INTRODUCTION

CHAPTER I

A change in lifestyles due to technological development has resulted into maximization of use of natural resources and subsequent addition into the generation of wastes and pollutants. Now there is hardly any life support system anywhere in the world, particularly in the developing countries like India, which is not adversely affected due to pollution of some kind. Most of the environment is exposed to a cumulative effect of multiple pollutants.

The pollution of water poses a threat to all countries, even more so in tropical countries where precipitation is high and the consumption of water is greater. The problem is more acute in a country like India where population is growing and more urbanization and industrialization are taking place. Sources of potable water, both surface and subsurface are increasingly polluted (Das, 2005).

Surface waters are most vulnerable to pollution due to their ease of access for the disposal of wastes. Both natural processes such as precipitation inputs, erosion, weathering of crystal materials and anthropogenic influences namely urban, industrial and agricultural activities, increasing exploitation of water resources together determine the water quality of surface water in a region. Rivers play a major role in assimilating or carrying-off different types of wastes (Sundaray *et al.*, 2006). From an environmental point of view, the river ecosystem can be considered as the geographic space of interaction between terrestrial and aquatic species (Adeniyi *et al.*, 2007).

Streams in agricultural and urban landscapes are affected by numerous natural and anthropogenic disturbances which may affect resource quality and availability, physical and chemical conditions, ecological integrity as well as disrupt ecosystem processes and biotic structures. Alterations to the land-use adjacent to water courses, increased nutrient levels from point and non-point sources, organic enrichment from domestic and industrial sewage play an important role in the stream physical characteristics and may affect macro-invertebrate assemblage (Buss *et al.*, 2002).

India is home to a wide range of water impoundments located in a diversity of climates, stretching from mountain conditions near the Himalayas in the north to tropical conditions in the south. The impoundments include natural lakes, wetlands and coastal lagoons as well as constructed reservoirs and tanks. Overall lakes all over the country are exhibiting varying degrees of environmental degradation caused by encroachments, eutrophication from domestic and industrial effluents and siltation. Only the degree of degradation differs between the reservoirs. The degradation itself is a result of lack of public awareness and governmental indifference. The high population density ensures that many water bodies are under severe and direct pressure from anthropogenic activities in their catchments.

Wastewaters from municipal and domestic effluents, toxic pollution from industrial effluents and storm water runoff are determining the water quality of water bodies. Also the non point sources of pollution such as nutrients through fertilizers, toxic pesticides and other chemicals mainly from agricultural run off along with organic pollution from human settlements contribute to the degradation of water bodies. Silting of lakes caused by increased erosion resulting from expansion of urban and agricultural areas, deforestation, road construction are also the important causes for impairment of lake basin.

Human settlements and public effluent sources are the chief factors for degradation of lakes, particularly in urban lakes in India. Infrastructure development, housing pressure and encroachments have caused all urban lakes to become hypereutrophic. For the surviving lakes, the drinking water supply has been substantially reduced or become totally non potable. Flood absorption capacity has become impaired, biodiversity threatened and the livelihood of fisher folk reduced.

The role of lakes as wildlife habitats and the goods and services they provide such as water supply and recreational opportunities have tended to be taken for granted. They form an essential component of landscapes in every continent. In their pristine state most water bodies have nutrient poor water as a result of efficient natural systems for nutrient conservation. Population growth and human activities in developed and developing countries have led to widespread eutrophication through the increased and continuous supply of nutrients to river and lake basins in the landscape (Madgwick, 1999). Pollutants are known to deteriorate the aquatic environments by affecting their dissolved oxygen, turbidity, pH, chemical contents, etc. This not only diminishes their auto regulating and buffering capacity but also influences the inhabiting biotic fauna (Gupta and Sharma, 2005).

Cultural siltation, in the form of immersion of idols during Ganesh and Durga festivals in India has been a source of serious pollution of lakes from heavy metals. Examples of such pollution include the Bhoj wetlands and the city lakes of Mumbai, Hyderabad and Banglore (Reddy and Char, 2006). Furthermore, for many lakes tourist pressure has resulted in disturbances to the biodiversity of lake related flora and fauna. Similar kind of pollution is also a cause of concern in Kolhapur district in Maharashtra.

