearby villages. It was also observed during the study period that elephants also use the Ganga canal for fulfilling their water requirements.

During the study period all the villages suffering from crop raiding have been investigated. The affected villages are Jagjeetpur, Mishrpur, Panjneri, Ajeetpur and Jaipota in the western side of the conservation area and all these villages are situated peripheral to river Ganges. Villages Kangri, Ghaziwali, Shyampur, Sajanpura, Pili and Rasiabad are located peripheral to forest area and national highway whereas villages Gaindikhata, Lahadpur, Chiriapur, Vasuchandpur and Naurangabad are also situated adjacent to the forest area and national highway on south western direction of the conservation area. The villages along the river Ganges are situated on land that was once part of the elephant's home range. Therefore, the increasing elephant – man conflict is unfortunate but inevitable. The electric fence erected along with these villages and river Ganges has presently got damaged due to lack of proper maintenance. It was observed and inferred from the present investigation that elephants are utilizing their traditional feeding grounds in few of the areas, which are presently denied to them and are replaced by human settlements.

Once, all of these villages were better known for sugarcane cultivation and production but from last four years few of them have totally bunged the cultivation of sugarcane mainly due to fear of loss by elephants. These crop raids are the indications of attempts by some of the elephants to use their traditional routes leading to their feeding grounds, which are now denied to them and are replaced by human settlements. Gujjars are still living in Hardwar forest division and it was observed that most of their deras (shelters) are present in this corridor area. Elephants generally follow Siddh shroth river stretch for interchanging the forest areas and most of the Gujjar deras were situated in this part. Besides, one temple was also located in this forest stretch and the pressure of workers and visiting devotees sometimes caused hindrance in animal movement.

In the adjoining areas of Hardwar – Bijnor National highway various stakeholders has constructed shopping complexes, check posts, shrines, etc. and all of these spots are working as a barriers as far elephant's movement is concerned. Elephant's movement was quite frequent near to east Ganga canal and irrigation road especially during night period because this area comprises of bushes of *Dendrocalamus strictus* (Bamboo), which is a favourite food item of elephants. Whereas during the day hour biotic pressure is quite more in this route and cattle grazing is also a very common phenomenon observed in this part.

Elephant's movement within riparian corridors

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

Elephants also use the Ghasiram and Mundal water streams for visiting to river Ganges especially when their local movement is frequent in and adjoining forest beats, which are attached to river Ganges along with few bridges, which are in Ganga canal of Chilla hydro electric power plant. Few of the groups were also reported to use the Shyampur and Chiriapur forest ranges of Hardwar forest division during rainy season as east Ganga canal is fulfilled with water during that period. It is interesting to mention here that currently only bull elephants are crossing this track whereas no groups were reported during last 2 years. As per last four year data, groups of the elephants were reported in the same area but rapid developmental activities has restricted the frequent movement of elephant's group towards river Ganga in this part.

Few of the major reasons affecting local movement of the elephants in rainy season are:

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

Impact of heavy floods during 2007

Initial observation suggested that a large extent of the Chilla forest was effected from the impact of heavy floods during July, August and September, 2007. On one side floods has affected the migrational pattern of elephants and on the other hand disrupts the natural regeneration potential of several grass species those grow especially in between dry river beds.

Effects on vegetation

Annual water stream beds inside the RNP area generally comprises of several fodder grass species like *Saccharum munja*, *Saccharum spontaneum*, *Desmostachya bipinnata* and *Cynodon dactylon*. All of these species are the favourite food item for elephants. Besides, few tree species those grow generally nearer to the Gangetic plains like *Acacia catechu, Dalbergia sissoo, Bauhinia variegata, Albizzia lebbek, Ehretia laevis* and *Lagerstroemia parviflora* were also damaged due to heavy floods. Mundal valley consists of larger feeding grounds (grassy patches) but this environmental event has destroyed whole of the area. Water flow has also caused damage to some extent in higher slopes of the protected area. Grass species which grow in profusion in higher altitude area like *Neyraudia arundinacea* were severely affected due to floods.

Effects on fauna

At the onset of winter (October onwards) elephant starts to migrate in lower forest pockets from high elevations but onservations suggested that elephant's migration rate may be slower as comparision to few previous years. It was revealed that few of the solitary bulls and groups, which utilizes whole of the area round the year, leaved the forest during the course of floods and migrated towards the adjoining forest of Garhwal forest division and Hardwar forest division. Even we have recorded the upper side movement of carnivores (*Panthera tigris* and *Panthera pardus*) and herbivores (*Muntiacus muntjak*, *Axis axis* and *Cervous unicolor*).

Developmental activities

1. *Agriculture expansion*

Increasing agricultural land use practices in the recent past have reduced the area that was once available to the elephants in Shivalik foothills. As wild foraging areas become increasingly less available to elephants, their depredations to the croplands is increasing regularly in many areas.

2. *Habilitation*

Human settlements in and around the park area have created the shrinking of elephant's natural paths. The human population around the RNP alone has doubled during past one decade and rapid urbanisation and industrialisation has resulting in the loss of many forestlands to townships and to various development related activities. Rapid enhancement in population born human settlement programmes includes the rehabilitation programme of the Tehri Dam evacuates, has lead to a very rapid, building construction work near to the forest area. And when a few constructions are build up, naturally the human beings inhabiting in these areas, starts clearing the nearby forest area for their biological, social, economic and aesthetic requirements.

3. *Fuel wood collection*

Collection of fuel-wood by villagers is one of the major problems, as they sometimes fell down the juvenile trees or shrubs.

4. *Grazing and fodder collection*

Grazing by the cattle of Gujjars (in few of the areas where they still exist) within the forest range and collection of the fodder species by them is also one of the major problem, as they lopped the tree (which are having seeds and young branches) even like *Ficus bengalensis, Ficus religiosa, Ficus rumphii, Emblica officinalis, Bridelia retusa, Anogeissus latifolia, Aegle marmelos, Dendrocalamus strictus, Bauhinia variegata, Grewia oppositifolia, Lagerstroemia parviflora, Oogeinia oogenensis*, *Garuga pinnata, Schleichera oleosa,* and *Lannea coromendelica*, which are crucial for frugivorous birds and mammals and sometimes they also fell down the small trees. Few of the villages those are peripheral to the park area also use the forest resources to meet out their energy requirements and their cattle also graze these feeding grounds frequently.

5. *Non-timber forest produce collection*

Collection of non-timber valuable forest production like *Eulaliopsis binata* (Bhabhar grass), *Kydia calycina* (Pula), *Aegle marmelos* (Bel) etc. are also the causative agents.

6. *Presence of unknown people*

At times unauthorized visits of local people were also reported loitering in the park area. Probably such people are of questionable identity and are responsible for acts of poaching. These types of anthropogenic activities are more commonly seen in those areas, which are attached to park boundary.

7. *Religious places within the protected area*

Religious places like Goddess Mansa devi, Chandi devi, Sureshwari devi and Bilkeshwar temple are in forest areas. The visiting devotees and workers of the above-mentioned temples hinder elephant's movement. Several times it was also seen that many people enter the prohibited areas of the park and indulge in nefarious activities. Sometimes they were reported to play cards, booze, roam here and there, burst crackers and throw stones to shoo away the elephants or other wild animals. There are many instances when religious banquets on large scale are organised. Hoards of visitors disturb elephants that came to drink water in the after noon hours. As during last decades the general economic condition of people has bettered, this has leaded to increase in the purchase power, social interactions, tourists and religious activities of the people at all levels (Joshi and Joshi, 2006).

More than 6-7 lacs people visit Mansa devi temple every year. In other temple more than 50,000 people visit annually. The crowd is seen especially in the Shivratri and Sawan Purnima fairs. As per a data more than twenty lack people has visited this year to Hardwar area on the occasion of Sawan Purnima fair. Many other religious places are also situated within the park area and it was inferred from present investigation that when elephant herd is on move nearer to these areas their frequent movement is hindered by presence

of local people. During the present investigation it was also observed that in few of the places elephant's time budget has changed, which has caused irregularity in their natural activities.

8. *Military complex*

Settlement of military complex (346.6 hectare) including an ammunition dump based at Raiwala in Dehradun district is situated in the center of the park and covered by park ranges from both the sides. This army complex is adjacent to the famous Chilla-Motichur corridor area of the park and presently this route is denied for elephant groups but occasionally adult solo bulls are known to use this area.

9. *Railway track*

Presence of railway track is another major problem, which impedes elephant migration and frequent movement within their home range. 19 elephants are killed due to train accidents since 1987 besides many other wild animals like leopard, spotted deer, python etc. This track is 16 kilometers long and comprises of sharp bends through which train drivers are unable to look the elephants from a safe distance and most of the accidents were occurred during night hours and in dry season (Joshi and Joshi, 2000).

10. *Increasing land for civil and developmental work*

The decrease in the extent to wildlife habitat is largely because of increase in human population and the resultant demand for more land. In many of the places those are peripheral to the park area, construction work is regularly on increase, which leads to the shrinkage of elephant's corridors.

