Our solar system originated through physical and chemical processes. After earth formed, organisms originated and evolved. The first organisms were microorganisms. The origin of earth and the evolution of life on our planet has been a long process. Microorganisms comprise most of the earth's biomass, maintain its environments, and hold the key in understanding the history of life on earth. They are the foundation of biosphere both from evolutionary and an ecological perspective. They were the first organisms on earth and have lived on this planet for a period of at least 3.7 billion years of the existence of the earth. Besides macroscopic plants and animals microbes are the major components of biological systems on this earth. They are ubiquitous in all ecosystems- in soil, water, air, inside our bodies and that of other plants and animals. They are present even at sites where no other life form could possibly exist such as deep inside the hydrothermal vents where temperature may be as high as 100°C, deep in the soil, under the layers of snow several metres thick and in the soil, acidic environments. They can perform activities in the zones where light cannot approach and thus photosynthesis cannot be the basic means to collect energy. In such zones, chemosynthetic microbes provide energy and carbon to the other organisms. Other microbes are decomposers, with the ability to recycle nutrients from other organism’s waste products. These microbes play a vital role in biogeochemical cycles. Microorganisms, being omnipresence, have impacted the entire biosphere.

1.1 Microbial biodiversity

New species of microorganisms evolved through the interactions of their genomes with the environment giving rise to great microbial diversity and altered ecosystem functions. The biodiversification of microorganisms has been occurring for over 3.85 billion years compared to only 600 million years for macroorganisms. In accordance with the Darwinian principles, mutation, genetic recombination and natural selection all played roles in evolution of new microbial species. Genetic variations and natural selection favoured the proliferation of some kinds of microorganisms and the extinction of others. The first lineages of bacteria (Aquificales and Thermotogales) appear to have been thermophiles that shared some physiological characteristics with Archaea, such as the presence of ether-containing lipids within their cytoplasmic
membranes. The Archaea evolved several important groups that could grow in hostile environments such as highly saline lakes and hot sulphuric pools.

1.2 Structure of microbial communities

Communities are usually characterised by a high state of diversity. The diversity of the community is related to the stability of the community. The diversity of microbial community can be described using diversity indices. Several mathematical indices called species diversity indices are used to describe the assemblage of populations within a community (Pielou, 1975). The two major components of species diversity are species richness and evenness. A widely used diversity measure of diversity is the Shannon-Weaver index (Shannon and Weaver, 1963). The diversity of the microbial community can be described using molecular analyses that reveal the genetic heterogeneity of the community. Species diversity tends to be low in physically controlled ecosystems; it tends to be higher in biologically accommodated ecosystems.

1.3 Ecosystems

The community and its abiotic surroundings compose the ecosystem, which is a self-sustaining ecological unit. Energy flows through an ecosystem and materials are cycled within the ecosystem by various populations of the community. Batch (Byers, 1963) and flow through (Monod, 1950) model systems permit the microbial ecologists to manipulate both abiotic and biotic parameters in a carefully controlled manner that allows for an understanding of how the components of the ecosystem interact. Substrate concentrations, temperatures, light intensities and other parameters can be manipulated to mimic particular habitats. In these model systems macroorganisms are excluded, permitting examination of the interrealtionships between microbial populations.