Kolhapur is the name of a famous city nested in the southwest corner of Indian state of Maharashtra. It is also known as Dakshin Kashi and is an important pilgrimage center. It is located approximately at 16.7° N and 74.22° E. Kolhapur is developed on the banks of River Panchganga with average height of 545 meters from the sea level. It is an irregular belt of Deccan plateau lying along the east of Sahyadri Ranges. The climate of Kolhapur is a blend of coastal and inland climate of Maharashtra. The temperature has a relatively narrow range between 12° C to 35° C. The city receives abundant rainfall from June to September due to its proximity to the Western Ghats. The average rainfall in the city is 1025 mm (Kolhapur District Gazetteer, 2004).

Kolhapur with geographical area 6682 hectares has population 4,85,183 as per the census 2001. For sustaining their livelihood, people have adapted to trade and commercial activities. Metal, textile, mining products, cash crops like sugarcane are the famous trade items of the city. Experts are of the opinion that Kolhapur is one of the cities of India which has got highest per capita income. The culture of Kolhapur is enriched and it is pinpointed in its treasures of varied items like food, jaggery, foot wears, jewelaries, etc.

Mahalakshmi temple is the landmark of Kolhapur. According to Puranas, Kolhapur was originally called "Karveera" from the Goddess Mahalakshmi using her hand in lifting her favored retreat from the water of the great deluge (Karveera Mahatmya Adhyaya, 6). Kolhapur originally was a cluster of six villages namely Bramhapuri, Uttareshwar, Kholkhandoba, Rankala, Padmala and Ravaneshwar. River Panchganga, one of the major reasons of prosperity in Kolhapur originates at Sahyadris and called Panchganga from Prayag Chikhali after the confluence of five rivers namely Kumbhi, Kasari, Tulshi, Dhamani and Bhogavati. The river flows towards eastern northern side and meets River Krishna at

Narsinhwadi. The entire catchment area lies in Kolhapur district. Panchganga Ghat is present to the northwest of Kolhapur city, on the banks of River Panchganga and has many ancient temples standing on it. This site is one of the greatest antiquity and many archaeological excavations have been made from time to time in this part.

Kolhapur was a city of lakes in true sense as more than 21 lakes were present in the city in the 18^{th} century. Number of beautiful gardens, hills with these lakes used to rule a healthy climate and ecology in the past. For the expansion of the town and developmental activities from year 1884 to 1954 most of these lakes were reclaimed and places used for other purposes like colony developments, markets, education societies, etc. Some of the names of those water bodies include Kapiltirtha, Varuntirtha, Susarbaug, Indrakunda, Padmala, Ravaneshwar, Petala, Sakoli, Khambale and Siddhala. With the end of these water bodies a rich ecological treasure also ended. Now only Rankala and Kotitirtha lakes are present in the city. But they are under a great threat of pollution.

The Rankala Lake is named after the God "Rank Bhairava" an incarnation of Lord Shiva. The origin of the tank was a quarry from which stones were removed to build various temples in the town. Later in the 8^{th} or 9^{th} century an earthquake is said to have enlarged the quarry and filled it with water. In 1883, considerable improvements were executed at a cost of nearly 3 lakh rupees and the lake assumed its present appearance of a very impressive and large artificial tank. Now the lake is an avenue of recreation and a tourist site.

The Kotiteertha Lake is one of the oldest lakes in the city. The year of its origin is not available. But the ancient story says that 33 crores of Gods and five demons had a terrible fight in this city. The Gods were defeated and only a crore ("Koti") survived. As per their appeal, Lakshmi with 9 crores female friends defeated the demons. The Gods bathed in the lake which was named "Kotiteertha" as per the demons' demand.

As the population of the town increased the demand for sources like water also increased. To overcome the insufficient source for irrigation Rajaram tank was constructed during the regime of Chhatrapati Rajaram Maharaj in the year 1928 at the cost of 3.25 lakhs of rupees. It was designed to store 38 million cubic feet of water. The depth of the tank is 11 meters while the length of the wall is 366 meters. The area of the tank is 21.6 hectares. Along with irrigation purpose this tank is now used for washing and other activities which are deteriorating the water quality of this tank.

With the nature's plenty of gifts Kolhapur is also bestowed with a religious culture. People of Kolhapur are always engaged in celebrating different cultural activities in every season and occasion. All the festivals are celebrated with their unique identity by different religions. Festivals like Diwali, Dushhera, Holi, Navratri, Ganesh Chaturthi and many more are celebrated by the different communities.

