

*Materials and
Methods*

MATERIALS AND METHODS

Description of the study area

The study area falls in the western parts of Gujarat (map 1). Two different sub areas have been chosen, Baroda and Savli. The distance between these two areas is ~35 km. Selection of sites in the sub areas is based on –

- 1) the level of anthropogenic activities
- 2) availability of earlier records describing vegetation
- 3) availability of vegetal cover

a) Baroda area

Geographically area of Baroda lies $22^{\circ} 0'$ & $22^{\circ} 30'$ North latitude and $73^{\circ} 0'$ and $73^{\circ} 30'$ East longitude and is more or less plain area at an altitude of 36.6 m above mean sea level. The river Vishwamirti flows right through the heart of the city in North South direction. The river Mahisagar, present in the North West of Baroda, forms the Western boundary of the area under study. The river Mini runs at Nandesari and ultimately meets Mahisagar towards Western region. The ravines formed by rivers Mahisagar and Meni at Vasad, Nandasari, Sindhrot and Bhimpura forms the Western and North Western sides of Baroda region

Baroda city earlier was a Princely State with little industrialization and a relatively smaller population. 1960 onwards the area saw an exponential increase in both industrialization and urbanization making great inroads into the area and altered the landscape. Presently the situation is very different as there is an exponential increase in anthropogenic pressure on the available natural resources. Improper land use without taking into consideration the capability of the land has led to the formation of sparsely covered and barren land cover. Large scale industrialization and

urbanization has altered the weather of the area. Changes in land use – land cover pattern have reduced available land for natural flora. All these stresses have a direct and /or indirect effect on the vegetation systems with respect to their quality and quantity.

b) Savli area

Geographically area of Savli lies between 22^o 30' and 22^o 55' North latitude and 73^o 0' and 70^o 20' East longitude at an altitude of 37.5 m above mean sea level and forms a part of Baroda district. River Mahisagar runs along the North West and Western boundary of the area. The Northern area is traversed by rivers Meshri, Kawach, Goma, Karad and their tributaries. River Meni and Vishwamitri flow through the Southern region.

Compared to the regions of Baroda, areas of Savli taluka are under fewer hassles laid by the anthropogenic pressures. It is more pristine. The whole area is either plain or ravinous with a few undulations in the northern region. The area is dominated by agricultural activities. Ravine area supports good variety of ground vegetation. There are a number of vital ponds distributed through out the area supporting a variety of hydrophytic and amphibious plant communities.

Climatic Conditions

Climatic conditions are considered similar for Baroda and Savli region as distinctive climatic data for Savli region is unavailable. The climatic data has been collected from the meteorological department, M. S. University of Baroda. The weather of study area is characterized by the three typical seasons, summer, monsoon and winter. Summer starts from March and extends upto June. The maximum temperature at times reaches 45^oC-47^oC. The average maximum and minimum temperatures experienced during the study period (2001-2003) were 45.33^oC and 10.00^oC respectively. The period from June to September is the monsoon season. Winds are generally light with some strengthening in force during late summer and early part of monsoon season. The variation in the annual rainfall from year to year is large (Table 1). About 95% of normal annual rainfall is received by the month of September. October and November constitutes post monsoon season. Winter starts from the month

of December to February. January is the coldest month with a mean daily maximum temperature of about 30°C and mean daily minimum temperature of 10.6°C. The average monthly temperature, humidity and monthly rainfall recorded for the study period (2001-2003) is given in (Table 2). The Baroda and environs are sometimes affected by cold waves in association with western disturbances passing across North India when the minimum temperature may approach near the freezing point of water. Winds blow from North or North East during post monsoon and early winter months.

Selection of Sampling sites

A preliminary survey was undertaken across the study area. Four sampling sites were selected in Baroda area based on the disturbance levels, variation in the habitat and similarity with the previous work done (Map 2)

1 Laxmi Vilas Palace Campus (LVP)

2 Nandesari

3 IPCL township

4 Timbi

Two sampling sites were selected in Savli area based on the variations in disturbance levels and the land use pattern (Map 3)

