1.0 Abstract.

The investigation (February 2000 to January 2002) of seasonal variation in the 20 physico-chemical (water quality) and 12 aquatic microbial variables revealed that the surface water of river Lakshmanatheertha followed by river Lokapavani are more polluted than the other three water courses studied. The initial hypothesis that, the four upstream tributaries might have caused pollution and thus might be responsible for the deterioration of water quality in the main river Cauvery was rejected, because, most of the microbial and water quality parameters studied were, significantly different in the river Lakshmanatheertha. In this investigation more than 1% i.e., upto 4% of the bacteria could be grown on the artificial nutrient agar media. The heterotrophic bacterial abundance in these five watercourses studied was largely controlled by physico-chemical (water quality) variables, notable are Temperature, Turbidity, PO$_4$, Calcium, TASA, Rainfall, COD, Conductivity, Chloride, CO$_2$, Chlorophyll-a, Temperature, Turbidity, TSS, SWV, NO$_3$, DO, LpH. The size of bacteria is an important trait in the predator prey relationship of aquatic bacteria and bacteriovorous protests. In this study, SO$_4$, DO, SWV, PO$_4$, TASA, BOD, COD, Conductivity, Chloride and Chlorophyll-a were potentially responsible for much of the bacterial cell-size variations. The SGR of heterotrophic bacteria was more significantly different in the river Lakshmanatheertha only. Comparatively more negative values of SGR of heterotrophic bacteria were noticed in the river Lakshmanatheertha, for e.g. Out of 50 determination 15 in the river Lakshmanatheertha, 3 in river Lokapavani, 2 each in rivers Harangi and Cauvery, and 1 in river Hemavathy. Over all investigation on micro-plankton revealed that the presence of phytoplankton species like, Gomphonema, Navicula, Cyclotella, Melosira, Nitzschia, Syneuda, Scenedesmus, Chlorella, Ankistrodesmus, Oocystis, Closterium, Actinastrom, Chlamydomonas, Gloeocystis, Crucigenia, Spirogyra, and Ulothrix, and zooplankton species like Paramecium, Strobilidium, Glucoma, Colpodium, Coleps, Colpoda, Cyclops, Daphnia, Keratella, Lepadella, Brachionus etc.
The spatial study along the full length of rivers Lakshmanatheertha and Lokapavani revealed that, most of the water quality and microbial variables were more significantly different in sites LT-4 and LT-5 in river Lakshmanatheertha, and in site LP-4 in river Lokapavani. However, the site LT-5 was entirely different significantly with respect to conductivity, turbidity, CO₂ and abundance of FLB, TB, CFU’s, % of CCFUs and SGR of heterotrophic bacteria. The final conclusions of spatial investigation revealed that the site LT-5 in river Lakshmanatheertha and site LP-4 in river Lokapavani were significantly different than the remaining sampling sites studied.

1.1 Outline of the thesis.

This thesis entitled “Microbial ecology of Lakshmanatheertha, Harangi, Hemavathy, Lokapavani and Cauvery river Ecosystems in south Karnataka, India.” Begins with abstract and brief preface. The thesis is then divided into two parts. The first part (Chapter 2-8) deals with a 24 months seasonal study which compares and contrasts the heterotrophic bacterial abundance, cell-size, specific growth rate of heterotrophic bacteria and abundance of micro-planktons (micro-phytoplankton, zooplankton and total plankton) and relevant environmental (water quality) variables in the main water course, the river Cauvery, with that of its four important upstream tributaries such as Lakshmanatheertha, Harangi, Hemavathy and Lokapavani. All the sampling sites of the main river Cauvery and its four important upstream tributaries are in Southern part of Karnataka state, India.

The second part of thesis (Chapter 9 and 10) deals with a spatial study (carried out on five occasions) along the river Lakshmanatheertha and river Lokapavani. This study includes the measurements of routine microbial and environmental, and also they were compared and contrasted for variations in the concentration of microbial and environmental variables between sites along the continuum of both the rivers under study, by using appropriate statistical test. The thesis concludes with a general discussion. This is followed by a list of literature cited in the text of the thesis and list of publications arising from this thesis.
1.2 Preface.

