Microorganisms exist on the earth in extremely diverse form – genetically, metabolically and ecologically. Microorganisms comprise most of the earth’s biomass. We have only just begun to discover the variety and abundance of microbial life on the earth. Despite our increasing knowledge of the scale of microbial diversity, most microbes observed in natural environments remain uncultivated. It can be stated that the functioning of the whole biosphere depends absolutely on the activities of the microbial world (Madigan et al, 2003). 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.

Water the most important resource of nature is increasingly becoming a scare resource. 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. 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. 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.

Microorganisms 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. Microorganisms and microbial products can efficiently remove soluble and particulate forms of metals, especially from dilute solutions, through bioaccumulation and therefore
microbe-based technologies provide an alternative to the conventional techniques of metal removal. 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.

The present study focused on the culturable bacterial diversity of Barak River system for its pollution status. We have assessed seasonal water quality of Barak River water with the use of bacteriological variables. The proposed research work involved the seasonal distribution of fungal diversity along with physico-chemical parameters of the river water. In this thesis attempts have been made to evaluate the status of heavy metal resistant bacteria isolated from Barak River water contaminated with paper mill effluent.

Findings of the experiments conducted are summarised as follows:

(1) Water samples were collected bimonthly from four different sites of river Barak namely, Panchgram, Katakhali, Annapurnaghat and Sadarghat from June, 2008 - June, 2010. The water samples from different sites and seasons were analysed for pH, dissolved oxygen, alkalinity, FCO₂, chloride content, nitrate, phosphate and total hardness.

(2) Microbial strains were isolated from the collected water samples by adopting serial dilution employing pour plate as well as spread plate technique. For enumeration of bacteria, serially diluted water sample was plated on nutrient agar and different selective media. All media plates were incubated at 37°C for 24 to 48h and final counts of colonies were noted. All isolations were performed in triplicate. Qualitative analysis was carried out by multiple tube fermentation technique using the three tube test with lactose broth (APHA, 1998).

(3) The cultural and morphological features of bacteria were studied by adopting standard methods (Collins and Lyne, 1989; Goodfellow, 1989). Different colony features such as configuration, elevation, margin, texture, consistency etc. were noted down by using a hand lens.
(4) For the recovery and isolation of fungi from the collected water samples, one ml of
diluted water sample was poured on Czapek Dox, PDA and Rose Bengal agar media in
sterile petri dishes under aseptic condition. Fungi were identified to genus level using
Barnett and Hunter (1999). Cultures were identified to species level using colony
diameter and spore measurement following references and monographs adopted by

(5) The distribution pattern of different fungi was studied by estimating their frequency,
density, abundance, relative frequency and relative density.

(6) Shannon index, Simpson’s index, Evenness and Equitability were analysed to study
the diversity pattern of fungal communities.

(7) In all the sites higher values of Dissolved oxygen (DO) was observed during
monsoon season which gradually declined attaining minimum during winter season. pH value of river water collected from different sites did not show significant variation
during the present study. The pH values ranged from 5.3 to 6.9; 6.4 to 7.75; 6.1 to 7.9;
6.1 to 7.9 in the site Panchgram, Katakhali, Annapurnaghat and Sadarghat respectively.
During monsoon season, the free carbon dioxide (FCO2) value was found to be high
whereas lower values of FCO2 was observed during summer and winter seasons in all
the study sites. In all four study sites the total alkalinity value showed a rising trend
during summer and winter season. In contrast, the values declined during monsoon
season. The observed values of total alkalinity were within the limit as outlined by

(8) The level of nitrate was high in monsoon season while low in winter season in all
the study sites. The phosphate level was also high in monsoon season as compared to
summer and winter season in all the study sites. Maximum level of hardness was
recorded during rainy season in the Annapurnaghat site and minimum during winter at
Panchgram. In all the study site the chloride concentration was also well within the
highest desirable limit of 250 mg/l as prescribed by WHO (1998).

(9) Twenty eight species of fungi belonging to sixteen genera were isolated and
identified from the water samples. The total colony forming units (cfu) of fungi was
relatively high during monsoon season and the lowest was observed in samples taken
during winter season. Members of the genus *Aspergillus* were common in almost all the study sites. *Aspergillus niger*, *A. Fumigatus* and *A. flavus* were frequently isolated.

(10) The genus *Aspergillus* was represented by eight species and had the greatest diversity of the isolated species as well as the highest fungal total count. The genus *Penicillium* was represented by *Penicillium citrinum* and *P. chrysogenum*. *Bipolaris* and *Nigrospora* were reported only in June, 2008 and April, 2009 from Sadarghat and Annapurnaghat respectively.