1 Goral

2 Mevli

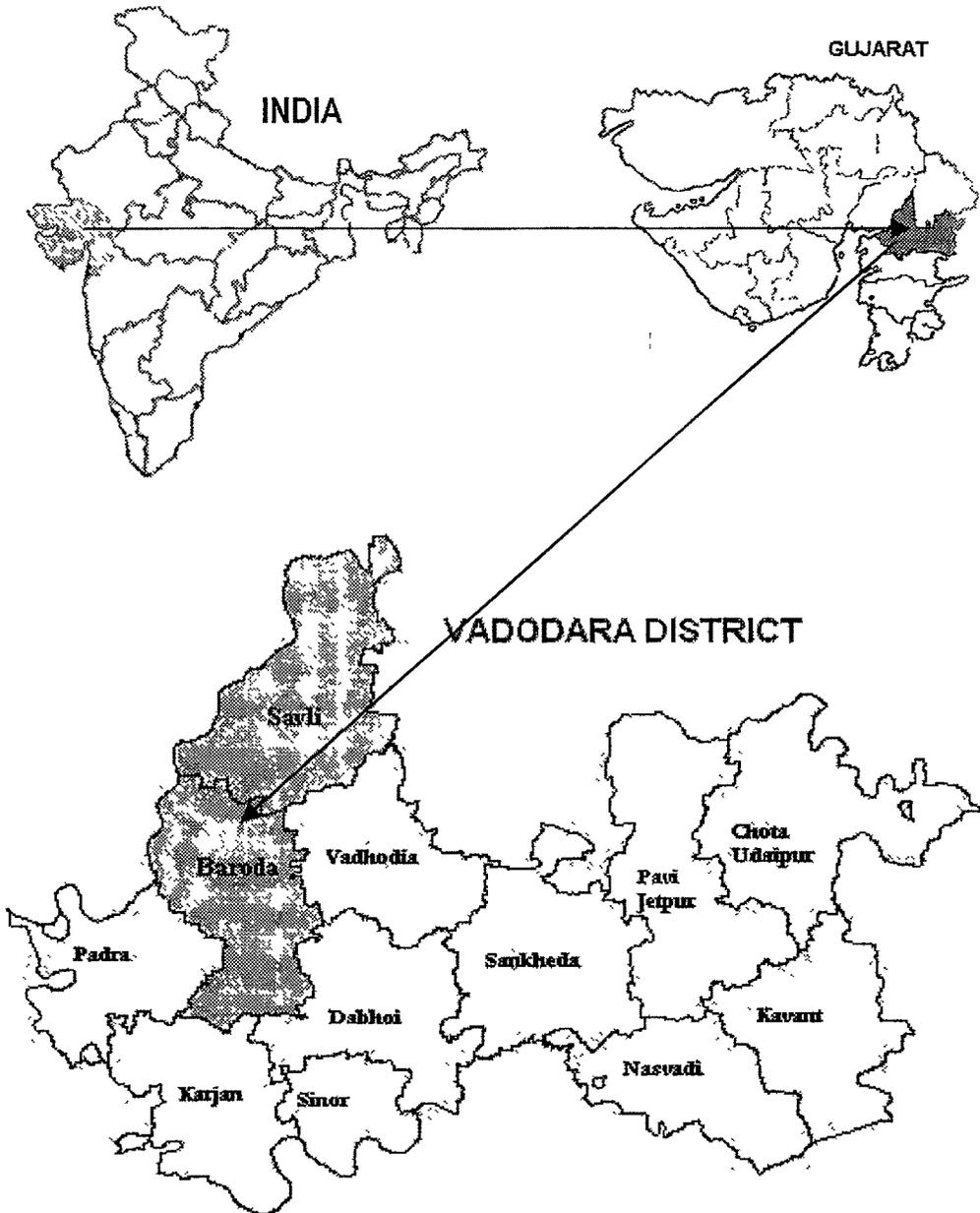
Description of Sampling sites

a) Baroda area

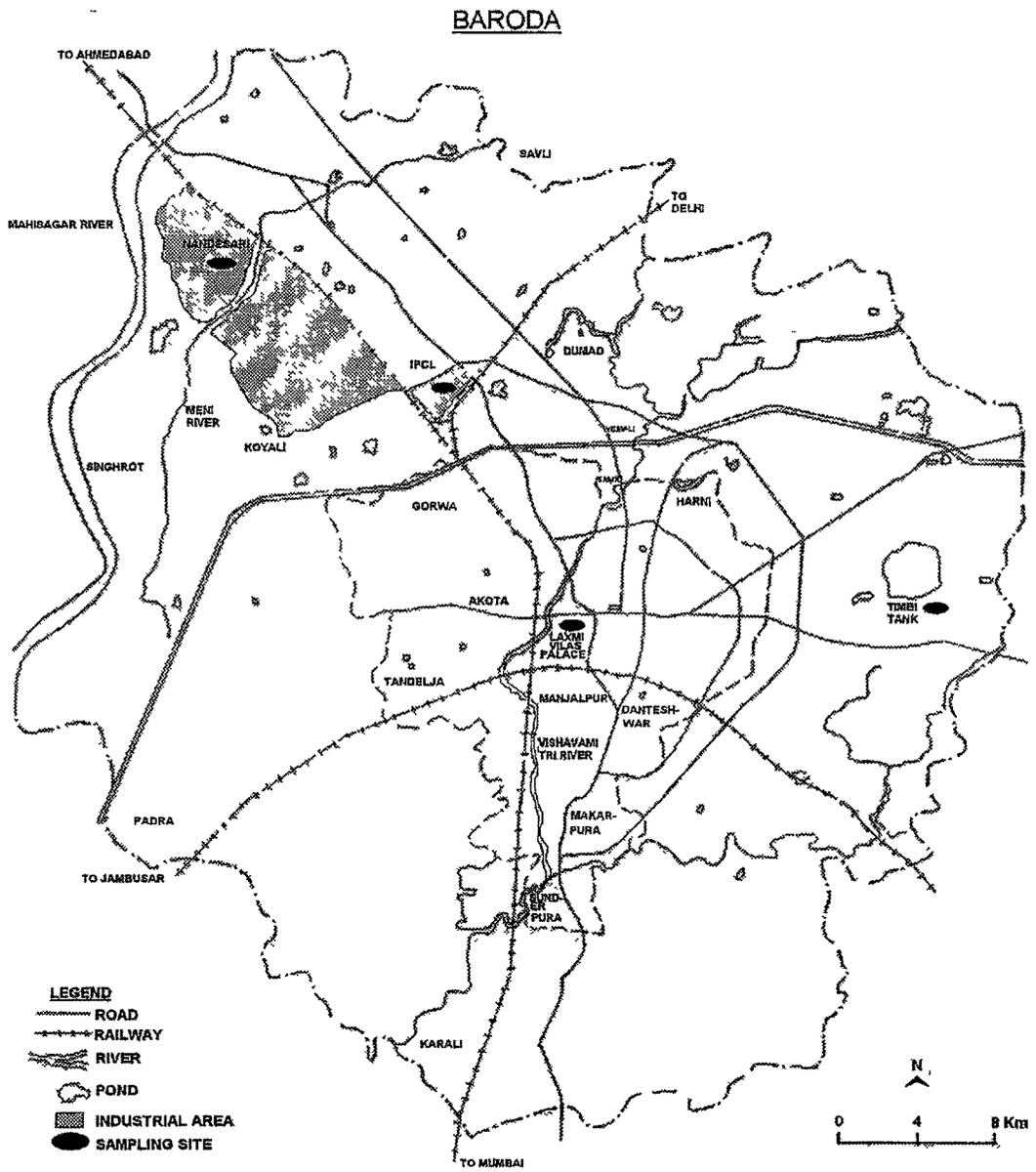
1. Laxmi Vilas Palace Campus (LVP)

The Laxmi Vilas Palace, the residence of Gaekwads, former rulers of Baroda, is situated on the west of the Baroda city. The area is restricted for local people to move in, as it is the private estate of Gaekwads. The boundary of the campus is screened by elevated iron fence, which makes the access of grazing animals difficult. Area is approximately 550 acres. Because of less interference of public and grazing pressures, a variety of wild vegetation thrives in the area

