Materials and Methods

Present work was carried out during June 2006 – January, 2008. The study area comprised major locations within Satara district. Satara district lies in the western part of Maharashtra state within Western Ghats. Now a days, Western Ghats are gaining importance due to their biological wealth (both flora and fauna). The region has been declared as one of the hot spots of biodiversity within the world. Satara district occupies important position owing to the important hill stations as well as "throne of king Shivaji". Satara lies between 17^0 50' and 18^0 11' North latitude and 73^0 31' to 74^0 75' East longitude along the Sahyadri ranges in Maharashtra state. It has an area of 10417 sq. km., with 11 administrative tahsils. The district has a compact shape with an east west stretch of about 144 km., and north south about 120 km. (See plate I) Residual hill ranges and a intermediate valleys, are well developed on a table land surface, form the main element of landscape in the Satara district. Several leading spurs pass east and south –towards east from the Sahyadri's. Within limits of these hill ranges there are several hills and hill – forts. The eastern region constitutes dry and plain land. Because of these geographical features the district enjoys all extremes of nature. The Satara city is bounded by Pune – Bangalore highway on east and situated at the base of fort of Ajinkyatara. A bold spur jutting northward from Yavteshwar and a small shoulder projecting similarly from Ajinkyatara fort from partial enclosures on east – west and south. Satara is a focus of upper Krishna basin. Climate of Satara is one of the best in Maharashtra. Minimum temperature ranges between 5 – 20^0 C and maximum between 26 – 38^0 C. The annual average rainfall ranges between 400 – 600 mm. Soil at Satara varies and consists of soft spongy easily friable ***murum*** overlaying the hard black rock. The main features in the Satara track are Ajinkyatara or Satara fort, Yavteshwar, Pateshwar and Jarendeshwar hills varying from 910 – 1215 meters MSL.

The collections were made after the post monsoon shower from temporary pools, puddles, ditches, permanent water reservoirs, newly constructed dams, small weirs and such other water bodies

Geomorphology of the area under study–

Soil - The soils vary from tract to tract. The soils in the Satara district belong to three main classes

1 Reddish brown soil on the hills

2 Black

3 Light coloured soil in the plains, depending on the nature of underlying rocks.

The typical lateritic soil found in Mahableshwar, Panchgani plateau and on the higher ridges of Sahyadri indicate a tropical one which is being soft and porous, forms a well drained sub soil. Rich black soil is found in Wai, Jawali, Patan and Karad Tahsils especially along the banks of leading streams. Medium to deep black alluvial soil is found in the central portion of Satara District.

Mineral Resources-

The bauxite occurrences are confined to the chains of plateau of Western Ghats. The major deposits are located in Koyna valley of Mahableshwar and Wai Tahsil of the district. Psilomelane nodules of manganese ore are found with lateritic fragments on the plateau of Mahabaleshwar, Yeruli, kas of Satara district. Kankary soil is also present in nala beds in Man and Bhima river sections.

Climate -

The climate of the district on the whole is agreeable. There are 4 seasons – cold season, hot season, the south – west monsoon and the post monsoon seasons.

Rainfall -

The rainfall varies in different parts of district. The monsoon rainfall mainly starts from mid June to September. The western parts receive high rainfall on account of Sahyadri ranges while the eastern part of the district has a low rainfall. The average rainfall for the district as a whole except Mahableshwar taluka comes to about 838 mm where as for Mahabalehwar taluka is concerned the average rainfall is about 6604 mm per annum. Western part includes Mahableshwar, wai, patan and Satara taluka having annual average rainfall of about 3175 mm while the Phaltan, Man, Mhasvad and Khatav talukas receive low to very low rainfall. The average rainfall is 508 mm per annum. There are winters as well summer rains. They occur frequently. The highest rainfall occurs during the month of July and August..

Table 1. Rainfalls occur in various regions in Satara District.

Rainfall in mm	Areas
Below 750	Dahiwadi (Man), Khandala
750 – 1250	Karad, Wai, Satara (East)
1250 -2000	Satara (West)
2000 -2500	Valleys of Krishna, koyna, Venna.