1.4 Microorganisms in their natural habitats

Habitats are the physical locations where microorganisms live. Because of their small size the habitats of many microorganisms occur on a microscale and are viewed as microhabaits. Autochthonous microorganisms fill functional roles called niches in their habitats. Other microorganisms are allochthonous, or foreign to the ecosystem; they may survive for varying periods of time but are viewed as transients that do not fill the ecological niches of that ecosystem.
The ecosphere where organisms live is divided into the atmosphere (air), hydrosphere (water), and lithosphere (soil) environments. The atmosphere is not known to support autochthonous microbial populations, but it serves as a medium for the rapid and global dispersal of many types of microorganisms. Many microorganisms produce spores that are transported through the atmosphere, their dormant state permits survival during air transport where they must exhibit resistance to desiccation and solar radiation. Compared to the atmosphere, the hydrosphere is far more suitable for microbial growth. The hydrosphere contains autochthonous microbial populations; most of which are motile and can grow at low nutrient concentrations. Large microbial populations typically are found in the neuston, which occurs at the air-water interface. Photosynthetic microorganisms are important members of the neuston and the underlying water column where light penetrates. Algae, bacteria, fungi and protozoa are characteristic autochthonous neuston microbiota. Algae and cyanobacteria typically occur nearer the surface, and anaerobic photosynthetic bacteria occur at greater depths of lakes. These photosynthetic microorganisms also occur in the sediments of the littoral zone, where light penetrates to the bottom. In deeper waters, decompositional acivity dominates below the compensation depth, which is the depth where photosynthetic productivity equals respiratory decomposition. Many lakes are characterised by thermal stratification with vastly different microbial populations inhabiting the epilimnion and hypolimnion above and below the thermocline, respectively.

Microorganisms play a key role both in lake productivity and in the transformation of organic compounds within a lake. Fungi and bacteria in freshwater ecosystems are largely responsible for the decomposition of allochthonous organic matter. The principal ecological functions of microorganisms in freshwater environments include decomposition of dead organic matter, liberating mineral nutrients for primary production; assimilation and reintroduction into the food web of dissolved organic matter; mineral cycling activities; primary productivity and supplying biomass as a food source for grazers.

The oceans occupy 71% of the earth's surface providing vast marine habitats (Rumney, 1968). Most marine bacteria are gram (-ve) and motile (ZoBell, 1946). Microorganisms are the principal producers and decomposers in the world's oceans. In the pelagic zone all primary production is carried out by microscopic algae and bacteria. In marine habitats planktonic microorganisms have a nearly exclusive role in primary
production. Freshwater mixes with marine waters in estuaries, which are characteristically more productive than either the ocean or the freshwater input.

Whitman et al (1998) completed a global survey of prokaryotic biomass in aquatic habitats, soils and subsurface sediments, and in the intestinal tracts of selected animals. Global survey of prokaryotes in aquatic habitats, exclusive of groundwater shows that cell densities in freshwaters (~10^6/ml) average approximately 20 times those of deep ocean water and twice that of the Upper Ocean and continental self-waters. Surface sediment layers of the ocean typically support high numbers of microbial cells (4.6 x 10^8 cm^3). Despite the low cell density in deep ocean water, this high volume habitat supports more than half of the global aquatic prokaryotic biomass.

1.5 Extreme habitats

There are some extreme habitats on earth where condition of temperature, pH, salinity, pressure, desiccation and other physical and chemical factors challenge the abilities of microorganisms to survive and to grow (Edwards, 1990). Microorganisms growing in such habitats evolved under conditions that permitted their survival and growth. They multiplied accordance with natural selection. For such adapted microorganisms the conditions of these habitats are not “extreme” but rather the normal physiological conditions for their growth in their natural habitats.

Cold environments prevail in the Polar regions and also through most of the world’s oceans. Most marine environments are at temperatures less than 5°C, and temperatures of -1.8°C, the freezing point of sea water are common. In these cold environments, microorganisms typically grow slowly and their populations usually are lower than in temperate regions. The cold benthic regions of the oceans exemplify habitats where microorganisms grow at slow rates with generation times of many hours or even days as opposed to less than half an hour for a typical enteric bacterium living in the gut of a warm blooded animal. Interestingly, in polar seas psychrotrophs rather than psychrophiles are the dominant bacterial populations. Physiological versatility, rather than a high degree of specialisation, appears to be critical for the long term survival of microbial populations in these cold habitats.

Soil temperatures in polar terrestrial habitats are even colder. Tundra soils are well under 0°C most of the year, with permafrost layer beneath the soils. Lichens and
other microorganisms adapted to slow growth and survival under extreme conditions predominate in these polar habitats. In these habitats many microorganisms are endolithic where they are partially protected from the hostile cold and desiccating conditions of those habitats. Yeasts with high lipid concentrations and endospore forming bacteria are commonly found in polar soils. Endospore forming bacteria are also commonly found in hot deserts. The ability to remain dormant during prolonged periods of drought allows these bacteria to survive.