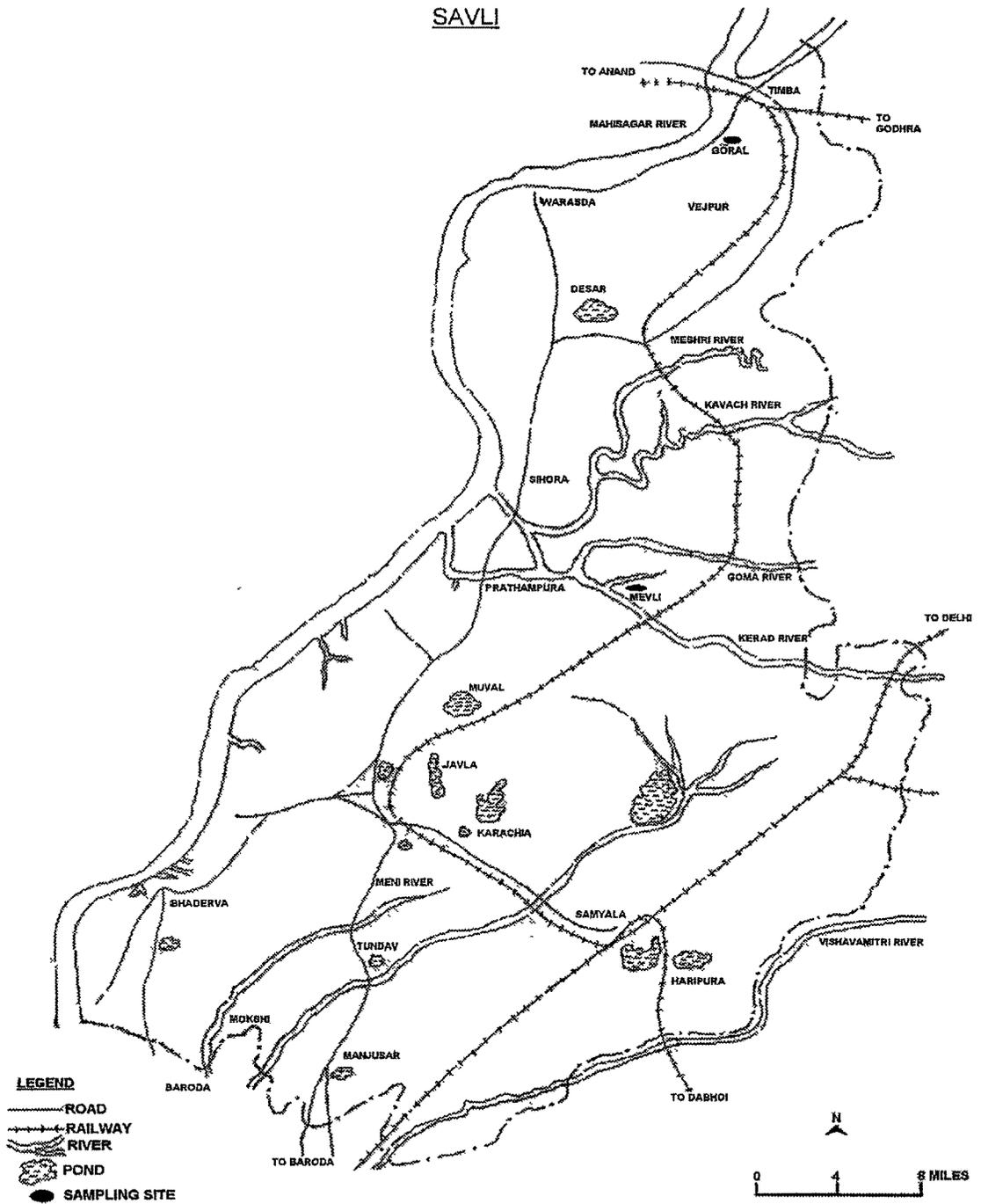
Map 1- Location of Vadodara District



Map 2- Map of Baroda



Map 3-Map of Savli



2 Nandesari

The area is situated in the north-west of Baroda region. Here, the major chemical industries are oil refinery, petrochemical industries, fertilizer complex, an alkalies and chemicals unit, gas based power plant and cyanide plants. Industries like Gujarat State Fertilizers Corporation Ltd., Gujarat Alkalies Company Ltd., Deepak Nitrate Ltd., Simalin Chemicals Ltd., etc. along with 239 small scale industries covering an area of 5 sq km area (the Nandesari industrial estate) are situated. These small scale industries are involved in manufacture of various chemicals, dyes, insecticides, pesticides, etc. In addition to above sources of pollutant, the National Highway No. 8 which passes through the industrial area with heavy vehicular traffic adds to the interference to wild vegetation. The vegetation is interfered by water and air pollution. With the advent of monsoon, number of annual and perennial herbs appears on the open areas and between the tree gaps. The vegetation is subjected to high grazing pressures created by the cattle of the near by villages.

3 IPCL township

The area is situated in the north west of Baroda region. The area is ~3 km from the industry. It has an area of ~ 28 acres earmarked for plantation. Different trees viz. *Acacia nilotica*, *Azadirachta indica*, *Ailanthus excelsa*, *Bauhinia racemose*, *Peltophorum pterocarpum*, *Pithecellobium dulce*, *Samanea saman*, etc. were planted ~ 25 years ago. The boundary of the green belt area is iron fenced but illegal accessibility of local people and wild-grazing animals is possible through the broken areas of fence. Vegetation is interfered by industrial pollution and grazing pressures.