13. *Running traffic*

The motor roads which are adjacent to the forests like Hardwar-Dehradun National Highway in Hardwar and Motichur forest ranges, Hardwar-Bijnor National Highway in Shyampur and Chiriapur forest ranges, Chilla-Rishikesh motor road and Hardwar-Laldhang motor road are few of the road networks, which consists of too much heavy traffic passes in between them. As both the sides of these road network comprises of protected area, therefore, elephants use these roads to inter change their traditional forest areas.

14. *Weed proliferation*

Few of the herbs and shrubs, which are replacing the forest vegetation rapidly are – *Parthenium hysterophorous, Lantana camara, Cassia tora, Cannabis sativa, Pogostemon benghalensis, Sida rhombifolia* and *Ageratum conyzoides*. These are never used by wild animals as their fodder, and on the other hand these are spreading very fast, reduces the other area in the forest. Weeds like *Parthenium hysterophorous* was more dominant in Motichur forest beat and distributed all over the site. In the month of October and November it shows flowering as well as fruiting stage, while in the month of December and January, it is in seed dispersal stage (Joshi et al., 2000).

15. *Forest fire*

Another major impact on the conservation of the elephant is forest fire. The periods from mid-March to June are called to be the fire season and this fire may be of natural or of anthropogenic origin. Sometimes burning cigarettes, biddies, matchsticks, electric fence etc. are also the causative agents of forest fire but sometimes this fire also took place naturally. When the upper surface of the land is too much hot the dry grasses like *Eulaliopsis binata* (Bhabhar grass) due to highly flammable nature sometimes catch fire on account of smallest negligence of human beings around. Few of the villagers are also responsible for this fire because they think that after burning of old vegetation new seedling of the trees come up quickly with better regeneration potential.

16. *Human-elephant conflict*

The factors that contribute to the killing of humans by elephants are the presence of villagers inside the forest area. Between the years 1980 to 1990, elephants in Rajaji – Corbett parks, killed 70 people. Such incidents appear to be increasing because 44 of these occurred between the years 1986 to 1990. Similarly, between years 1988 to 1999, elephants has killed 39 persons and injured 5 persons in and around the RNP area. In Hardwar forest range of the park elephants has killed 24 persons and injured 11 persons in between year 1985 to 2001.

17. *Impact of heavy flood during 2007*

The heavy floods of July, August and September, 2007 that impacted the riparian wildlife corridors and movement related activities of Asian elephant (*Elephas maximus*) represent a catastrophic environmental event in Shivalik foothills.

18. *Presence of Gujjars*

Gujjars are the nomadic community whose members stay inside the forest in huts. Gujjar rehabilitation programme is still ongoing in the RNP and till today out of nine, five forest ranges are completely freed from them. But Gujjars are still existing in the Hardwar forest division and are currently utilizing all the waterholes and fodder resources frequently for their routine requirements as the result of which few forest patches are rapidly replaced by toxicious weed like *Parthenium histerophorous* (Gajar grass) and *Lantana camara* (Lantana / Kuri jhari). Elephants must scarify the ground in order to feed on the short grasses due to domestic buffaloes being grazed. In this type of situations elephants are diverting themselves to the peripheral forests or they are performing their outside movements towards the agriculture fields, which generally lead to crop depredation by elephants.

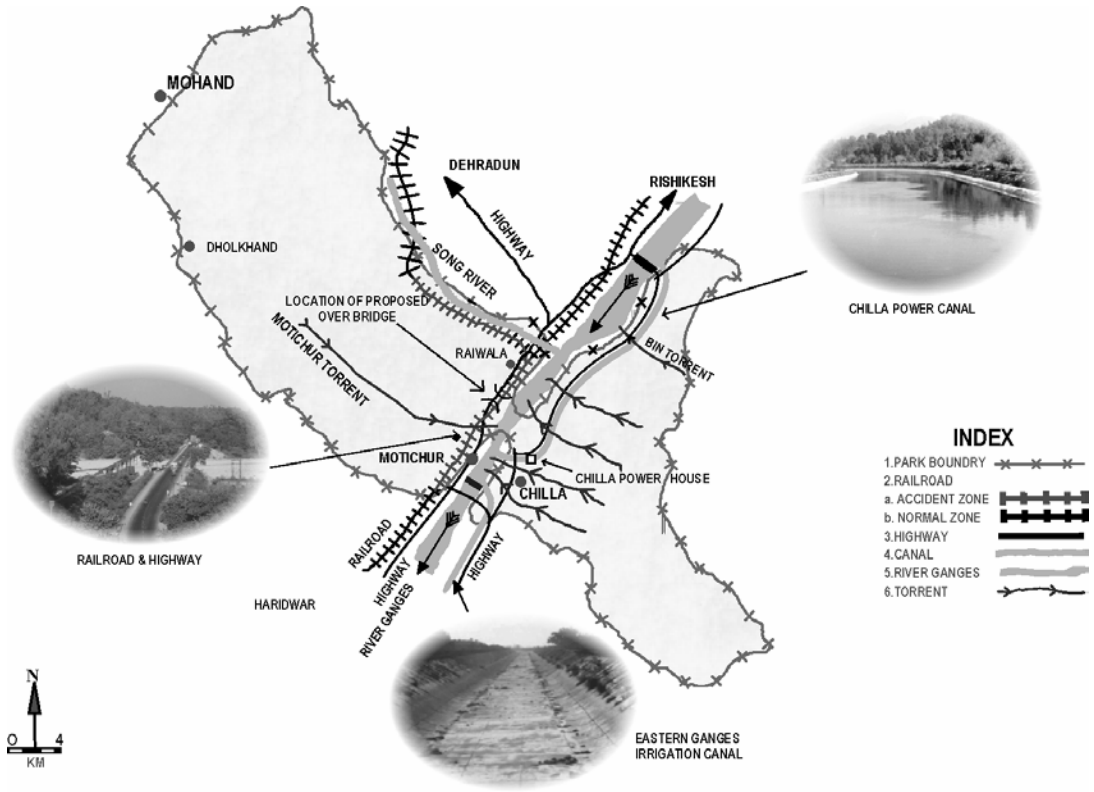

Figure 1. Map of the study area.

Figure 2. Bull elephants walking along the Hardwar – Bijnor National Highway at Shyampur forest.

Discussion

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

The population of wild Asian elephants has a discontinuous distribution in the northern, eastern and southern forest ranges in India. In the past the elephant population of the north used to migrate freely from one end to the other from the river Yamuna to the river Brahmaputra, traveling a maximum distance of approximately 1,300 kilometers as per their requirements in the foothills of Himalayas (Singh and Sharma, 2001). All the major habitats of elephants are very close to perennial rivers that fulfill their water requirements. This entire belt has comprised of all those fodder plant species that elephants like. Elephants do not have a fixed territory, but they show various territorial behaviours according to the season and availability of natural food and water. RNP and its adjoining forests are playing an important role in the conservation of elephants especially after the rehabilitation of Gujjar community.

The Chilla – Motichur corridor and Khara – Anjani corridor linking the Chilla forest with Motichur forest and Shyampur forest are subjected to severe anthropogenic pressures. Livestock grazing, fuel wood collection and movement of people are the major activities observed in both corridors. These anthropogenic activities have substantially affected the movement of elephant within their home range and have led to the loss of forests connectivity. Grazing by cattle has altered the feeding grounds and has led to increase in exotic weeds. Fishermen were also reported within these corridors in early morning and evening hours when elephants are more active. They used to cross the Ganges with the help of rubber tube and stay inside the island while moving inside the forest.

Corridors are important conservation tools and need to be preserved to ensure the genetic flow between the populations. If these corridors has got turned to shrink, inter-breeding will be replaced by intra-

breeding, which may led to loss of genetic material even for wide ranging wildlife species. Land use conflicts have intensified especially in those areas where wildlife movements are more common outside the protected areas. Human habitation and expanded agricultural activities between both of these corridor areas have already increased the number of incidents of conflicts between local communities and elephants. Similar phenomena are very likely to occur in few other internal corridors. Uncontrolled fishing, collection of fuelwood, grazing by cattle and encroachment along the forest edge and river Ganga will ultimately prevent the movement of elephants and other wild animals in this part. The long-term effects will include genetic isolation, habitat degradation within different reserves and intensify the conflicts between villagers and wild animals.

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

The long-term effects will include genetic isolation, habitat fragmentation within the same forest and enhancement in the human-elephant conflict in adjoining areas. Genetic isolation of elephant populations may also increase the chances of replacement of interbreeding to intrabreeding, and thereby reduce the population persistence even for wide ranging wildlife species.

The creation of corridors between two reserves has been proposed to minimize the genetic effects of isolation (Diamond, 1975; Dendy, 1987). Large and medium sized herbivores and carnivores largely depend on corridors for exchange of genes between populations and to search for seasonal foraging grounds and water. Loss of forest cover due to agriculture expansion and construction related work is responsible for the loss of forest connectivity between forested areas in the Nilgiri Biosphere Reserve (Sukumar, 1990). Although the importance of corridors has been accepted widely for management of wide ranging species, only a few studies have been carried out on the impact of human interferences on habitat corridors in India (Johnsingh et al., 1990).