Thermophilic bacteria and hyperthermophilic archaea also grow in very hot habitats, including areas of volcanic activity such as the deep sea thermal vents, and thermal springs where temperatures can be well above 100°C in the deep sea habitats. Extremely thermophilic archaea grow optimally at such high temperatures; some such as *Pyrodictium brockii* and *Pyrococcus furiosus* have optimal growth temperatures of 105-110°C. Some archaea such as *Sulfolobus* and *Acidianus* species, use inorganic sulphur compounds and chemolithotrophic metabolism to grow in these habitats. Extremely thermophilic bacteria such as *Thermus aquaticus* also grow in hot spring pools. *Bacillus stearothermophilus* is often the dominant bacterial species in hot springs in temperature zones of 55-70°C, but many others thermophiles, including thermophilic cyanobacteria and algae, also occur in such hot spring habitats.

Alkalophilic bacterial and archaeal populations live in habitats growing optimally at pH values above 8.5. Some alkaline lakes are also characterised by high salt concentrations. Extremely halophilic and alkalophilic archaea, such as *Natronobacterium* and *Natronococcus*, grow optimally at pH 10.0 or above. Nonalkalophilic archaea, such as *Halobacterium, Halococcus, Haloarcula* and *Halofax* grow in saline lakes and soils at pH values near neutrally.

Other extreme habitats include those lacking oxygen, those with very low nutrient concentrations and those with high levels of toxic heavy metals or high intensity radiation. The environmental conditions of each of these habitats limit the diversity of microbial populations that can survive. Yet in each, some microbial populations have evolved adaptations that permit their growth, so that virtually all habitats on earth, including the extreme habitats, have their characteristic microbial populations.
1.6 Water quality testing

The public health importance of clean drinking water requires an objective test methodology to evaluate the effectiveness of treatment procedures and to establish drinking water safety standards (Bonde, 1977). The most frequently used indicator organism for faecal contamination is the normally non-pathogenic *Escherichia coli*. Positive tests for *E. coli* do not prove the presence of enteropathogenic organisms but establish the possibility of such a presence because *E. coli* is plentiful and easier to grow than the enteropathogens, the test has a built in safety factor for detecting potentially dangerous faecal contamination.

Safe drinking water has been one of the proud achievements of developed countries and an important milestone that less developed countries are trying to achieve. According to rough estimates, more than 15 million deaths worldwide result annually from waterborne infections. Diarrheal diseases of small children alone result in more than 2 million deaths annually in developing countries (Young, 1996). Prevention of river requires effective monitoring of physico-chemical and microbiological parameters (Ramteke et al, 1994). Thus detection and enumeration of indicator organisms are of primary importance for the monitoring of sanitary and microbiological quality (Gunnison, 1999). The bacteriological examination of water has a special significance in pollution studies, as it is a direct measurement of deleterious effect of pollution on human health. For assessment of water quality is not only the physico-chemical characteristics of river conform to prescribed standard of microbiological water quality (APHA, 1998).

Physico-chemical quality of water also regulated the microbial growth. The elevated turbidity is often associated with the possibility of microbiological contamination, as high turbidity makes it difficult to disinfect water properly (Van Loon, 1982). High nitrate level also stimulates the algal growth (WRC, 2000). Total coliform (TC) and faecal coliform (FC) counts are the most widely used bacteriological procedures for assessment of the quality of drinking and surface water (McDaniels et al, 1985). Human faecal material is generally considered to be greater risk to human health as it is more likely contain human enteric pathogens (Scott et al, 2003). Typhoid fever, cholera, infectious hepatitis, bacillary and amoebic dysenteries and many varieties of gastrointestinal disease can all be transmitted by water. The most important
aspect of water quality is its freedom from contamination with faecal matter. The use of bacteria as water quality indicators can be viewed in two ways, first the presence of such bacteria can be taken as an indication of faecal contamination of the water and thus as a signal to determine why such contamination is present, how serious it is and what steps can be taken to eliminate it; second, their presence can be taken as an indication of the potential danger of health risks that faecal contamination possess (Baghel et al, 2005).