Traditionally, Diwali was a festival of lights, when houses were decorated to propitiate Goddess Lakshmi and for attainment of health, wealth, wisdom, peace, etc. However over the years the festival has lost its sanctity and has turned into a festival of pollution, noise, crackers, artificially coloured sweets and serious health hazards. In Diwali, cities turn into gas chambers as burning of crackers increases toxic fumes and gases like carbon dioxide, sulphur dioxide and nitrogen dioxide as well as suspended particulate matter in the air. Crackers cause throat and chest congestion and are likely to aggregate problems for those already suffering from coughs, colds and allergies. An increase in noise pollution above 125 dB is above the tolerable limits and can cause deafness. An

increase in incidents of respiratory diseases such as acute asthma, bronchitis and heart attacks (Toxic Links, 2000). In present generation these crackers have become an integral part of festival celebrations like Navratri, Ganesh Chaturthi, etc.

Holi is a festival of vibrant hues that brings with it a variety of colours of happiness and unity has also changed its way of celebration. The seemingly harmless, "pleasing to eye" colours are synthetic and toxic because of the presence of cheap materials like mica, acids, alkalis which not only induce skin disorders like abrasion, irritation, itching but can impair vision, cause respiratory problems and also cancer.

Ganesh festival is celebrated in the state of Maharashtra all in a big way as a social and community activity. Idols of Lord Ganesha are worshipped in the month of Bhadrapada at household and community level for 5 or 10 days and then along with the offerings and decorative articles these idols are immersed in different water bodies like rivers, lakes, reservoirs, estuaries and open beaches. Traditionally, the Ganesh idol was sculpted out of earth taken from nearby one's home. After worshiping the divinity in this earth idol, it was returned back to the earth by immersing it in a nearby water body. However, increase in population and scarcities of the water resource do not allow the past trend of immersion activity in any water body because of the pollution burden due to different human activities. Also as the production of Ganesh idols on a commercial basis grew, the earth or natural clay was replaced by Plaster of Paris which is harmful to aquatic ecosystems.

When Ganesh festival started by Lokmanya Tilak, the objective of the festival was mainly to bring people together and promote freedom movement. Now with the passage of time, the celebration of the festival has changed in terms of its scale as well as involvement of the people. These activities also cause concerns in terms of water, air and noise pollution. The crackers and loudspeakers used in the immersion procession create problems of air and noise pollution. Crores of rupees are spent erecting the pandals and providing light decoration. In Kolhapur itself about 5 crores of rupees were spent in Ganesh festival in the year 2007. At the time of Durga Pooja in Navratri similar problems of pollution occur. The practice of immersion of Ganesh and Durga idols is causing severe water pollution as thousands of idols having different sizes get immersed in water bodies. In Orissa alone 5000 Durga idols are immersed in water bodies (CPCB report, 2004).

In Kolhapur city, Ganesh festival is celebrated in big way and idols are immersed in various lakes like Rankala, Kotiteertha, Rajaram and in river Panchganga. The large size idols above 12 feet are immersed in a quarry near the Rankala Lake named as "Irani Khan". These idols are made up of Plaster of Paris (PoP) or typical clay called "Shadoo" supported by small iron rods and are coloured with different types of paints such as varnish and water colours (Reddy and Vijaykumar, 2001). When these idols are immersed these coloured chemicals dissolve slowly in water bodies leading to significant alteration in the water quality. The colours used for decorating the idols contain different shades with mica for luster. These colour pigments are having heavy metals such as Lead, Copper, Zinc, Chromium and Iron. The other constituents of the idol, like bamboo, flowers, cotton, clothes and other pollutants such as eatables like 'Prasad', coir, plastics, etc. increase the nutrients in the lake leading to eutrophication. The water column is disturbed completely during the idol immersion altering the physico-chemical parameters like pH, turbidity, dissolved oxygen, total solids, biochemical oxygen demand, chemical oxygen demand, hardness, nitrates, phosphates, etc. along with heavy metals (Dhote *et al.*, 2001). Bottom sediments are known to act as a sink for metals introduced to surface waters from both natural and anthropogenic sources.

The fate of these idols is always sinking at the bottom of the water bodies and different chemicals get dissolved in water affecting the aquatic ecosystem. Plaster of Paris is chemically Calcium sulphate which is white odorless powder with molecular weight 136.15. Though toxicity data is not available related to physiology, Plaster of Paris may cause gastrointestinal discomfort when ingested and may cause irritation to skin, eyes and respiratory tract when come in contact.