(11) In Panchgram site, the Shannon diversity index was found to be maximum (H= 2.2) during June, 2008. Shannon diversity index was maximum for the Katakhal site (H= 1.48) during June, 2008. In Annapurnaghat site, the Shannon diversity index was observed maximum (H= 1.67) during August, 2008. In Sadarghat site, the highest value of Shannon index was observed during April, 2009 (H= 1.30).

(12) Simpson index was found to be maximum (D= 0.88) during June, 2008 in Panchgram site. In Katakhal site Simpson index also was found to be maximum (D= 0.74) during June, 2008. Simpson index was observed maximum (D= 0.79) during August, 2008 in Annapurnaghat site. Simpson index was observed maximum (D= 0.71) during April, 2009 in Sadarghat site.

(13) Evenness and equitability for the site Panchgram presented maximum values (e= 0.98; J=0.98) during February, 2009. The highest values of evenness and equitability were observed maximum (e= 0.98; J= 0.98) during February, 2010 in Katakhal site. The values of Evenness and Equitability were observed maximum (e= 0.99; J= 0.99) during December, 2009 in Annapurnaghat site. The values of Evenness and Equitability were observed maximum (e= 0.97; J= 0.98) during June, 2010 in Sadarghat site.

(14) The bacterial isolates were tested for their resistance to different heavy metals by growth in nutrient broth tubes containing various concentrations of heavy metals (0.1, 0.5, 2.0, 4.0 mM). The metals selected for the present investigation include Ni, Co, Cd, Cu and Cr. Except Cr the rest of the metals were used as their chloride salts. The bacterial growth was determined by measuring the optical density using spectrophotometer at 540 nm. Relative growth of the isolates was expressed as the percentage of those obtained in untreated control which was taken as 100%. 15 pure
cultures of bacterial isolates showing resistance to different heavy metals were isolated in pure form.

(15) It was observed that all the isolates were resistant to heavy metals showing growth at lowest concentrations. But with the increase of metal concentrations, the percent relative growth of the isolates decreased. Based on their growth in different concentrations of Cr$^{6+}$, isolates D (2), K(6)PA6, E (3) and E (4) were showing 34- 49% of growth at a concentration of 4.0 mM. of Cr$^{6+}$. The four selected isolates were then subjected to chromium reduction assay.

(16) For chromate reduction assay, nutrient broth containing 100 $\mu$m of Cr (VI) was inoculated with isolates and incubated under continuous shaking up to 72 h at 37$^\circ$C (Park et al., 2000). Samples of inoculated medium were collected during the incubation period after every 24 h interval. Growth was also determined by measuring the optical density at 540 nm. No isolate showed significant reduction after 24 h of incubation. After 72 h of incubation, the isolate E(4) showed highest reduction (34.38%) followed by E(3) and K(6)PA6, both showed 28.75% reduction and then D(2) (27.5%).

(17) Morphological and biochemical characteristics of the selected chromium resistant bacterial isolates were carried out. Based on comparison of these characters with standard descriptions in Bergey’s Manual of Determinative Bacteriology (9th ed. 1994) and further molecular characterisation, the isolate K(6)PA6 was identified as Bacillus cereus.

(18) The 16S rDNA gene sequence of the isolate K(6)PA6 was submitted to the NCBI Genebank database under the accession number JN202315. Comparison of morphological and biochemical characteristics with standard descriptions in Bergey’s Manual of Determinative Bacteriology (9th ed. 1994), isolates E(3), E(4) and D(2) were identified as bacteria belonging to a group known as “coliform” that exist in intestine of warm blooded animals.

(19) Data revealed that except K(6)PA6, all the isolates were gram(-)ve. Carbohydrate utilisation of all the isolates showed positive response for glucose and negative response for sucrose, rhamnose and lactose.
The chromium resistant isolates were tested for their sensitivity to 10 different antibiotics. Isolate E (4) appeared to be most susceptible being inhibited by 8 antibiotics and resistant to penicillin G and ampicillin. The isolate E (3) was resistant to as many as five antibiotics and showed susceptible responses to the rest of the antibiotics. Both the isolates K(6)PA6 and D (2) were resistant to four antibiotics and showed intermediate to susceptible response to the rest of the antibiotics.

From the results of the investigation presented in this thesis it can be concluded that the river water of Barak near Hindustan Paper Corporation may be contaminated with chromium. Reduction of chromium by bacterial isolates can be considered as an effective tool for bioremediation of chromium.

The present investigation also clearly indicated that most of the study sites of Barak River were not suitable for domestic purpose with respect to the maximum permissible limits of total coliform and total faecal coliform as per the standards of National River Conservation Directorate (NRCD), India. From the results of the bacteriological investigation of the Barak River it can be concluded that the river water was polluted by sewage, faecal contaminants and industrial wastes and the water of river Barak is not suitable for drinking and other recreational purposes.