Water the most important resource of nature is increasingly becoming a scare resource. Rivers are playing an important role as major water resource in this planet. Unfortunately, river is being polluted by indiscriminate disposal of sewage, industrial waste, which affects its physico-chemical properties and microbiological quality (Koshy and Nayar, 1999). In the recent past, expanding human population, industrialisation, intensive agricultural practices and discharge of massive amount of wastewater into the river have resulted in deterioration of water quality. Water is playing an important role in the transmission of human disease. Free from contamination with faecal matter is the most important criteria of water quality assessment as the faecal matter contain human enteric bacteria (Scott et al, 2003). Coliforms are the major microbial indicator of monitoring water quality. Therefore, bacteriological assessment, particularly for coliforms, the indicator of contamination by faecal matter is routinely carried out to ascertain the quality and potability of water. Total coliform and total faecal coliform counts are the most widely used bacteriological methods for assessment of the quality of drinking water. The presence of such bacteria can be taken as an indicator of faecal contamination of the water and thus, to determine why such contamination is present, how serious it is and what steps can be taken to eliminate.

The World Health Organisation (WHO, 1998) provided guidelines for drinking water quality and recommended that no faecal coliform should be found in drinking water. Presence of faecal coliforms as *E. coli* serves as an indicator for the possible presence of other disease causing pathogens. Faecal coliforms are selected members of the coliform group of bacteria which are able to ferment lactose at 37°C and fairly specific for the faeces of warm blooded animals. The bacteriological parameters of different river systems have been studied by various groups (Badra et al, 2003; Arvanitidou et al, 2005). The bacteriological examination of water has a special
significance in pollution studies, as it is a direct measurement of deleterious effect of pollution on human health.

Among the microorganisms, fungi are probably more important, especially in all but the late stages of decomposition. The allochthonous species in freshwater habitats may be either through living or dead plant or animal material, soil or litter, having been in contact with water. Several workers have reported the occurrence of terrestrial fungi in freshwater resources. Works also had been conducted on the distribution of aquatic fungi in relation to physico-chemical factors of fresh water habitat (Rajashekhar and Kaveriappa, 2003). The autochthonous fungi including those of strictly aquatic habitats and those of terrestrial habitats are commonly found in pools, ponds, lakes, rivers and streams. Aquatic fungi are significant components of the structure-functional organisation of biohydrocenoses. In aquatic ecosystem hydromycetes participate in turnover of organic substances of energy (Park, 1972b). Fungi contribute to the energy flow and productivity of an ecosystem by their presence as a source for meeting the basic requirements of organic carbon and higher trophic levels.

The classic test for the determination of water quality is the standard Most Probable Number (MPN) technique which involves the three tube test with lactose broth. Fermentation tubes were inoculated with 10 ml, 1ml, 0.1 ml aliquots of the sample in accordance with standard methods APHA (1998). The tubes were incubated at 37⁰C for 24h. Positive tubes producing acid and gas were used in estimating the presumptive MPN/100ml.

Confirmed test was carried out by transferring a loopful of broth from a positive tube into brilliant green lactose bile (BGLB) broth, followed by incubation at 37⁰C for 24-48 hrs. The tubes were observed for gas formation. Completed test was performed by plating a loopful of broth from a positive BGLB tube onto an Eosine Methylene Blue (EMB) agar plates. The plates were incubated at 37⁰C for 24h and observed for dark red colonies and metallic green sheen. Stock cultures of the colonies were prepared on nutrient agar slants and colonies were used for gram staining and biochemical tests. Final fecal coliform or E. coli count as MPN/100 was calculated based on the completed test.

The drinking water standard does not absolutely exclude the possibility of ingesting enteropathogens bit attempts to reduce this possibility to a statistically
tolerable limit. Enteropathogens are likely to be present in much lower numbers than faecal coliforms, and a few infective bacteria are usually unable to overcome body defenses. A minimum infectious dose from several hundred to several thousand bacteria is necessary for various diseases to establish an actual infection. Drinking water supplies that meet the 1/100 ml coliform standard have never been demonstrated to be the source of water borne bacterial infections (Olson and Nagy, 1984).