The idols made up of Plaster of Paris remain intact in most of the lakes, tanks as water must be remaining quiet except the underwater motion imparted by movement of fishes and other animals. The idols must be getting disintegrated in creeks or rivers or in coastal marine environment due to water waves, tides and surfacewater turbulence generated by wind action (Asolekar, 2007).

The main objective of the present work was to study the alteration in the water quality after immersion of Ganesh idols and to observe the biological effect of colour pigments used for colouring the idols on the freshwater bivalve, *Lamellidens marginalis*. As the selected animal is the representative of lentic as well as lotic water bodies the study also reveals the results for both types of water bodies.

Degraded water quality affects macro-invertebrates including benthic organisms by eliminating many of them and those which remain become abundant seemingly due to decreased competition and increased tolerance for the adverse conditions. Macro-invertebrates and macro-benthic organisms can, therefore, serve as excellent diagnostic indicators for measuring extent of pollution of aquatic ecosystem (Gupta and Sharma, 2005). Among pollutants non-essential heavy metals are the major cause of concern for aquatic environment because of their toxicity, persistency, and tendency to accumulate in organisms and in undergoing food chain amplification (Agarwal, 1992).

The aquatic organisms are constantly exposed to different concentrations and combinations of toxicants and mixture toxicity experiments can yield information relating to actual pollution status of aquatic ecosystems in a realistic way. pH of the aquatic habitat is known to affect the toxicity of pollutants by influencing the rate of degradation, which in turn affects the chemical speciation and bioavailability. The presence of one metal influences the metabolism of another metal. Metallothionein synthesis is induced by heavy metal ions such as Cu, Zn and Cd. Mobilization of metals from watershed, resolubilization of metal from sediment and joint action of these metals with H^+ will stress aquatic life (Nair, 2005).

Zooplanktons, worms, mollusks and fishes suffer from various ill effects like mass mortality, chronic changes in behavior, low survival rates and morphological changes in different organ systems (Jadhav, 1995). Non-biodegradable toxicants like heavy metals bioaccumulate in biotic components. These toxicants change the natural condition of aquatic medium in turn causing behavioral responses in mollusks, fishes and other aquatic organisms. Therefore, toxicity testing is an essential component of evaluating water pollution. Presence of large number of heavy metals in ecosystems and their toxicity manifestation, ranging from mildly harmful to lethal has been reported by several researchers (Fugare and Deshmukh, 2004).

Mussels are widely used as bioindicators of aquatic pollutants in freshwater, marine and estuarine ecosystems and as a direct measure of metal bioavailability. Mussels can readily accumulate metals in freshwater ecosystems by direct transport of water across gills and from ingestion of suspended particles and bottom sediments. They are sedentary, long-lived, widely distributed and tolerant of high trace metal concentrations. As a result, their metal body burdens can reflect the contamination history of a certain environment (Jamil *et al.*, 1999). Bivalves form an important component in the food web of an ecosystem and also are of commercial importance (Gokhale, 1990). They have an inherent ability to act as sedentary filter feeders which absorb and accumulate metal ions in their tissues. Therefore, they serve as an index organisms providing information on the extent of metal on contamination in aquatic environment. Bivalves' maximum area is exposed to pollutants, primarily damaging the gill structure, reducing respiration mechanism and thereby compromising energy generation system (Fugare and Deshmukh, 2004).

The bivalve molluscs have a ciliary mode of feeding and gills also serve respiratory function. Among environmental parameters, particularly for freshwater species, temperature and hydrogen ion concentration strongly influence ciliary activity than does concentration of any other ion (Muley and Mane, 1995). Industrial effluents containing heavy metals, drained into rivers may affect the aquatic fauna through various ways. Individual and combined action of heavy metals produces different responses on animals. The action of cilia present on the gills of the mussel creates currents of water and this serves respiration and feeding. Combinations of certain heavy metals may be synergistic or antagonistic in action. Zn gets concentrated by certain mollusks and increases ciliary activities while Cu, Cd decrease the ciliary activities.