The results from this study provide a sketch of the extent and likely development of human-elephant conflict in Shivalik foothills, major threats with some management measures. The RNP and its adjoining area is an important biological area and has great potential for wildlife and its conservation. People are also increasingly utilizing these old hamlets and as a result management of crop raiding by elephant is often uncoordinated and complicated. It is therefore appropriate to develop a scientific based protocol for conducting in depth analysis of these traditional corridors and serious human-elephant conflicts.

Management measures

1) During the course of elephant movement nearer to the National Highways, traffic should be stopped at a safe distance. At the same time people are not allowed to deter the elephants.
2) In Chilla forest elephants interchange the forest through a small bridge over to Ganga canal commonly known as Sony shroth. The bridge should be widened to some extent so that elephants may cross easily.
3) Ghasiram shroth is a traditional corridor in Chilla area and elephants utilize this track especially during dry months. During that period traffic of the Chilla – Rishikesh road should be stopped in evening hours.
4) Dudhia forest beat (island) and the islands situated in between river Ganges should be restored from any anthropogenic disturbances.
5) Grazing may be banned at least from the crucial areas of the corridors.
6) Habitat restoration may be planned to enhance forest cover in degraded areas. Besides, plantation of fodder plant species is also required.
7) Artificial water holes must be created, spread within the park area at short distance. For solving the problem water uplifting pumps will be used to uplift the well water during day hours, which will help during dry periods. Waterholes can also be connected with Ganga canal and management related practices should be carried out regularly.

8) As the park area mainly comprises of Dehradun / Hardwar region so it is proposed that the time of the night trains be shifted approximately half an hour earlier than the present schedule time. By employing this method the train could be made to move slowly and can be easily stopped in emergency, through the park area up to Hardwar.
9) Relocation of villages and Gujjar deras those are in the corridor area.

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Effects Of Organic Manure Types On Root-Gall Nematode Disease And African Yam Bean Yield

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ABSTRACT: Evaluation of the effects of different organic manures on root-gall nematode disease on African yam bean was conducted in a sandy loam soil naturally infested with a root-gall nematode, *Meloidogyne incognita*. The experiment was conducted in a randomized complete block design with four replications. African yam bean plant were treated with sawdust, municipal garbage, swine, compost, poultry and farmyard manures at 2.5 ton/ha. Results showed that severe root galls occurred on plants treated with sawdust and rare galls on those treated with poultry and farmyard manures. Growth and yield characteristics of the plant were also affected by root-gall damage at the various organic manure treatments. [The Journal of American Science. 2008;4(1):76-79]. (ISSN: 1545-1003).

Keywords: African yam bean, organic manures, root-galls, growth and yield, Owerri, Imo State, Nigeria

INTRODUCTION

Plant parasitic nematode problems have most commonly been managed by chemical soil treatments and crop rotation (Murphy *et al*, 1974). Although both practices improve yields of many crops, there are undesirable features associated with each. Crop rotation designed to reduce a specific nematode species often does so at the expense of increasing other species to damaging levels (Brodie *et al*, 1970; Daulton, 1963; Minton and Donnelly, 1971). Chemical soil treatment, which generally improves crop yields, rarely reduces nematode population densities for more than 2 – 3 months, resulting in post harvest population densities greater than preplant densities (Brodie and Good, 1973).

Considerable interest has however developed in the possibility of using organic manures to reduce nematode populations and increase crop yields (Johnson, 1962; Mankau and Minteer, 1962; Lear, 1959). This innovation satisfies the undesirable features of chemical soil treatments and crop rotation (Ismaila *et al*, 1973). Little is however known about the relative efficacy of different organic manures in the control of root nematodes. This study evaluated different organic manures for effective control of root-gall nematode disease on African yam bean (*Sphenostylis stenocarpa*).

METHODS

The study was conducted in the cropping seasons (April – September) of 2005 and 2006 at the Teaching and Research Farm of Federal University of Technology, Owerri, located between latitudes 5^0 20'N and 5^0 27'N and longitude 7^0 00'E. The soil was sandy loam (91.44% sand, 3.44% silt and 5.22% clay) and naturally infested with a root-gall nematode, *Meloidogyne incognita*. The nematode population densities was estimated by modified sieving and Bearman's Funnel Technique (Viglierchio and Schmitt, 1983).

Before planting, the land was cleared, ploughed, harrowed and made into 28 seedbeds (1.2 x 8 m) at 7 seedbeds/block. The seedbeds were treated by incorporating no manure, sawdust, municipal garbage, farmyard, compost, swine and poultry manures at 0.024 tons/seedbed (i.e. 2.5 tons/ha) in a randomized complete block design with four replications. Percentage organic carbon, ammonium, potassium, phosphorus and carbon/nitrogen ratios in the organic manures were respectively analysed using Walkley-Black method (Black, 1965), Colorimetric determination method (Anderson and Ingram, 1993); Flame Photometry and Vanado-Molybdate methods (AOAC, 1970);and Fumigation and Extraction method (Wander and Traina, 1996). All seedbeds were repulverized 7 days later and immediately seeded with African yam bean (Benue brown cv) at 0.8 x 0.8 m, a population density of

Table 1: Approximate percentage of organic carbon, ammonium (nitrogen form), potassium, phosphorus, carbon/nitrogen ratio of organic manures (dry weight basis)

Constituent	Sawdust (%)	Poultry (%)	Farmyard (%)	Municipal garbage (%)	Compost (%)	Swine (%)
Organic carbon	60.0	20.0	50.0	40.0	40.0	45.0
(c)	0.1	6.5	4.0	0.5	1.3	1.5
Ammonium (N)	600:1	4:1	12:1	80:1	30:1	30:1
C:N ratio (C/N)	5.0	1.0	2.0	4.0	3.0	2.0
Potassium (K)	2.0	1.0	1.5	2.0	3.5	1.0
Phosphorus (P)						

15,625 plants/ha. The plants were staked with two plants/stake and hoe weeding done 40 and 75 days after planting. At 14 day intervals from 30 to 100 days after plantings, leaf area index (area of leaf materials divided by the ground area over which it is displayed), leaf area growth rate (cm^2/day), number of shoots, seed bearing pods and leaves/stand and plant heights were recorded. Pods and 1000 seeds dry weights were also recorded at harvest (150 days after planting). Root-gall incidence (percentage of infected plants) and severity were measured. Root-gall severity was scored using the following scale: 1 = no root-galls, 2 = 1 – 25%, 3 = 26 – 50%, 4 = 51 – 75% and 5 = 75 – 100% roots galled. Twenty cores (2.5 x 15 cm) of soil were also collected from the centre rows of each seedbed and processed for nematode population density according to the methods of Viglierchio and Schmitt (1983). All data were subjected to analysis of variance (Steel and Torrie, 1981) and means separated by Fisher's least significant difference (Fisher, 1948) at P = 0.05.

RESULTS AND DISCUSSION

Results of the study showed that incidence and severity of root-gall nematode disease on African yam bean varied with different organic manure treatments. Highest disease incidence and severity occurred on sawdust treated African yam bean plants. These were followed by those on plants treated with municipal garbage. Moderate root-galls occurred where swine or compost manure was applied. Plants treated with poultry manure were rarely galled. The same was true for farmyard manure but with higher disease incidence. Nutrient constituent analysis (Table 1) showed that the different organic manures varied significantly in amounts of potassium, phosphorus, ammonium (in form of nitrogen), organic carbon and carbon/nitrogen ratios. Amounts of these nutrients influenced root-gall nematode damage on the African yam bean. This was as evidenced by severe root-galls associated with sawdust which had high (5%) potassium and very low (0.1%) ammonium contents. Also, poultry manure with high (6.5%) ammonium and low (1%) potassium contents gave rare root-gall damage. These observations agree with that of Otiefa (1959) which stated that root-gall nematode damage on cabbage increased with amounts of potassium available to the host plant because potassium increased the rate of reproduction of the nematode. Huber (1980) also reported that root-gall nematode damage on Lima bean decreased with increased ammonium supplied to the plant.

Growth and yield characteristics of the African yam bean were affected by root-gall nematode damage at the various organic manure treatments (Table 2 and 3). Leaf area index, leaf area growth rate, plant height, number of shoots, leaves and seed bearing pods and weights of dry pods and seeds were significantly reduced at high disease severities resulting from sawdust, municipal garbage and no manure applications. Plants treated with poultry and farmyard manures gave significantly higher yields than those of other organic manures. This was because rare root-galls occurred at poultry and farmyard manure applications. Plants with fewer root-galls would translocate more nutrients to vegetative organs than heavily galled roots (Otiefa and Elgindi, 1962). The C:N ratios of poultry and farmyard manures were also very narrow (Table 1). Miller and Donahue (1990) reported that organic residues with C:N ratios of 20:1 or narrower have sufficient nitrogen to supply to the decomposing microorganisms and also to release for plant use.