Enteroviruses and the cysts of some pathogenic protozoa are somewhat more resistant to disinfection by chlorine chloramines or ozone than bacteria, and occasionally active virus particles or live cysts are recovered from water treated to meet faecal coliform standards (Olson and Nagy, 1984). The considerable uncertainty about the hazards posed by enteroviruses in water supplies and recreational waters is due to the difficulty of detecting and enumerating viruses have to be grown in suitable tissue culture cell lines and detected by immunological processes.

1.7 Microbial accumulation of heavy metals

Various heavy metals other than mercury, including tin, cobalt, chromium, nickel, cadmium, and thallium are used in metal alloys or as catalysts. Their mining, smelting and ultimate disposal cause heavy metal pollution problem. These metals are substantially toxic in higher concentration to plants, animals and microorganisms. Release of Cd, Cr, Pb and Ni into the environment has increased in the recent years at an alarming rate. Cadmium discharged into the environment in large amounts as an industrial waste has led to its current rank as a major anthropogenic pollutant (Cunningham & Lundie, 1993). It enters the human food chain through plants, smoking materials and diet (Kalcher et al, 1993). Cadmium is a potent carcinogen, embryogen, teratogen and mutagen and it may cause hyperglycemia, reduced immuno-potency and anemia, due to interference with iron metabolism (Sanders, 1986). Cadmium and Pb, though non-essential and non-beneficial, are considered highly toxic to plants, animals and microbes (Ajmal et al, 1998). Chromium (VI) species are also extremely toxic and exhibit mutagenic and carcin-ogenic effects on biological systems (McLean & Beveridge, 2001). Microbial reduction of Cr (VI)) to Cr (III) is a potentially useful process for remediation of Cr (VI) affected environments (Michel et al, 2001; Ganguli & Tripathi 2002; Camargo et al, 2003). Microbes showing resistance to Cr (VI) have
generally been isolated and characterized from serpentine soil (Pal and Paul, 2004), from river water contaminated with pulp paper mill effluent (Rajkumar et al, 2012). Nickel, another toxic metal, reduces growth and development in plants and is teratogenic (Chen & Lin, 1998). Microbes showing resistance to Ni have generally been isolated and characterized from anthropogenically polluted sites (Grass et al, 2001; Pal et al, 2004). Lead, a major pollutant that is found in soil, water, air and is highly toxic to humans, animals, plants and microbes (Low et al, 2000).

Microorganisms are considered to be the best indicators of changes in environmental conditions (Bezverbnaya et al, 2005). In general, they are very sensitive to low concentration of heavy metals but rapidly adapt to the specific habitat conditions. An alternation of their activity may support the plasticity of communities and in some cases it ensures the possibility of melioration of the environment. However, it has been experimentally demonstrated that bacterial adaptation to heavy metal contamination in marine environment, can be accompanied by the spread of R-factors than by mutation and selection (Bhattacherjee et al, 1988). In plasmids, these heavy metal resistance genes may be linked with genes of antibiotic resistance and genes responsible for pathogenic properties (Bezverbnaya et al, 2005). Thus, individual bacterial strains have developed the capacity to survive under toxicological stress which is sometimes hazardous to human and animals.