4 Timbi

Area is situated in the eastern part of Baroda. The area is at a distance of 20 km from the proper Baroda city. Timbi is natural irrigation pond having water almost through out the year, water levels recede in summers. The area supports a wide variety of vegetation, which includes aquatic, amphibious, and wetland communities.

b)Savli area

1. Mevli

The area is situated ~ 45 km from the proper Baroda city. Mevli is situated in the central part of Savli region. Area is ravinous from by river Karad. The area is adjacent to the main Godhra highway road. River Karad is perennial but during summer water level decreases to large extent. Surrounding areas are dominated by the agricultural activities. The area experiences fair amount of grazing pressures. Here the tree and shrub cutting activity is noticed by the local village people for fuel purpose. The area experience less pressures created by air and water pollutions. The area supports good variety of ground vegetation

2 Goral

The area is situated ~ 85 km from the proper Baroda city (map 3) It is the Northern most part of the Savli area bordered on Northern side by the river Mahisagar, which retains water throughout the year. The place is approachable from Baroda city through local bus/private jeeps upto Savli city area. From Savli city area, one can reach upto Vejpur (7 km away from the village area) through local bus/jeep. From Vejpur, the village can be reached by walking or by bullock cart (if available). The area is cut off from the main roads (from Vejpur) at a distance of 7 km. The area experiences almost negligible vehicular pollution. Government buses run twice a day (one in early morning and one in afternoon) for local public plying upto proper Savli town area. The local village population is of ~ 500 people. The river Mahisagar is situated at a distance of ~500 m from the village. The area is agricultural dominated and experience almost negligible pollution and anthropogenic pressures. The Northern area is ravinous formed by Mahisagar river. Majority of land is in wild state and supports good variety of wild vegetation

Quantitative assessment of herbaceous vegetation

A preliminary survey was conducted for the entire study area looking into :

1. distribution of herbaceous vegetation
2. coverage/area of occupancy
3. species diversity

Subsequently based on the observations of the survey, specific sites/localities have been marked for a detailed analysis. Background of floristic studies published (Chavan & Mehta, 1955; Phatak & Joshi, 1956; Phatak & Oza, 1957; Phatak & Satakopan, 1957; Chavan & Sabnis, 1958; Chavan & Mehta, 1958; Phatak & Oza, 1958; Chavan & Padate, 1960(1); Chavan & Padate 1960 (2); Chavan & Sabnis, 1960 (1); Chavan & Sabnis, 1960 (2); Chavan & Oza, 1961; Chavan & Padate, 1961; Chavan & Sabnis, 1961; Sabnis & Pathak, 1961; Chavan *et al.*, 1962; Chavan & Padate, 1962; Chavan *et al.*, 1963; Sabnis, 1967; Padate, 1973; Patil, 1980) was also considered for demarcating the study area. Minimum area of the quadrat, and number of quadrats to be laid as replicates was determined from the standard protocols (Kreb, 2000). Quadrat size was determined by (Smith, 1990; Sutherland, 1996). Quadrat number was determined by species accumulation curve (Kreb, 2000).

First sampling was done after the appearance of herbaceous vegetation. Herbaceous vegetation starts appearing after 5 to 10 days of first monsoon showers. At this phase, the vegetation appears in seedling stage. The first emerging leaves are cotyledonous leaves. At this stage, the species identification is quite difficult. Therefore, the first sampling was initiated in the second week of July when the plants were resolute to be identified in their vegetative stage

Field observations

The sites were visited early in the morning at around 7:30 – 8:00 am. One field assistant used to accompany during the course of the work. Equipments viz. measuring tape, scale, thread, pair of scissors, vernier calipers, field lenses, knife, carry bags, news paper, file, field diary, wooden stick, etc. along with few first aid measures were carried along with during the field survey. Ten (1 x 1 m) quadrats were randomly laid at each sampling site. Approximately 40 m

distance was maintained between each quadrat. The quadrats were scattered in the area. Two samples, similar in size and vegetal characteristics were maintained at each site in order to overcome any kind of eventuality such as disturbance caused by grazing pressure, etc. while sampling. Permanent signs were kept to mark the quadrats. Total number of species, total individuals of each species and their diameter were recorded in the field diary.

The flowering plant specimens were collected in quadruplicates in the field (away from the quadrat-demarcated area), instantly pressed in the newspaper and were piled up in a file of hard cardboard. Field observations concerning the phenological conditions, dimensions of plant parts, major associates and seasonal changes were also noted down. Variation observed in reproductive period amongst the regions of Baroda and Savli were noted down. Digital photographs of each specimen were clicked. Photographs taken with high magnifications and resolutions provide information pertaining to minute details of the flowers like anther number, shape, petals shape, etc. Also true colors of the subject are clearly demarcated in the picture, which makes identification of plant an easy process

The sampling study was repeated at an interval of 15-20 days until the termination of life cycle of the herbaceous plant species. Special visits in the evening/late afternoon were made at various localities to take the observations and photographs of the plants which used to flower in the late afternoon and during the evening period.