Conclusion: Application of sawdust as a soil amendment aggravated root-gall nematode damage on African yam bean in a sandy loam soil. This was as opposed to significant reductions in root-gall nematode damage caused by municipal garbage, swine, compost,

poultry and farmyard manure applications. Poultry and farmyard manures most effectively controlled the disease and improved African yam bean growth and yield.

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Table 2: Growth and yield characteristics of African yam bean as affected by root-gall nematode (*M. incognita)* disease at various organic manure applications in 2005

Organic manure (2.5tons/ha)	Mean										
	Disease		Leaf area		Number of		Plant height (cm)	Number of seed bearing pods/stand	Dry weights of		Nematode counts/ 200cc of soil at harvest
	Incidence (%)	Severity (1 – 5 scale)	Index	Growth rate (cm^2/day)	Shoots/ Stand	Leaves/ Stand			Pods (gm)	1000 seeds (gm)	
No manure	65.20	5.00	2.40	166.00	8.50	143.18	218.12	7.00	31.20	122.30	28.90
Sawdust	64.90	5.00	3.20	180.04	9.00	152.40	220.24	6.68	33.10	120.00	32.44
Swine	47.80	3.40	4.96	256.06	11.70	198.02	251.15	15.06	80.40	168.15	10.43
Poultry	14.90	2.20	7.36	340.03	13.36	239.13	286.07	22.40	138.60	201.10	3.95
Farmyard	28.60	2.40	6.80	324.05	14.56	242.22	280.04	25.80	144.00	220.40	2.75
Municipal garbage	46.40	4.30	3.52	270.01	11.58	201.19	231.23	11.24	45.18	134.23	18.27
Compost	44.80	3.20	6.23	313.08	13.06	228.04	248.14	18.15	92.70	178.63	8.97
$LSD_{0.05}$	3.40	0.90	1.29	18.54	2.10	11.16	22.40	2.98	8.68	22.42	1.50

Table 3: Growth and yield characteristics of African yam bean as affected by root-gall nematode (*M. incognita)* disease at various organic manure applications in 2006

Organic manure (2.5tons/ha)	Mean										
	Disease		Leaf area		Number of		Plant height (cm)	Number of seed bearing pods/stand	Dry weights of		Nematode counts/ 200cc of soil at harvest
	Incidence (%)	Severity (1 – 5 scale)	Index	Growth rate (cm^2/day)	Shoots/ Stand	Leaves/ Stand			Pods (gm)	1000 seeds (gm)	
No manure	62.78	5.00	2.62	154.48	7.70	139.04	198.44	9.00	36.36	136.76	31.07
Sawdust	67.03	5.00	2.90	168.17	7.23	144.30	194.05	8.64	32.14	128.26	36.56
Swine	44.54	3.01	4.14	244.38	12.18	167.15	232.43	13.42	68.82	155.24	12.04
Poultry	20.15	2.12	7.76	322.12	12.04	231.18	256.48	23.01	55.22	218.00	2.84
Farmyard	22.63	2.43	7.22	312.44	13.24	236.14	261.22	24.61	161.78	226.18	3.01
Municipal garbage	45.19	4.16	4.14	258.62	10.28	198.42	220.13	10.04	54.06	121.67	21.54
Compost	44.16	3.12	6.06	301.12	12.44	222.06	240.33	15.10	84.21	164.18	10.18
$LSD_{0.05}$	4.86	0.52	1.12	20.01	20.01	8.15	12.02	3.18	12.23	18.86	2.07

The Truth about Global Warming

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Abstract: The author is an expert in the subject of global warming. The earth is a self-contained biosphere. This article describes the global warming topics from the earth orbit aspect. [The Journal of American Science. 2008;4(1):80-82]. (ISSN: 1545-1003).

Keywords: earth; global warming

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

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

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

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

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

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

warming, when they realize they are wrong, and their plans are not working. Please don't let them do that, time is running out! Remember there is a point of no return.

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Pravastatin Preserves Vasomotor Response in Atherosclerotic Arteries After Balloon Angioplasty

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Abstract: Background: Vasodilation response to pharmacological challenge is inhibited following balloon angioplasty. Pravastatin, a 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor, has been demonstrated to enhance endothelial cell production of nitric oxide and reduce low-density lipoprotein cholesterol. This study was conducted to evaluate the effect of pravastatin on vasodilation following balloon angioplasty in normal and atherosclerotic arteries. **Methods:** Three normal and three atherosclerotic New Zealand White rabbits were used. Atherosclerosis was induced by feeding a high cholesterol diet. Rabbits were sacrificed and carotid arteries were isolated and placed in a dual perfusion chamber. Both arteries from each rabbit were perfused with oxygenated physiologic buffered solution at 37^{o}C and 60 mmHg. One artery was exposed to pravastatin (100 μM) and the other served as control. Balloon angioplasty (BA) was performed in both arteries using a 2.5 × 15 mm balloon catheter inflated to 10 atm at 3 different sites for one minute each. Pharmacological challenge was given using acetylcholine (2×10^{-5} M) and sodium nitroprusside (2×10^{-5} M) in norepinephrine (2×10^{-6} M) preconstricted arteries. Vessel diameter was measured by a computer planimetry system. **Results:** After BA in normal rabbit arteries, acetylcholine did not demonstrate significant difference in percent lumen dilation between control and pravastatin (25.5±10.4 vs 16.6±7.5, p=ns) while atherosclerotic arteries had significantly preserved vasomotor response with pravastatin (16.9±7.2 vs 33.6±18.2, p<0.005). Similar results were noted with nitroprusside in normal arteries (29.0± 14.5 vs 18.0±10.5, p=ns) and atherosclerotic arteries (18.6±7.4 vs 38.4±19.8, p<0.003). **Conclusion and Discussion:** Pravastatin preserved vasomotor response in atherosclerotic arteries following BA when compared to normal arteries. This effect may be due to an enhanced production of nitric oxide in atherosclerotic arteries. However, pravastatin also appears to influence vasomotor response by either non-endothelial dependent or a combination of endothelial and non-endothelial dependent mechanism. [The Journal of American Science. 2008;4(1):83-89]. (ISSN: 1545-1003).

Keywords: artery; atherosclerosis; pravastatin; rabbit

Abbreviation: Ach: Acetylcholine; EDTA: Ethylenediamine-tetraacetic acid; G-6-Pase: Glucose 6-phosphatase; HDL: High-density lipoprotein; HMG-CoA: Inhibiting 3-hydroxy-3-methylglutaryl-coenzyme A; LDL: Low-density lipoproteins; NE: Norepinephrine; PBS: Physiologic buffered saline solution; SN: Sodium nitroprusside

Introduction

Hypercholesterolemia is a recognized independent risk factor for coronary heart disease. Drug therapy is recommended for patients whose low-density lipoprotein (LDL) cholesterol concentrations are not adequately lowered by dietary modifications. Statins (atorvastatin, cerivastatin, fluvastatin, lovastatin, pravastatin and simvastatin) are the most effective agents currently available for lowering plasma levels of LDL cholesterol by inhibiting 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, a key enzyme in the synthesis of cholesterol. HMG-CoA reductase is a rate-controlling microsomal enzyme that converts HMG-CoA to mevalonic acid, a precursor of cholesterol. HMG-CoA reductase inhibitors are the primary hypolipidemic drug treatment in most countries and statins are the mainstay of therapy for

hyperlipidemia (Illingworth, 1994). Pharmacological lowering of LDL cholesterol concentration has been shown in several primary and secondary intervention trials to decrease the occurrence of coronary heart disease and to prevent or delay coronary heart disease progression and the regression of atherosclerotic lesions has been demonstrated in some patients (Levy, 1984). Pravastatin is a new HMG-CoA reductase inhibitor for the treatment of hypercholesterolemia, which reduces LDL cholesterol and increases high-density lipoprotein (HDL) cholesterol (Jungnickel, 1992).

Pravastatin is formed by microbial transformation by the *microorganism Nocardia autotrophica*. The structural formula of pravastatin is shown in Figure 1 along with those of lovastatin and simvastatin. The dihydroxyheptanoic acid moiety is the substrate analogue that interacts with the active site of HMG-CoA reductase. The decalin ring interacts with the binding site. Pravastatin sodium is chemically designed as [1S-[1α (βS*, δS*), 2α, 6α, 8β(R*), 8α]]-1,2,6,7,8,8a-hexahydro-β,δ,6-trihydroxy-20methyl-8-(2-methyl-1-oxobutoxy)-1-naphthaleneheptanoic acid, monosodium salt. Its empirical formula is $C_{23}H_{35}NaO_7$ and its molecular weight is 446.52 (Arai, 1988). Pravastatin is a hygroscopic, crystalline powder that is freely soluble in water (>300 mg/ml) and methanol, slightly soluble in isopropanol, and practically insoluble in acetone, acetonitrile, chloroform and ether (Watanabe, 1988). Atherosclerotic plaque disruptions with subsequent arterial thrombosis are critical causes for acute coronary ischemic syndromes. Pharmacological protections of artery are under searching. We suppose that pravastatin will preserve the vasoactivity of the atherosclerotic artery by its affectation of lowering LDL cholesterol levels.