Temperature -

The temperature variation occurs from season to season. The cold season starts by the end of November and continues to about middle of February, December being the coldest month. In this season the mean daily maximum temperature in the plains is 28 .4 ^{0}C while the mean daily minimum temperature is 13.2 ^{0}C. During the period from the middle of February to end of May there is continuous increase in the temperature. The rise in temperature is more marked in the plains than on the hills. The maximum temperature in the plains are 38.5 ^{0}C and minimum temperature in the plains are 22.3 ^{0}C. The onset of the south – west monsoon in the first or second week of June brings down the day temperature appreciably. After the withdrawal of the south – west monsoon day temperature show an increase in October. The overall temperature ranges up to 37 ^{0} C in summer while Mhasvad and Khatav areas are hot.

Humidity-

Presence of water vapor makes air humid. It refers to that state of the atmosphere in which water vapor is present. It changes from 31% to 83% in the morning, 21% to 79% in the evening (Gazetteer of Satara district 1991). In the plains the dryness is more marked than in the hills.

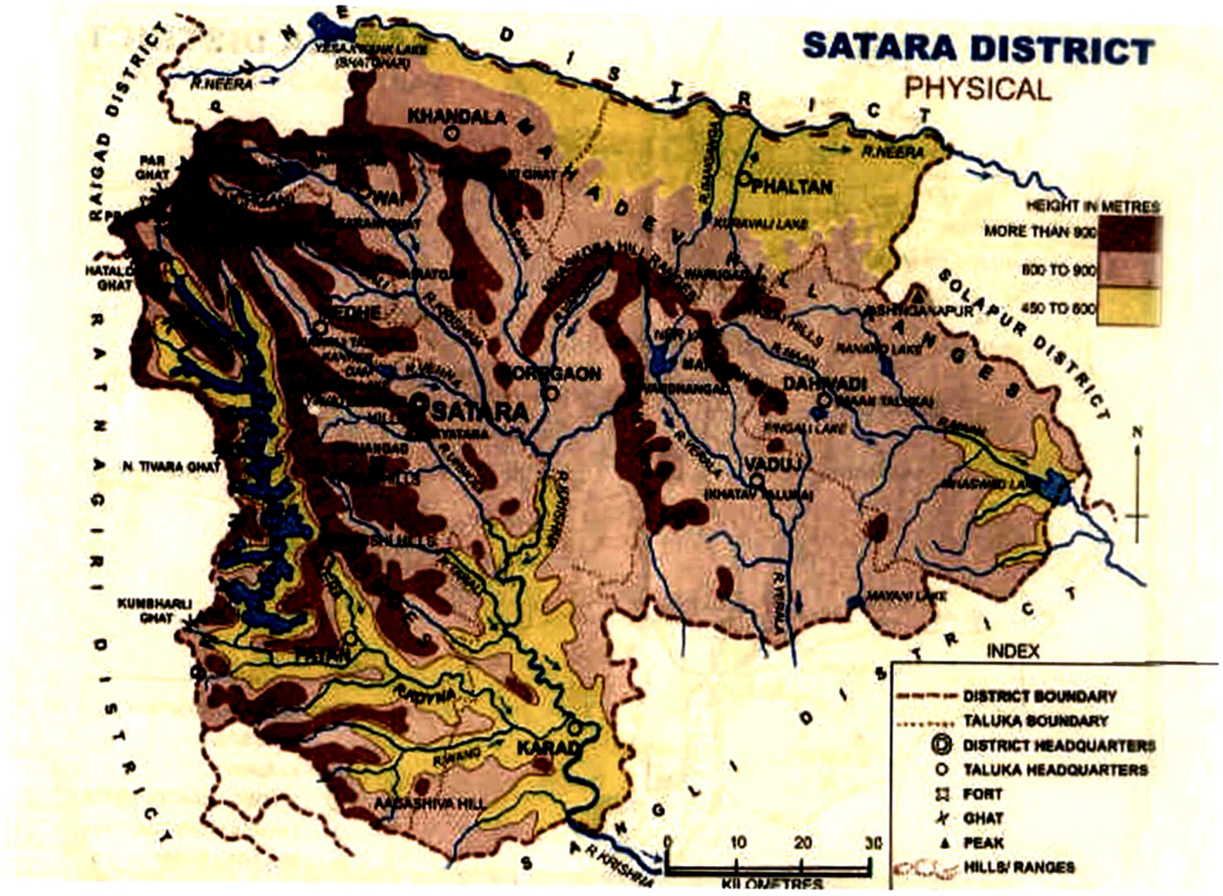
The study area is very unique area as it represents every type of habitat known to support the growth of the Charophytes. The following localities were screened for the collection of the Charophytes.