Conventional methods as precipitation, oxidation/reduction, ion exchange, membrane filtration and evaporation, though capable of eliminating these toxic metals from the environment, are extremely expensive and inefficient for metal removal from dilute solutions ranging from 1 to 100 mg of dissolved metal per liter (Volesky, 1990). Microorganisms and microbial products have been reported to efficiently remove soluble and particulate forms of metals, especially from dilute solutions, though bioaccumulation and therefore microbe-based technologies provide an alternative to the conventional techniques of metal removal/recovery (Ozdemir et al, 2004). Microbes are capable of accumulating toxic metal ions by two well defined processes viz: (i) biosorption: an energy independent binding of metal ions to cell walls and (ii) bioaccumulation: energy dependent process of metal uptake into the cells. Both live and inactivated
Microbial mass of bacteria, fungi and algae are utilized for removing toxic metal ions (Li et al, 2004). Microorganisms, owing to their large surface-to-volume ratio and high metabolic activity, are important vectors in introducing heavy metals and radionuclide pollutants into food webs. At neutral to alkaline pH, heavy metals in soils and sediments tend to be immobilised by precipitation and adsorption to cation exchange sites of clay minerals. Microbial production of acid and chelating agents can reverse this adsorption and mobilize these toxic metals. Microbial metabolic products that can chelate metals include decarboxylic and tricarboxylic acids, aromatic hydroxy acids and some specific chelators such as the enterochelins and ferrioxamines. Mobilization is often followed by uptake and intracellular accumulation of the heavy metals both by microorganisms and by plant roots. Filamentous fungi were shown to transport heavy metals and radionuclides along their hyphae. Microbial accumulation of radionuclides, with clear human health implications, has been demonstrated in some Arctic areas (Hanson, 1967). Lichens are extremely effective in concentrating the nuclides such as $^{90}$Sr and $^{137}$Cs from atmospheric fallout.

The vast array of microbial activities and their importance to the biosphere and to human economies provide strong rationale for understanding their diversity, conservation and exploitation for society. Microorganisms thus merit more respect from the conservationists and ecologists due to the recognition of the current massive economic values of the products of microorganisms that are already cultured, the vastness of the currently untapped genetic resources and their potentialities (Hawksworth, 1994).

Most of the rivers in urban areas of the developing world are the end points of effluents discharged from the industries. Paper pulp industries are the sixth largest effluent generating industries of the world (Ugurlu et al, 2007). These effluents have been found to contain approximately 700 organic and inorganic compounds (Tambekar et al, 2008). Some of these substances have been classified as carcinogenic and mutagenic (Karrash et al, 2006). Microorganisms by virtue of their wide degree of metabolic adaptability have shown resistance to several heavy metals and many of them have the potential to sequester metal ions from aqueous solution (Kappo). The microorganisms...
have acquired a variety of mechanisms for adaptation to heavy metals. Among the various adaptative mechanisms, metal sorption, mineralisation, uptake and accumulation, extracellular precipitation and enzymatic oxidation or reduction to a less toxic form and efflux of heavy metals from the cell have been reported (Joshi-Tope and Francis, 1995). Reports are available on antibiotic and heavy metal resistant bacteria isolated from different polluted environment (Rajbanshi, 2008; Ezaka and Anyanwu, 2011) however, report is available from Barak River contaminated with paper mill effluent. The present research work was an attempt to evaluate the status of heavy metal resistant bacteria isolated from Barak River water contaminated with paper mill effluent.

The present study focuses on the culturable bacterial diversity of Barak River system for its pollution status. The study assesses seasonal water quality of Barak River water with the use of bacteriological variables. The proposed research work involves the seasonal distribution of fungal diversity along with physico-chemical parameters of the river water. The present study was also an attempt to evaluate the status of heavy metal resistant bacteria isolated from Barak River water contaminated with paper mill effluent. The bacterial isolates were tested for their resistance to chromate by growth in nutrient broth tubes containing various concentrations of chromium. Chromate reductase activity of the bacterial isolates was assayed for selective bacterial isolates. The resistance of the selected isolates against other heavy metals was also tested in the nutrient broth. The isolated bacterial strains were characterised morphologically and biochemically and screened for heavy metal tolerance. Antibiotic sensitivity profile of the selected bacterial isolates was carried out against different antibiotics. The 16S rDNA gene sequencing of the selected isolates was carried out.

1.8 Objectives

Present investigation was broadly carried out with the following objectives:

- Seasonal microbial load from the water samples of the Barak River.
- Seasonal estimation of the physico-chemical variables of water samples.
• The structural composition of microbial communities viz, fungi and bacteria present in the river Barak.

• Ecological and genetical diversity of microbes in the Barak River in Cachar.