Different habitats falling in the boundaries of study area were visited twice or thrice in every season for documentation of existing species. The sites included Sunderpura Reserve forest, Vasad Ravines, Sindhrot area, Gorwa area, Chhani area, Sama area, Old padra area, Por area, Makarpura, Harni Pond, Lalbag Pond, Kamlanagar Pond and Danteshwar Pond of Baroda region and Desar, Vejpur, Wasanpura, Muval, Tundav, Manujsar, Prathampura, Sihora, Samalaya, Haripura, Bhaderva, Savli Pond, Muval Pond, Desar Pond, Manujsar Pond, Tundav pond, and Haripura pond of Savli region. The density status of the species which do not used to fall in the sampled quadrats were visually interpreted. These species were normally the plants that were growing on the roadside hedges, agricultural hedges, agricultural weeds, aquatic and amphibious plants, plants growing on abandoned walls, wells, etc. The field

study was carried out for three consecutive years from 2001 to 2003 to test the validity of the first year's results.

Laboratory observations

a) Identification and preservation of plant specimens

The collected plant specimens were brought to the laboratory and the field identification was confirmed by dissecting the floral parts of the plant and confirmed from The flora of the Presidency of Bombay (Cooke, 1908), The flora of Gujarat State (Shah, 1978), Modern Plant Taxonomy (Subrahmanyam, 1997) and Aquatic and wetland plants of India (Cook, 1996). The plant specimens collected during the excursions were processed and preserved following Lawrence (1951) and Singh (1999) with few modifications wherever necessary. The recent nomenclature was checked with the CD ROMS - ENVIS, BRAHMS, BIOSIS, IOPI, and The flora of Maharashtra (Sharma *et al.*, 1996; Singh & Kartikeyan, 2000 and Singh *et al.*, 2001). The collection has been deposited at the herbarium of dept of Botany, M.S. University of Baroda.

b) Comparative study

The purpose of comparative study was to look for the probable reasons responsible for decline in the density of few plant species and to document the morphological/phenological changes that have cropped up due to changing environmental conditions

Comparative study was initiated after the collection of first and second year's data of the study tenure. The selection criterion was based on focusing the plants that have shown remarkable decline in their densities as compared to earlier records. Phenological and morphological details of the selected plant species were gathered. The plants were collected from the areas which were earlier mentioned as wild and undisturbed lands, now slowly and gradually getting urbanized. The plants were preserved in the form of herbarium. These herbariums were then compared with the selected herbariums of the past years (1960's and

1980's) of similar localities. Comparisons also were made with previously published reports.

Initially the herbariums were visually compared documenting the details concerning morphology of the plants, flower number, flower size, fruit size, seed number, etc. The dried flowers of the past herbarium specimens were removed carefully and were dipped in warm water for 5-7 minutes. This treatment allowed the dried flowers to soften. The flowers were dissected; the floral parts were carefully separated and mounted on a clean slide. Similarly, the flowers of recently made herbariums were separated and given similar treatment. The dissected parts were match up with former specimens with an aid of stereo microscope at 4x magnification and comparative measurements were noted down. The size, shape and texture of the seeds were examined with the aid of compound and simple microscope and the dimensions were noted by the use of ocular and stage micrometer. Care was taken to pick up intact specimens from herbariums. Observations were compared with the notes depicted on the herbarium sheets and the details specified in the corresponding year's thesis.

The observations were made for five herbarium specimens of past and present years. Positioning of the plant parts selected for observations were the same to avoid biased observations. The study was repeated for the plant collection of third year's data.

Phenotypic Plasticity

The observation of the vegetation growth during the first years study resulted in the affirmation that the few of the morphological features and the reproductive output of good number of species of wild herbaceous plants varied amongst the two regions (Baroda and Savli). During the second year of the study, 5-10 individuals of each plant species (for which the variation was noted in the first year's study) in the Baroda and Savli areas, were marked as permanent and their growth was monitored periodically. Measurements of height, leaf size, and reproductive output were done periodically. A check on the phenological observations was also continuously monitored. This was continued until the

termination of life cycle of the plant. The study was repeated for the third year to confirm the results of the second year's observations.

Statistical analysis

The raw data of all the sampled sites from the field diaries of the three consecutive years were transferred in an electronic format in spreadsheet layout (Microsoft excel). The data was finally analyzed to calculate Importance Value Index for each species from all the sampling sites. The species were arranged in tabular form in order of their importance value indices (Kreb, 2000). The data was subjected to calculation of diversity indices, the output of which can describe the complex ecosystem and assist in comparisons of different sampled sites in terms of diversity richness and evenness.