1. **Vairatgadh fort:** - This ruined fort is in Wai taluka which lies 26 km. north of Satara on a spur of main Sahyadri ranges. It is 1300 mt. above sea level. It is a prominent object east of Wai city between Khambataki ghat. The fort is about 60 mt. above plateau. Fort has a vertical scarp of black rock 10 mt. high and about 3 mt. long loop- holed for musketry. The lower parts of the wall are of larger rectangular unmortared stones. The upper part is mortared and of smaller material. Inside the fort are five small ponds having near about 14 mt. diameters and outside the fort at lower level there is one cave pond. In all these ponds there was growth of charophytes.
2. **Paddy fields Satara:-** These paddy fields are situated near to tunnel. The tunnel is cut through the base an offshoot of the Yavteshwar hill to the south of town for securing communication with the roads leading to Karad.
3. **Parali:-**It is in south west, the burial place of Ramdas Swami.

4. **Ozarde:-** (Tal. Wai, Satara) The village is located towards north of Satara at 36 km. distance along the Pune Banglore highway. The road side puddles in rainy season showed abundant growth of charophyta.
5. **Kavathe:-** (Tal. Wai, Satara) The village is located towards north of Satara at 30 km along the Pune Bangolare highway. In the rainy season outside wel-water body a cannal was found which was full of charophytes.
6. **Vele: -** (Tal. Wai, Dist. Satara) This locality is situated at the foot – hills of Sahyadri ranges near Khambataki pass about 35 km. from Satara towards north.
7. **Dahiwadi:-** (Tal. Man, Satara) 17 40'N and 74 30' E. It is 50 km. east of Satara. The river banks are low and the village is spread along the sides for about a kilometer. The soil around village is calcarious.
8. **Masur:-** It is in Karad taluka, on the Chiplun- Pandharpur sub – highway.
9. **Pateshwar lake:-** It is a peaked hill rising above rest of the ranges about 11 kms south – east of Satara. The Pateshwar hill is famous for its cave temples. At the base of hill there is a permanent water body, from which water percolates out down on clay wall furnishing good growth place for Charophytes.
10. **Kas:** - It is situated between 17 41' N and 73 56' E in Javali taluka. It is a border of Javali and Satara. There lays a huge water reservoir occupying 15.68 sq.km. area. It is part of sahyadri ranges. It is situated at 1300 mt. from the sea level. There is typical lava topography at flat tops. The soil is lateritic and basaltic in origin. It is brownish black and rich in humus. Average rainfall is in between 3810 – 4570 mm. Major rains come from south – east monsoon from June to September. Summer and winter rains are frequent. The humidity is 100% in the month of July and August. The overall climate is temperate with cool nights, vegetation is evergreen type mixed with deciduous species.
11. **Ajinkyatara fort:-** the steep sided and flat topped hill fort of Satara lies to the south of Satara city and at the end of one of the many sahyadri spurs. It rises rather abruptly to a height of about 900 mt. Fort is surrounded by a wall with an entrance in north – west and second blocked entrance in south – east. The sides are steep and bare with a little scrub. There remains on the top two small water reservoirs which harbor good algal growth.

Plate -I

Maps of Satara district showing physical features and collection sites of charophytes



Methodology -

For the present study specimens were collected from above various localities within Satara district. Live specimens of the taxa, well immersed in pond water were brought to the laboratory in polythene bags. For the cytological work young tips bearing antheridia were washed with tap water and transferred to different concentrations of 8 – hydroxyquinoline, p-dichlorobenzene and colchicines. Then the tips were stored at 10 – 12 ^{0}C in refrigerator for two hours. These pretreated tips were washed thoroughly with tap water to remove excess solution and fixed into Carnoy's acetic alcohol solution (1: 3, acetic acid – alcohol) for 24 hours. Then these tips were transferred to 70 % alcohol for future use. Different chemicals like colchicines, 8 – hydroxyquinoline and para - dichlorobenzene were tried for the pretreatment with various concentrations. However, best results were obtained with 8 – hydroxyquinoline solution at lower temperature.