The effects of statin drug therapy on the cardiovascular system extend beyond their anti-hyperlipidemic properties. Many studies showed that statins have a pronounced antioxidant effect as well as well documented endothelial protective effect. Statins are 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors, a key enzyme in the synthesis of cholesterol. They have been shown to decrease significantly the risk of cardiac events in the setting of primary prevention, secondary prevention or during the acute coronary syndrome (MIRACL trial). Since the effect of statin therapy is so diversified we elected to study the effect of pravastatinon the vasoreactivity of the endothelium following acute physical injury mimicking the current intravascular interventions.

Materials and Methods

Six, male, New Zealand White rabbits (Harlan-Sprague Dawley, Inc., Indianapolis, IN) weighing between 2.8 and 3.2 kg were used in this study. The control group consisted of three normal rabbits that were fed a regular diet (Harlan-Sprague Dawley, Inc., Indianapolis, IN) for six months. To induce atherosclerosis, another three rabbits underwent balloon-induced arterial injury, then were maintained on a 1% cholesterol diet (Harlan-Sprague Dawley, Inc., Indianapolis, IN) for one month followed by another month of alternated regular diet and the two months diet cycle was repeated for three times to keep the rabbits for total of six months. Balloon-induced arterial wall injury of the aorta was performed with a 4F Fogarty Arterial Embolectomy catheter (0.9 × 40 cm, Baxter Healthcare Corporation, Irvine, CA) introduced through the right femoral artery cutdown. The catheter was advanced in a retrograde fashion to the aortic valve and then withdrawn 3 cm. The balloon was inflated with 1.5 cm^3 of air, and the catheter was retracted down to the iliofemoral artery. This was repeated three times in each rabbit as described previously (Abela, 1985). Rabbits were anesthetized with ketamine (50 mg/kg, IM, Fort Dodge Animal Health, Fort Dodge, Iowa) and xylazine (20 mg/kg, IM, The Butler Company, Columbus, Ohio) in this surgery process (Abela, 1995).

After intravenous administration of heparin sulfate (1000 IU/rabbit) (Elkins-Sinn, Inc., Cherry Hill, NJ) to prevent postmortem clotting, rabbits were anesthetized by injecting nembutal sodium solution (pentobarbital 50 mg/ml, 1 ml/kg rabbit) (Abbot Laboratories, North Chicago, IL) through a marginal ear vein. Both carotid arteries from each rabbit were removed immediately after the rabbits were sacrificed and were immersed in oxygenated physiologic buffered saline solution (NaCl 119 mM, KCl 4.7 mM, $CaCl_2$ 2 mM, NaH_2PO_4 1.2 mM, $MgSO_4$ 1.2 mM, $NaHCO_3$ 22.6 mM, glucose 5.5 mM and Na_2EDTA 0.03 mM) (PBS). Then the arteries were perfused in a dual organ chamber under 60 mmHg flow pressure and 2.5 ml/min flow rate at 37°C and the artery diameter vasodilation was measured instantly. Balloon angioplasty was performed in both arteries using a 2.5 × 15 mm balloon catheter inflated to 10 atm at 3 different sites for one minute each. One artery was served as non-pravastatin control and the other artery was exposed to

pravastatin (100 μM). After norepinephrine (NE, 2 × 10^{-6} M, Sigma Chemical Co., St. Louis, MO) preconstriction, pharmacological challenge was done with acetylcholine (Ach, 1 × 10^{-5} M, Sigma Chemical Co., St. Louis, MO) and sodium nitroprusside (SN, 1 × 10^{-5} M, Sigma Chemical Co., St. Louis, MO) (Table 1).

The data were calculated according to the formulas: (PBS-NE) (%)=(PBS-NE)/NE × 100, (Ach-NE) (%)=(Ach-NE)/NE × 100 and SN-NE (%)=(SN-NE)/NE × 100 separately, where Ach, NE, PBS and SN represented the average diameter (mm) of the arteries that were perfused by the PBS containing a corresponding chemical. Balloon angioplasty was performed in both arteries using a 2.5 × 15 mm balloon catheter inflated to 10 atm at 3 different sites for one minute each. Vessel diameter was measured by a computer planimetry system (Figure 1). Procedures were performed according to Michigan State University's Animal Care and Use Committee approved protocol.

Table 1. Artery Perfusion Step for Diameter Measurement

Treatments	Perfusion Steps	Perfusion Steps - Abbreviation in Figures	Perfusion Time (min)
Cycle 1: Before balloon injury Without pravastatin	Buffer 1	B1	10
	NE 1	N1	10
	Ach 1	A1	10
	SN 1	S1	10
	Buffer 2	B2	10
Cycle 2: Before balloon injury Half of arteries with pravastatin (100 μM)	Buffer 3	B3	10
	NE 2	N2	10
	Ach 2	A2	10
	SN 2	S2	10
	Buffer 4	B4	10
Cycle 3: After balloon injury Half* of arteries with pravastatin (100 μM)	Buffer 5	B5	10
	NE 3	N3	10
	Ach 3	A3	10
	SN 3	S3	10
	Buffer 6	B6	10

* Same arteries as in cycle 2

Statistical analysis: With Jandel Scientific program, SigmaStat (Sigma Chemical Co., St. Louis, MO) was used for data statistical analysis. $P<0.05$ was considered statistically significant difference. Measured data were reported as mean±SD. The student t-test was used for different studies.

Results

From the observation of aorta arteries, all the rabbits were atherosclerosis with the balloon-induced injury and maintained on a 1% cholesterol diet for one month alternatively up to 6 month feeding.

Myocardial infarction in human cases a triggering activity such as physical exertion precipitates the acute onset of the disorder (Mittleman, 1993; Muller, 1989; Tofler, 1990), but it is difficult to be studied in human. Therefore, a suitable animal model is important for the research in this field. This study demonstrated that atherosclerotic rabbit can be induced with balloon induced arterial injury surgery combined with 6 months of alternative 1% cholesterol diet (Abela, 1995). The rabbits which were balloon induced arterial injury and then were maintained in an alternative 1% cholesterol diet for a total of 6 months clearly caught atherosclerosis. This model is a useful method to get atherosclerotic animal for the related scientific research purpose.

In this experiment, the vasoactivity of both normal and atherosclerotic rabbit carotid arteries was

measured using NE preconstriction and pharmacological challenge with Ach and SN (Figures 2, 3, 4). The measurements were performed with the steps of a perfusion cycle as showed in Table 1.

For normal rabbit arteries under PBS perfusion and pharmacological challenged by Ach and SN to NE, there was no significant difference in percent vasodilation between control and pravastatin in both before balloon injury and after balloon injury cycle (25.5±10.4 vs 16.6±7.5 for Ach, p=ns; 4.5 vs 18.0±10.5 for SN, p = ns). However, compared to control, pravastatin demonstrated a significantly greater percent vasodilation on atherosclerotic arteries after balloon angioplasty (16.9±7.2 vs 33.6±18.2 for Ach, p<0.005; 18.6±7.4 vs 38.4±19.8 for SN, p<0.003) (Figure 2, 3, 4).

The vasodilatation range of ratio to NE of PBS and pharmacological challenge with Ach and SN was 30-50% for before balloon injury and 20-40% for after balloon injury. Pravastatin enhances the vasodilation response in atherosclerotic arteries following balloon angioplasty. Pravastatin may influence vasodilation by a combination of endothelial and non-endothelial dependent mechanism.

Pravastatin enhances both endothelium dependant and independent vasoreactivity of carotid arteries in the setting of acute balloon injury. This was true in the presence of atherosclerosis. The mechanism of this effect is unlikely to be due to the lipid lowering property of pravachol. This might be due to its antioxidant effect or to some other unidentified process through a direct endothelial process, or the activation of some receptors or the induction of some signal that ultimately relates to the genomic makeup of the endothelium. Its effect on the NO system is being established and might be part of the puzzle.