The diversity indices were calculated by the aid of computer soft wares –

- a) Species Diversity & Richness - Version 2.65
www.irchouse.demon.co.uk
- b) Biodiversity Professional – Version 2
www.biodiversitypro.nhm.ac.uk

The following indices were estimated for the collected data-

1. Species accumulation Curve

The plot of the cumulative number of species, $S(n)$, collected against a measure of the sampling effort (n) is termed the species accumulation curve.

2. Shannon Wiener diversity indices

A function devised to determine the amount of information in a code or signal

$$H = - \sum (p_i)(\ln p_i)$$

H = Shannon Wiener diversity indices

p_i = Proportion, or relative abundance, of each individual species to the total (measured from 0 to 1)

$\ln p_i$ = Natural logarithm of p_i

3. Simpson diversity indices

A diversity index proposed by Simpson (1949) to describe the probability that a second individual drawn from a population should be of same species as the first.

$$D = 1 / \sum p_i^2$$

D = Simpson index

p_i = Proportion, or relative abundance, of each individual species to the total (measured from 0 to 1).

4. Equitability analysis

Equitability refers to the pattern of distribution of the individuals between the species.

$$J = H / \log(S)$$

J = Equitability

H = Shannon Wiener index

S = the total number of species in the habitat

5. McIntosh Diversity Measure

McIntosh (1967) suggested the dominance index

$$D = N - U / N - N^{1/2}$$

D = McIntosh diversity measure

N = total number of individuals in the sample

$$U = \sum (n_i^2)^{1/2}$$

n_i = the number of individuals belonging to the i^{th} species

6. Fisher's α

This is a parametric index of diversity that assumes that the abundance of species follows the log series distribution:

$$\alpha x, \alpha x^2/2, \alpha x^3/3, \dots \dots \dots \alpha x^n/n$$

where each term gives the number of species predicted to have 1,2,3,.....n individuals in the sample

7. Beta diversity

β diversity measures the increase in species diversity along transects.

Whittaker's β_w

$$\beta_w = S / \bar{\alpha} - 1$$

S=Total no. of species

$\bar{\alpha}$ = Average species richness of the samples

8. Cluster analysis

Measurement of similarity between samples from communities. The analysis is performed by the aid of the soft ware Biodiversity Professional – Version 2

Database development

Bioinformatics is a newly emerging interdisciplinary research area which is defined as the interface between biological and computational sciences. This field deals with the computational management of all kinds of biological information, whether it may be about genes, whole organisms or even ecological systems. It is the field of study that relates to the collection, organization and analysis of large amounts of biological data using networks of computers and databases. A database can be defined as a data structure that stores metadata, i.e. data about data. More generally, an organized collection of information with a better retrieval facility.

A digital database has been prepared for the existing herbaceous vegetation of the area undertaken for the study. The core motive was to endeavor in building up an easy methodology, which helps in quick and correct identification of herbaceous plants and along with that edifice a devise that stores the inventory of the existing flora of a region at one place that can be easily accessible to every one interested. The database layout, structural backdrop and the data format are developed from the pattern followed by the following websites-

1. www.plants.usda.gov - Tropical Plant database
2. www.plantsindia.org - Plants of Western Ghat (India)
3. www.botany.hawaii.edu - Hawaii Plant Database
4. www.ildis.org - International Legume Database
5. www.neoflora.com - Neoflora – The World's Largest Plant Database
6. www.plantsdatabase.com - Plant Database
7. www.hotiplerx.gardenweb.com - Hortiplerx Plant Database
8. www.ansci.cornell.edu - Cornell University Poisonous Plants Database
9. www.rain-tree.com - Tropical Plant Database
10. www.evergreen.ca - Evergreen Native Plant Database
11. www.csdi.tamu.edu - Vascular Plant Image Library
12. www.calflora.org - Database on wild plants in California
13. www.species2000.org - Enumeration of all known species of organisms on earth as the base line database for studies of global biodiversity
14. www.anbg.gov.au - Database on Australian plants
15. www.gbif.org - Global biodiversity information facility

After a detailed study of the available online databases of plants, it was discerned that the databases provided substantial information on the geographical distributions, detailed data on the various regional vernacular names, the information of citation in worldwide floras, etc but were lacking in the information pertaining to the detailed taxonomic and morphological portrayal. In addition, a lacuna was observed in the actual field observations and ecological data. Hence, based on the collected information in the surveyed localities of the study area, a digital database in HTML format (for herbaceous vegetation of Baroda and its environs) is prepared which includes detailed taxonomic & ecological information, medicinal uses if any, and high resolution digital photographs of almost every specimens, which makes the identification an easy process. Special features of the species are digitally highlighted.