The reaction and survival of aquatic organisms under toxic conditions depend on several factors such as toxicity and concentration of the toxicant and temperature, salinity, DO, pH, etc. in addition to the type and time of exposure to the toxicant. Impairment of aerobic oxidation in *Lamellidens marginalis* due to toxicity of pesticides is also reported (Muley and Mane, 1987). The freshwater mollusks face the problem of endosmotic water gain which needs to be eliminated to balance the internal ionic concentration. In an aquatic medium pollutants interfere with a number of physiological processes. Bivalves exhibit a wide variety of responses, one of the most significant of which is manifested through osmoregulation (Patil, 2003).

Fe and Mn have been normal constituents of the hydrosphere; small increase above the normal level of them is toxic to biota. Enzymes are very sensitive macromolecules and are easily affected by even small changes either in internal or external medium. Metals bind with enzymes in organisms exposed to them and seriously impair the activity of enzymes involved in the synthesis or breakdown of tissue components (Ingle *et al.*, 1994).

The accumulation pattern in animals is dependent not only on uptake but also on the depuration rate which was reported in some mussels to be an exponential function of exposure time. Lamellibranch molluscs are known to concentrate heavy metals considerably. However, there was some loss in body weight implying physiological stress on the animal. High metal concentration reduces the filtration rate of the bivalves. Metal uptake via the gill was a major contributor to body burden. The gill has a very large surface area for filtration. The size variability of Cd accumulation with *Lamellidens* was attributable to age and size specific growth dependent metabolic activities (Das, 2003).

Sub lethal concentrations that usually prevail in water bodies are known to cause adverse biological effects manifesting as subtle physiological, biochemical, anatomical or behavioral changes in the exposed organisms. Such changes can be employed as early warning signals showing up as negative responses of impacted species at concentrations below acute thresholds (Oyewo and Pedro, 2006).

Some metals like Zn, Cu, Fe and Mn which are required for metabolic activity in organisms lie in the narrow "window" between their essentiality and toxicity. Other heavy metals like Cd, Hg, Cr and Pb may exhibit extreme toxicity even at low levels under certain conditions, thus necessitating regular monitoring of sensitive aquatic environments. The study of organisms as pollutant monitors has several advantages over the chemical analysis of abiotic components. Some ecological factors such as dissolved oxygen, salinity and detritus have a significant effect on both desorption and bioaccumulation of metals (Karadede and Unlu, 2007). Heavy metals affect the biologically active molecules such as lipids, proteins, amino acids and co-enzymes. Zn in traces is essential to sustain biological processes such as optimum body growth, development and reproduction; however, this essential micronutrient is also toxic to the organisms; if exposed to higher levels (Meenakshi, 1998).

The fate of chemicals which are released into the aquatic environment is determined by the interaction of processes such as bioconcentration, sorption, dissolution, volatilization, etc. In numerous studies exchanges of chemicals between two compartments have been studied as isolated processes (Schrap , 1991). As pollution may induce certain biochemical changes in fishes before the drastic cellular and systematic dysfunction manifest themselves, appropriate biochemical parameters could be used effectively as sensitive indicators (Agarwal, 1992).

It is necessary to establish relationships between the concentrations of chemical contaminants in the environment and in the tissues of biota and cause-effect relationships between contaminant concentration and the resultant biological effects. The combination of chemical analysis of contaminant levels in the body tissues of mussels and the measurement of biological effects in terms of physiological energetics is ideally suited to such a "cause-effect" framework. Physiological energetics measurements not only provide information of the key processes of energy acquisition, energy expenditure and thus energy available for growth and reproduction, but also reflect some of the major mechanisms of toxicity. Mussels and other bivalves readily accumulate hydrophobic organic contaminants in their tissues with minimal metabolic transformation.

Major mechanisms of toxicity reflected in physiological energetics include non-specific necrosis affecting the ciliary feeding activity of bivalves, uncoupling of oxidative phosphorylation causing an increase in respiration rate, inhibition of oxidative metabolism thus reducing respiration rate and toxic effects of membrane structure and function affecting processes of food digestion and absorption. Energetics offers a common currency i. e. energy enabling the consequences of primary toxic mechanisms at the cellular level to be translated into effects on growth, reproduction and survival at the individual and population levels. The ultimate effects at the higher levels of biological organization are thus more readily interpreted and understood. Field and mesocosm studies have provided confirmation that the long-term consequences to growth and survival of individuals and population can be predicted from measured effects on energy balance observed at the individual level (Widdows and Donkin, 1991).