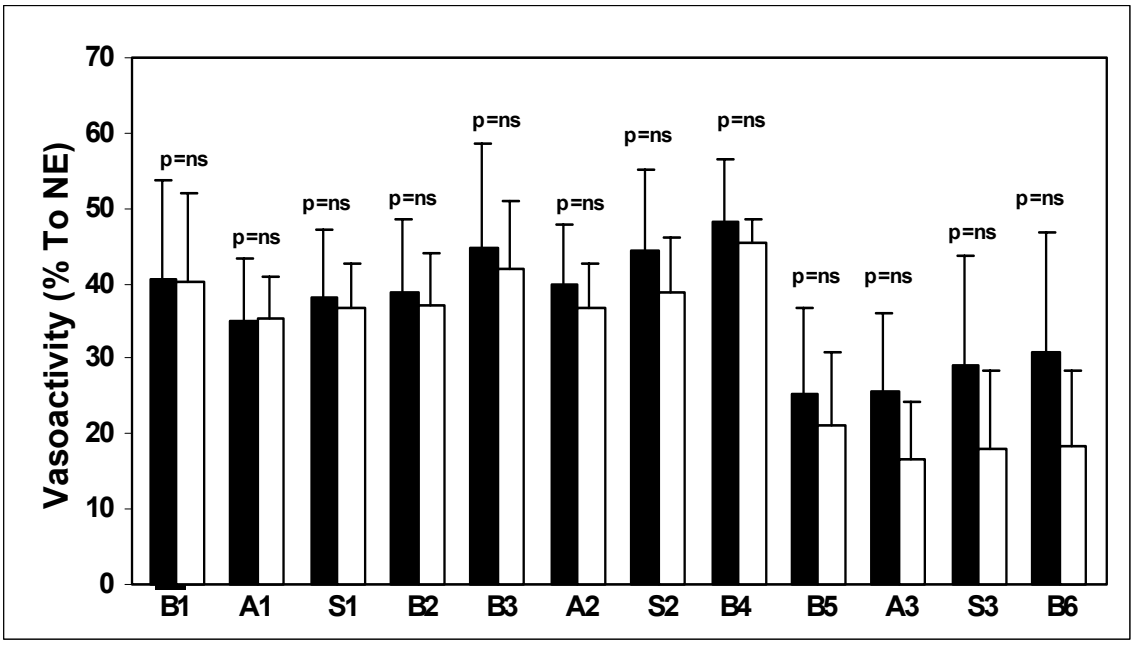

Figure 2. Vasoactivity (% to norepinephrine) of control rabbit.
▬: Control; ▭: Pravastatin. Letter meanings are shown in Table 1.

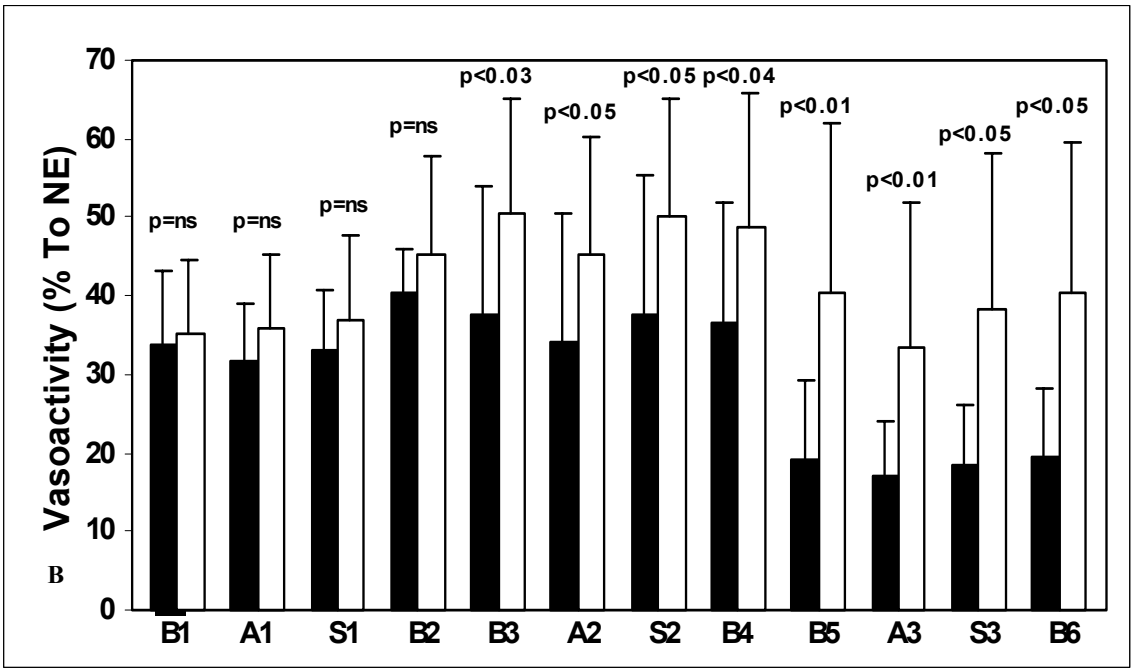

Figure 3. Vasoactivity (% to norepinephrine) of atherosclerotic rabbit.

: Control; : Pravastatin. Letter meanings are shown in Table 1.

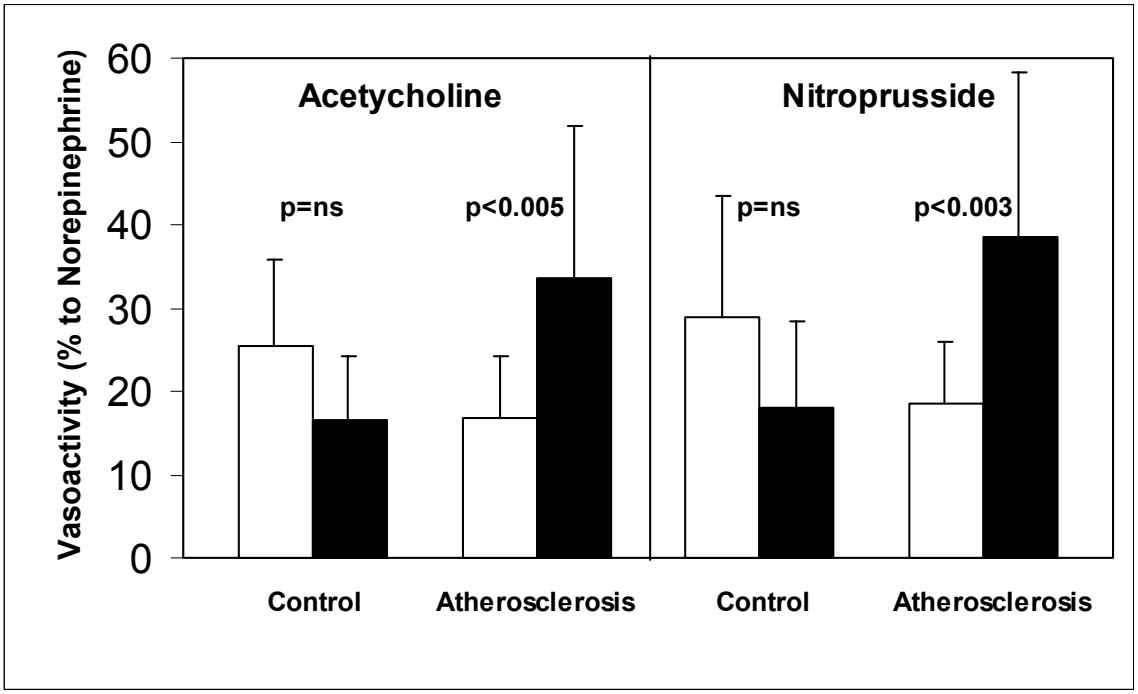

Figure 4. Vasoactivity (% to Norepinephrine) of rabbit carotid balloon injured. Perfused by buffer; Perfused by buffer plus pravastatin.

Discussions

Atherosclerosis, or "hardening of the arteries", is the process that causes heart attacks and most strokes. It is currently believed that cholesterol, especially the LDL, must be modified or oxidized before they can be taken up to cause foam cells. When cells use oxygen for energy, they produce by-products called free radicals. Free radicals damage cells and tissues during a process called oxidation - a factor in many chronic illnesses, including some forms of cancer, cataracts, arthritis and cardiovascular disease. LDL, known as the "bad cholesterol", is actually a protein that carries cholesterol throughout the body. The cholesterol carried by LDL deserves its bad reputation, however. It often ends up in our arteries, causing clots that can lead to heart attacks. Oxidation of LDL-cholesterol contributes to the plaque build-up in arteries, a process called atherosclerosis that can cause blockages and reduced blood flow. The process also plays a role in the loss of elasticity in arteries.

Antioxidants help neutralize free radicals and prevent them from causing cellular damage. Once oxidized, the cholesterol is less apt to be expelled by the body's cleaning mechanisms and more likely to be stored in arteries.

One of the problems for atherosclerotic rabbits is that their free radical and oxidation conditions are changed under the disease. Free radical modification of serum that is not the solely increased level of lipoprotein oxidation products in blood lipoproteins is an important cause for cholesterol accumulation in cells, and apparently for their transformation into foam cells during atherosclerosis.

Once altered by free radical oxidation, plasma lipoproteins undergo dramatic change, both in the manner in which they can interact with cells and in the ways in which they influence cell functions (Chisolm, 1991). Pravastatin preserved vasomotor response in atherosclerotic arteries following BA when compared to normal arteries. This effect may be due to an enhanced production of nitric oxide in atherosclerotic arteries. However, pravastatin also appears to influence vasomotor response by either non-endothelial dependent or a combination of endothelial and non-endothelial dependent mechanism. Pravastatin play protection function on vascular activity may through anti-oxidation.