Database layout

The digital database is prepared in HTML format through Microsoft front page. The background template design is adapted from the default formats provided in the Microsoft front-page software. A template is a pre-designed page that can contain page settings, formatting, and page elements

The main page consists of information about Baroda and Environs pertaining to climatic conditions, topography, general vegetation structure, etc. The main database page is provided with two searches

- by family name
- by scientific name.

The family name search opens a page with a list of families, which are alphabetically arranged. A search by scientific name opens a page with the entire list of plants arranged alphabetically. Each family name is again linked to page containing list of alphabetically arranged members of that particular family each hyperlinked to separate pages containing detailed information of that particular plant.

The main page of individual plant contains its correct botanical name along with author citation and classification of plant as followed by Bentham and Hooker. The top of the page contains three separate links giving information about taxonomic description, ecology and medicinal uses of that particular plant. The taxonomic detail contains information about habit, habitat, and detailed morphology. Relevant photographs of plant habit, flower and fruit are provided in thumbnails form followed by respective titles. The photographs get magnified by double clicking it. The ecological details include the current status of the plant in study area, distribution, major associates, phenological period and the duration of flower opening. The medicinal uses mentioned are only quotation of the diseases for the remedy of which the plant is useful (the information is being provided by Prof. M.H. Parabia, S.G. Univ., Surat, from the work done on medicinal plants of Gujarat by Uma Devi (1988)). This is just for the purpose to highlighting the importance of the plant. Information about the clinical trials, the chemical constituents and doses are not included as similar information is available in good number of online databases. Inclusion of the same would be a mere repetition.

All the linked pages on the top are provided with home link, which is directly hooked up to the main search page with family and botanical names.

Digital photography

The photographs are clicked with the aid of a digital camera model Kodak easy share DX 4330, 3.1 Mega Pixel resolution, 10X zoom (3X optical, 3.3X advanced digital). All the photographs are snapped directly in field conditions. Photographs are clicked in the best quality mode (camera mode). Habit photographs are taken in the normal mode and close up photographs are clicked in the magnification ranging from 4X to 9.2X depending upon the original size of flower and fruit. 9.2X magnifications were used for the flowers of very small size, similar to that of *Hedyotis corymbosa*. Cloudy atmosphere was preferred for the photographs. Use of flash was avoided while taking photographs. If the intensity of the natural light was too high then artificial shadow was provided to specimen for photography.

The photographs displayed in the database hold the original color of the specimen and modification in color contracts in photograph by any software is strictly avoided. For few grass and cyperaceae species, photographs are taken in the herbarium format as the glumes were of inconspicuous nature and possessed a high blending color characteristic to the surroundings. Photographs of few submerged hydrophytes were taken replacing them in separate container with clear water, keeping submerged in it. The size of all the photographs in the database is maintained to 700 width (in pixels) and 465 height (in pixels).

Intricacies faced during the tenure of the work

1. During the tenure of first two years of field work at Laxmi Vilas Campus, a difficulty was faced in maintaining continuity in quadrat study as there used to be frequent clearing and burning of wild vegetation by the campus guards due to the panic of snakes and wild animals inhabiting in the campus area and for also for their easy mobility for security purpose. During the third year of the study, a great difficulty was felt in continuation of quadrat study till the termination of life cycle of herbaceous plants as almost 60% of the campus area was converted into an international golf ground and a wide area; rich in wild vegetation; had undergone deracination of ground vegetation. This has also resulted in totally desertion of few herbaceous plant species which were exclusively available in the campus area.

2. Every year in Timbi, the heavy rains in the month of August – September resulted in raising the water level of the pond upto 11 feet until the month of October/November and a marked area for the sample study used to get submerged underneath the water. Hence, the repetition of vegetation monitoring was executed for 3-4 times per year.

3. Heavy rains during September 2003 resulted into water logging for a prolong duration in the IPCL green belt area and it decayed the ground vegetation. Hence, the repetition of vegetation monitoring was executed for 4 times in the year 2003.

4. Many a times the heavy rains washed away the muddy roads connecting the field area in Savli village, which made it intricate to reach the field destination.

5. Certain plant species documented during the first year of the field study tenure were unable of being relocated in the later years due to their extreme scanty population. Since digital camera was procured during the last year of the work tenure, photographic evidences of some plants were unable to capture which were documented during first two years of work phase .