Ecosystems are under the pressure of complex mixtures of contaminants whose effects are not always simple to assess. Biomarkers, acting as early warning signals of the presence of potentially toxic xenobiotics, are useful tools for assessing either exposure to, or the effects of these compounds providing information about the toxicant bioavailability (Picado *et al.*, 2007). Biomarkers are now used routinely in environmental monitoring to examine the toxicity of chemicals to non-target species and have been successfully applied in a wide range of species including fish, birds and invertebrates (Agrahari *et al.*, 2006).

Proteins are the important organic cellular constituent forming a major part of the cell boundaries along with lipids. During stress conditions the protein synthesis and inter-conversion between amino acids, glucose and fatty acids to liberate energy get affected. This depletion may constitute as a physiological mechanism and play a comprehensive role under stress, to provide intermediates to Kreb's cycle. Under proteolysis, enhanced breakdown dominates over synthesis, while in case of anabolic process; increase protein synthesis dominates the protein breakdown (Chaudhari, 1998).

Glycogen and glucose play a key role in carbohydrate metabolism of animals which are used for the intermediate metabolic requirements to meet the energy demand caused by stress (Karuppasamy, 2000). Cholesterol is about $1/3^{rd}$ of the total sterols existing in many species of Eulamellibranchia (Lomate and Chaudhari, 1990). Cholesterol has multiple effects on biological membrane and can modulate its fluidity, phospholipids spacing, enzyme activity within the membrane (Vijayalakshmi and Motlag, 1998).

The importance of metabolic profiles involving the estimation of different energy metabolites and the enzymes seemed pertinent in toxicological studies as they contribute to metabolic scope of animals. Phosphatases are considered as important hydrolytic enzymes involved in hydrolysation and active transport of metabolites for yielding energy. Phosphatases being non specific to substances they can catabolise proteins and nucleic acids besides carbohydrates. Hence their activity could reveal the turnover of metabolites at times of stress (Ramlingam, 2002). Among all the transaminases the most ubiquitous are those which catalyze the exchange of amino groups between glutamate and aspartate and between glutamate and alanine. Glutamic Oxaloacetic Transaminase and Glutamic Pyruvate Transaminase are important links between carbohydrate and protein metabolism, particularly during stress. Activities of Glutamic Oxaloacetic Transaminase and Glutamic Pyruvate Transaminase are considered as an index of gluconeogenesis (Tilak, 2002).

Studies addressing impact at histological and cellular levels of organization are particularly important to establish the cause and effect relationships between exposure to contaminants and adverse health of organisms. Histopathological indicators are beneficial in that they show the net effect of biochemical and molecular changes in the organism resulting from exposure to a contaminant. Light structure of tissues and organs is altered when levels of the contaminant are still at low levels. Therefore histopathological studies provide a valuable screening method of an ecosystem before severe ecological damage occurs. Histopathological biomarkers are also helpful as they can specify the target organs, tissues, cells and organelles of a single or group of toxins.

In the present study, the impact of idol immersion on water quality of different lakes in Kolhapur namely Rankala, Kotiteertha and Rajaram and river Panchganga was studied by analyzing the water samples from different sites of the said water bodies before, during and after the Ganesh festival for the two subsequent years 2006 and 2007. Also the sub lethal effects of the colour pigments used for decorating the idols on the freshwater bivalve, *Lamellidens marginalis* were studied after chronic exposure. The physiological effects were observed by comparing the biochemicals such as proteins, glycogen, cholesterol and lactic acid with the control ones. The impact on the enzymes like ACP, ALP, GOT, GPT, ATPase and LDH were also studied along with heavy metal accumulation in *Lamellidens marginalis* after exposure to the sub lethal concentrations. Light microscopy was used as a biomarker for studying changes at cellular level after exposure of the animal to the colour pigments. A laboratory level experiment was carried out by immersing the idols and exposing the animals in the same water for 15 days. The observations were recorded for changes in the water quality and changes in the biomakers in the bivalves.

The present study is presented in five chapters. The first chapter gives a detailed introduction of the topic. The second chapter gives detailed materials and methods used in the investigation. The third chapter deals with detailed observation of all parameters studied. The fourth chapter deals with discussion of all parameters studied. The fifth chapter deals with the summary, precise conclusion and a brief plan of future line of work. The dissertation also gives a detailed bibliography of the references which are directly and indirectly referred for the co-relation of the present investigation.