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Free Radical-Induced Copolymerization of Norbornene and Methacrylate

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Abstract: Various chemically functional monomers are formatted copolymerization and feed different ratios that they affect the yield, molecular weight, glass transition temperature, and transparency. In this investigation, a series of norbornene (Nb), and methacrylate (Ma) were synthesized by catalytic amount of *N*,*N*'-azobis-isobutyronitrile (AIBN) as the initiator. Addition to all of these mainly properties of poly(norbornene-*co*-methacrylate)s were depended on the ratio of methacrylate (Ma)/ norbornene (Nb). Moreover, not only were glass transition temperature but also its transparency, increased with decreasing methacrylate (Ma)/ norbornene (Nb) ratios. Further, these series of targeted copolymers of compositions are identified by ^{1}H NMR spectra analyses, and calculated molecular weights approaching the end groups of main copolymers by GPC are also described in this paper. [The Journal of American Science. 2008; 4(1):90-96].

Keywords: Norbornene; Poly-(norbornene-*co*-methacrylate)s; *N*,*N*'-azobis(isobutyronitrile); Transparency

Introduction

Optical polymers have several advantages over inorganic material in terms of lower price and opportunities [1]. However, they also have some disadvantaged properties such as modest yield of production, formatting the targeted adducts which own highly purity and lower thermal stability. Copolymerization of norbornene with ethylene leads to cycle olefin copolymer (COC) with unique physical property has been reported [2-8]. Addition to all of these products have own highly optical transparent, high glass transition temperature (Tg), and high decomposition temperature (Td). Further, it potentially replaces polycarbonate on the field of engineering plastic [9-15]. Moreover, there are no polar functional groups on the skeleton of COCs, which COCs are not compatible with solvents or which the other resins [16-18]. Consequently, mathacrylate serves to incorporate polar functionality into the polymer, providing necessary adhesion and solubility properties that required for optical polymer material also. In this investigation, all of these targeted polar COCs were resulting from the polymerization of norbornene (Nb) and methacrylate (Ma) using catalytic amount *N*,*N*'-azobis-isobutyronitrile (AIBN) as the initiator. These Poly(norbornene-*co*-methacrylate)s are typically soluble in organic solvents such as toluene, chloroform and tetrahydrofuran (THF), and that thin films have the properties of highly transparency. It is strongly believed that these optically copolymeric materials have usefully potential properties in the future.

EXPERIMENTAL SECTION

General Procedures and Materials

NMR spectrum was recorded on a Bruker 500MHz instrument. DSC analysis was recorded on a Perkin Elmer DSC-7 instrument. TGA analysis was recorded on a Perkin Elmer Pyris 1 instrument. Gel permeation chromatographic (GPC) analysis utilized a Polymer Standards Service column (10 μm gel. SDV linear, 60cm), a Knauer HPLC Pump 64, and a Waters differential refractometer (R 401). All GPC analyses were performed in solutions contain toluene (0.4-0.8 g/dL). Calibration was based on six polystyrene standards ranging from Mn 1300 to 580000 (Mw/Mn < 1.1). UV-VIS spectra (HP-8453A photodiode array spectrophotometer) were measured for thin copolymers films that spin coated on glass substrates from concentrate chloroform solution. The scan range and scan rate were 200-600nm and 2nm/s, respectively; THF was dried over sodium benzophenone ketyl and distilled.

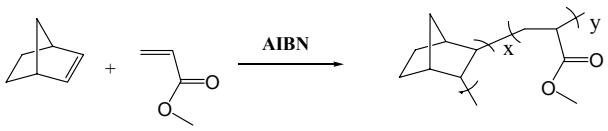
General procedure of the polymerization reaction

The polymerization reaction was operated by adding through a Schlenk flask and charged with norbornene and mathacrylate (the molar feed ratio for norbornene to mathacrylate were 100:0, 80:20, 60:40, 40:60, 20:80 and 0:100) and 2 mole% of *N*,*N*'-azobis-isobutyronitrile (AIBN), and using vacuum-transfer 10-ml THF. Under the condition of 16 hr at 70 ℃, these copolymers were isolated by precipitation with methanol that products were dried over 80℃ for 24 hr. In the copolymerization the molar feeds ratio for norbornene to mathacrylate were 100:0 (poly-1), 80:20(poly-2), 60:40(poly-3), 40:60 (poly-4), 20:80(poly-5), and 0:100(poly-6) gaining these targeted materials of white powder and that yield from 32% to 85% were obtained.

RESULTS AND DISCUSSION

Copolymerization of norbornene and methylacrylate have been synthesized by using catalytic amount of *N*,*N*'-azobis-isobutyronitrile (AIBN) as the initiator giving random polymeric adducts were similar to the copolymer of norbornene-ethylene is shown in Scheme I [12-17].

Scheme I



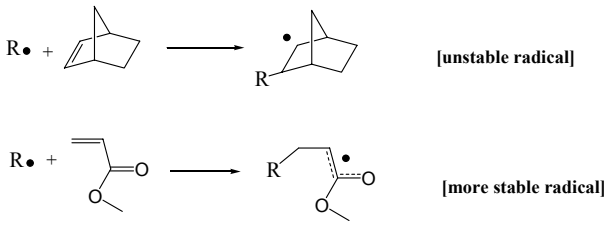
A Schlenk flask was charged with norbornene and mathacrylate (the molar feed ratio for norbornene to mathacrylate were 100:0, 80:20, 60:40, 40:60, 20:80 and 0:100) and 2 % mole of *N*,*N*'-azobis-isobutyronitrile (AIBN) then added THF by vacuum-transfer. After 16 hr at 70℃, and these copolymers were isolated by precipitation with methanol giving targeted products(the molar feed ratio for norbornene to mathacrylate were, 100:0 (poly-1), 80:20 (poly-2), 60:40 (poly-3), 40:60 (poly-4), 20:80 (poly-5), and 0:100 (poly-6), dried at 80℃ for 12hr. All of these results of the experiments are summarized in Table **1** also.

Composition of norbornene and mathacryl- ate copolymer

Poly-3 has strongly peak of chemical shift at δ3.7-3.4 ppm for three methyl protons and chemical shift at δ 2.3-0.8 ppm for three ethylene protons, and ten norbornene protons with the ratio of 1:2.14 integral also. This result indicates that the copolymeric composition is 27:73 for norbornene to mathacrylate that it is shown in Fig. **1**. Additionally, the copolymerization of the molar feeds ratio for norbornene to mathacrylate were 80:20, 60:40, 40:60, and 20:80. The composition of the obtaining Poly(norbornene-*co*-methacrylate)s were founded 37:63, 27:73, 18:82, and 9:91 for norbornene to mathacrylate repeat unit in basing on the analysis of ^{1}H NMR spectra that is shown in Table **1**.

Consequently, according to ^{1}H-NMR spectra analysis, the composition of Poly-(norbornene-*co*-methacrylate)s is increased by the ratios of methacrylate/ norbornene. In 1971, Koch and Gleicer reported that bridged allylic hydrogen on the norbornene did not have chain-transfer in appreciable extent due to the resulting unstable radical[18]. On the other hand, mechanically, as the methacylate has own an ester of electron-withdrawing group which radical is stabilized of delocalization, and that is shown in Scheme II.

Scheme II



Number-averaged molecular weights and yields of the copolymeric mathacrylate/norbornene materials

These copolymers associate with number-average molecular weight ranging from 6000 to 29000 (Mw/Mn from 1.43 to 2.69), and 32%~85% yields were typically obtained. An increase in methacrylate monomer feeds ratio resulting in an increase in the number-average molecular weight. However, a simultaneous increase in yield was also observed for poly-1 (polynobornene), and poly-6 (polymathacrylate) [19] that are shown in Table **1**.

Poly-1 (PNB:Polynorbornene) was synthesized in higher yield (71.5%) and broader Mw/Mn (2.69) from the rigid property of structure and higher number-average molecular weight (28878) from bridged allylic hydrogen property.[18] On the other side, poly-6 (PMA：Polymethacrylate) was synthesized in modest yield (42%) and higher number-averaged molecular weight (27307) from the softly structural property and lower molecular weight; it was also soluble in methanol.

The ^{1}H NMR chemical shift of poly-3 shows peaks at δ 6.15 ppm and δ 5.93 ppm with the ratio of 1:1 integral. It is assigned to the C=C$\underline{H}_2$, that is the end group of Poly-3, and that it is shown in Fig. **1**. There are two protons in the end group and three methyl protons with the ratio of 2:236.4 integral. Consequently, this result is calculated that the number-averaged molecular weight showing 9505 for the copolymer and approximate GPC experimental value 9461.

Thermal and optical properties of mathacrylate and norbornene Copolymer

Those analyses of differential scanning calorimetry (DSC) were shown that the glass transition temperature (Tg) of copolymers fall in the region of 142.3~28.8℃, which it is shown in Fig. **2**. Additionally, as methacrylate has soft structure that is an increase in monomer of methacrylate feeding ratio resulted in a decrease in the glass transition temperature (Tg). Moreover, Fig. **3** shows the decomposition temperature (Td) by thermogravimetric analysis (TGA). The decomposition temperature (Td) of the copolymer was around 360-388℃, and monomer feed ratio was not significant.