The process of cell division in charophytes has been restricted usually to the apex of the axes, nodal cells, antheridia and oogonia. However, the easiest access for the mitotic division has been found with the antheridial filament. Formation of antheridial filament is a part of consecutive divisions in the antheridial initial cell. Development of globule starts with antheridial initial cell. The antheridial initial cell divides transversely and 2 cells are produced. Basal cell is pedicel and upper antheridial mother cell divides transversely and periclinally. Its division results into formation of outermost shield cells, middle manubrial cells and innermost primary capitulum cells. From each capitulum cell six secondary capitulum cells are cut off inside the globule. On the secondary capitulum cells the initials of antheridial filaments are formed. Each antheridial initial develops into a branched or unbranched antheridial filament. The ultimate antheridial filament consist 15 – 20 cells. Individual cell is uninucleate, isodimetric and devoid of reserve food material as well as pigments. Some authors claim that the easiest way to count the chromosome number is to observe the divisions in pedicel itself. However, during our course of investigation we gave more stress on the antheridial filament as the number of cells in the filament is more and the most important feature is synchronized division of the cells. This gives more probability of getting good spread over metaphasic plates

For the cytological preparation the antheridia were dissected to remove antheridial filaments in 45% Acetic acid and stained with 1% Acetocarmine (Godward, 1948). In order to remove the overlaps tapping gently with blunt heads was found suitable than any

other technique. It is essential to bring all chromosomes in plate in one plane so that the counting becomes easier and measurements can be made easily. Well separated plates were used for initial count as well as immediate camera lucida sketches. Sealing the coverslips with wax and keeping it overnight gives good staining and more contrast is obtained to take microphotographs with Sony digital camera (model DSC-W5). Some slides were also made permanent by passing through (n–butanol: acetic acid) grades. However, the percentage of retaining good plates is very less. Measurements of chromosomes were taken with occular micrometer and also from the photographs.

Observations were made on young antheridial filaments with respect to interphase and dividing nucleus. Maximum divisions were observed in the material fixed in the morning. (usually between 8.00 am to 9.00 am). The cells were 14 µm. – 16 µm. in length and 8 µm. – 10 µm. in breath.

Interphase

The cells were uninucleate in composition. The dividing cells showed distinct appearance from those adjacent nondividing cells in their nuclear organization. The dividing cell nucleus was generally larger, occupying nearly three fourth of the cell volume. Nucleus was oval in outline uniformly or discontinuously stained and surrounded by distinct nuclear membrane usually single nucleolus was present in the nucleus.

Prophase

At the inception of prophase the nuclei were seen increasing their volume. At the early stage few darkly staining threads like structures (chromatids) appeared near the nuclear membrane and later on thick chromatins were seen appearing around the periphery of the nucleus. As the prophase advances the chromosome appeared to be thick rods. Slowly the nuclear membrane disintegrates and the chromosomes, as thick rods get dispersed within cytoplasm. The staining of the chromosome was discontinuous giving them an appearance of beaded structure. The nucleolus and the nuclear membrane disappeared at the end of prophase.

Metaphase

Before arranging themselves at equatorial plane the chromosomes remain suspended around the center of the cell. Usually the metaphase plates get arranged at a perpendicular plane to the long axis of the antheridial filament. These plates were more in

length than the breadth of the cell. Sometimes due to curvature of the filaments the plates appeared to be diagonally placed. The metaphase chromosomes varied in their thickness. They still undergo condensation.

Anaphase

During anaphase various shapes of chromosomes like L, J, V, I, etc. were observed. These shapes clearly indicated the varied centromeric position within different chromosomes. At early anaphase the two anaphasic plates separating from each other remained parallel. But during later period they moved at different planes.

Telophase

A slight change in the axes of anaphase plate appeared prominent during telophase. The result was placing the two groups of chromosomes side by side instead of one above the other. Immediately after the daughter nuclei enter into resting condition, the nuclear membrane reappeared and the nucleoli were seen within the two nuclei. The septum which separated the two nuclei was seen developed diagonally within the antheridial cell. The two daughter nucleoli in the daughter cell again occupy the central place in newly formed cells.

Plants were also preserved in 4 % formaldehyde solution for morphological observations. Identification was made using monographs by Wood and Imahori (1965), Zanneveld J.S. (1940), Pal and Kundu (1960), D.Subramanian (2002)