Thus, the copolymer of thin films of the UV absorption spectra is showed in Fig. **4**. Consequently, in the range of 300-600 nm that is an increase in methacrylate monomer feeding ratio and resulted in a decrease in the transparency that is shown in Table **1**. Typically, thin films were spin-coated on 1×1cm quartz substrate and film thickness which was controlled 500±20 nm that transparence are around 7-60% at 220 nm. Poly-6 is methacrylate homopolymer (PMA：Polymethacrylate), and that only transparence 7% at 220 nm. Last, but no means least, methacrylate is an unsaturated molecule containing carbonyl group undergo n→π* (ca. 205nm) transition [20]. In a word, norbornene homopolymer (Poly-1；PNB：Polynorbornene) does not contain unsaturated functional group which means it has highly transparence 60% at 220 nm.

CONCLUSIONS

To sum up, the materials of polar COCs were synthesized in this work that the targeted copolymer of properties are not only highly transparence, decomposition, and temperature, but they also they are readily soluble in organic solvent, such as toluene, methylene chloride, as well as tetrahydrofuran. In addition, the synthetic monomer feed ratio that affect the yield, molecular weight, glass transition temperature, transparency and composition of copolymer of norbornene and methacrylate have been investigated for free radical reaction. Consequently, the composition of copolymer is identified from ^{1}H NMR spectra analysis and the calculated molecular weights approach by GPC analysis from the end group. Last, but no means the least, the copolymer of the effort has indicated that yields, molecular weights, and compositions are resulting in increasing the ration of methacrylate (Ma) and norbornene (Nb). In a word, the glass transition temperature and transparency increased with decreasing methacrylate (Ma)/ norbornene (Nb) ratio are described in this publication.

Table 1. Norbornene(Nb)/Methacrylate(Ma) Copolymerizations were catalyzed by AIBN[a]

NO	feed molar ratio Nb/Ma	yield (%)	Tg(℃)	Td(℃)[b]	Mn	Mn/Mw	copolymer ratio	transparency (%)[d]
molar Nb/Ma[c]								
Poly-1	100/0	71.5	142.3	380	28878	2.68	100/0	60
Poly-2	80/20 32	96.7	367	6441	1.43	37/63	54	
Poly-3	60/40	53	71.3	387.9	9461	1.98	27/73	48
Poly-4	40/60	71	41.3	370.7	16310	2.02	18/82	38
Poly-5	20/80	85	28.8	366.9	27742	2.01	9/91	12
Poly-6	0/100	42	29.4	360.5	27307	1.83	0/100	7

[a] Conditions: AIBN = 2 mol%, T = 70℃, solvent = THF. [b] 95% weight lose. [c] for ^{1}H NMR spectra analysis. [d] film thickness

was controlled 500±20nm and transparent at 220 nm.

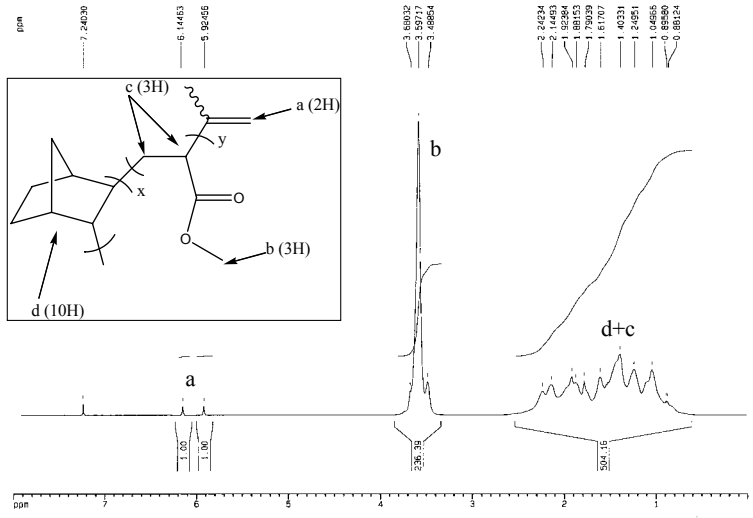


Fig.1 ^{1}H NMR spectra of the copolymer Poly(norbornene-*co*-methacrylate) (27:73)

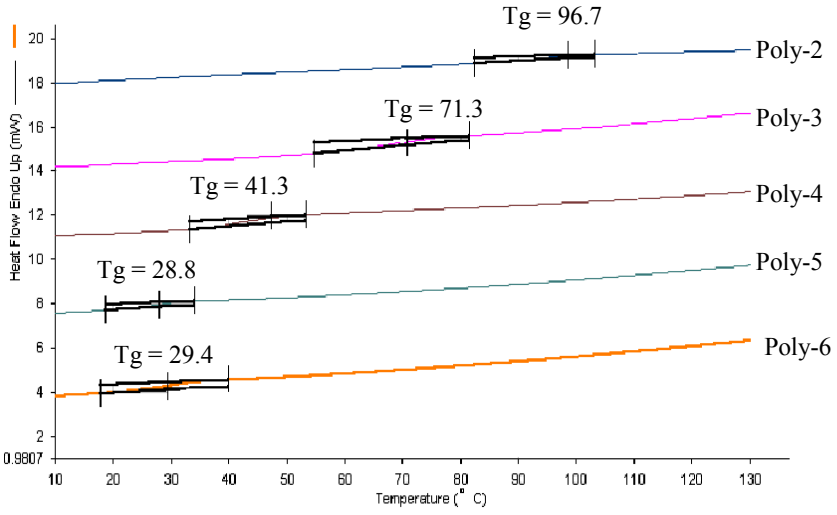


Fig.2 DSC curves for Poly(norbornene-*co*-methacrylate)

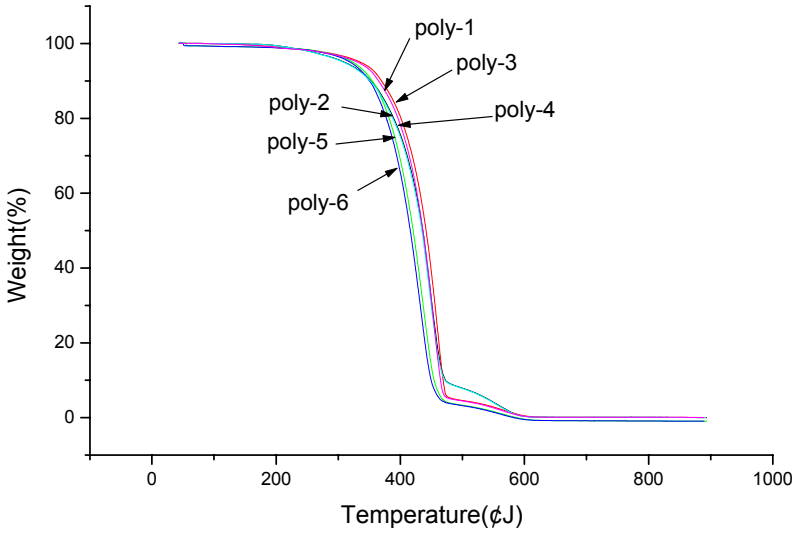


Fig.3 TGA curves for Poly(norbornene-*co*-methacrylate)

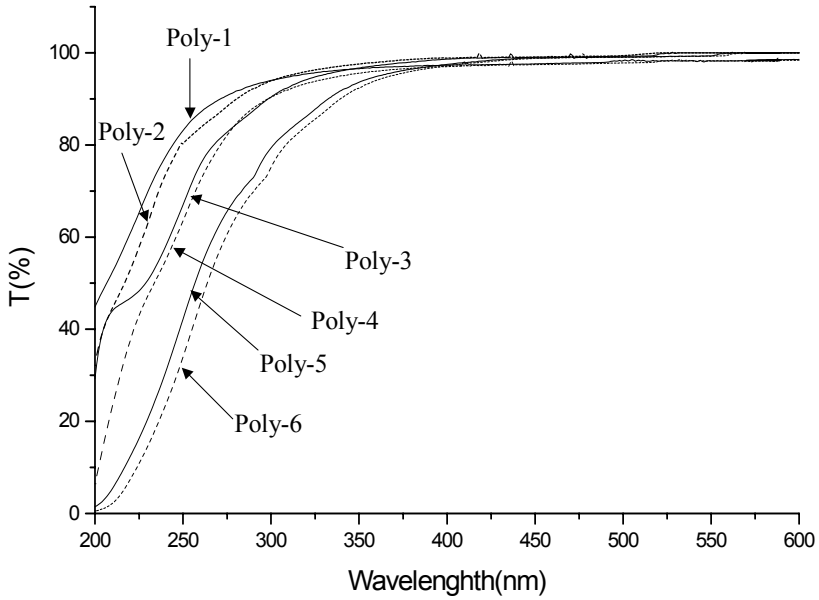


Fig.4 UV absorption spectra of the Poly(norbornene-*co*-methacrylate)s' thin films.

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

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

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

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

MAASTRO lab has a vacancy for a

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

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

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