Water is aptly described as the 'Mother of life', because the life evolved millions of years ago first in water, and is the most essential and basic component of all living things (both plants and animals including microorganisms), as it supports the life processes by providing vital or essential nutrients to living organisms. As most of the bio-chemical reactions that takes place through the metabolism and growth of living (both unicellular and multicellular) organisms involves water, without water no life is possible to sustain on this planet Earth. Water is also known as 'Natural Liquid Gold' because it is the most valuable and precious liquid of the natural resources available. It is called 'Universal Solvent' as most of the inorganic compounds; all-natural elements and many organic chemicals are dissolved in water.

Water occupies 71% of planet Earth's surface, of this 71% of water covering the Earth's surface, ocean and inland seas contains about 97%, the icecaps of mountain and glaciers is about 2.15%, surface and ground water resources about 0.65% and the atmosphere about 0.02% to 0.05%. This distribution shows that, out of all the available water on the Earth surface, only 3% constitute fresh water which is present in the form of icecaps, glaciers, rivers, lakes, ponds, streams and ground water resources. Out of this 3% of fresh water only 0.6% is suitable for human consumption (Neill, 1993). Fresh water habitat occupies relatively small portion of the Earth's surface as compared to marine and terrestrial habitats, but their importance to man is far greater, because fresh water habitat is the convenient and cheapest source of fresh water for domestic and industrial needs. Fresh water habitat may consist of lentic (standing or stationary water bodies like lakes, ponds, puddles etc.,) or lotic (running fresh water bodies like river and streams) ecosystems. Lotic ecosystems are affected directly by using them as waste water devices and indirectly by contaminating the soil. The river waters act as a chief and dependable source of water, but recently in India, due to an increase in population, urbanization and rapid phase of industrialization (all of which might) have
caused pollution of rivers by indiscriminate disposal of sewage, domestic wastes, agricultural runoff, industrial effluents and plethora of other human activities. Water the greatest gift of nature, a plentiful supply of clean water is essential for the survival of the human being, plants, and other animals, including micro organisms. In India, supply of potable water is a serious problem, because of population growth. After 50 years of independence, India may have attained the status of nuclear power, but still more than 80% of Indians across the country do not have access to potable water supply. Thus, the water which is regarded as the soul of nature, its pollution will perish / destroy the entire biotic community. Pollution of rivers first affects its physico-chemical quality and then systematically destroys the microbial and plankton communities, thus imbalancing the delicate microbial food web which in turn affects the food chain of that ecosystem (Pelczar, 1993).

The term ecology is derived from the two Greek words, ‘oikos’ meaning ‘household’ or ‘dwelling’ and ‘logos’ meaning ‘study’ or ‘law’ or ‘discourse’. Thus, ecology is the law of the household or the science that explores the interrelationships between organisms and their living (biotic) and non-living (abiotic) environments. Aquatic ecosystems (consisting of oceans, lakes, ponds, pools and lotic waters) play an important ecological role on a global scale, as the greater part of many natural microbial conversions occur in water. Further, the micro organisms occupy a key position in the orderly flow of materials and energy through the global ecosystem by virtue of their metabolic abilities to transform organic and inorganic substances. Heterotrophic bacteria in fresh waters are important in the processing of natural organic matter and in bio-purification of water, which receives organic pollution. Degradation of organic matter contributes to the purification of the ecosystem and is therefore a major process controlling water quality (Servais and Garnier, 1993). The heterotrophic bacteria are accounted for important proportion of decomposers, and also represent substantial nutrient resources available to support the higher trophic level, because by virtue of their abundance, ability to use dilute substrate, high assimilation efficiencies and rapid growth rates represent a significant resources available to support the next higher trophic level
(Thomas, et al., 1990; Hessen, 1998, Travnik, 1998; Choon Weng Lee, et al., 2001; Kritzberg, et al., 2004), and are responsible for much of the respiration in large rivers; they may also affect the amount and quality of carbon transported by large rivers to the oceans (Findlay, et al., 1992, Benner, et al., 1995, Castillo, et al., 2004). In order to understand the regulation of bacteria by ‘top-down’ (Grazing activities by protozoans and other microorganisms) and ‘bottom-up’ (Nutrient supply) forces, it is useful to study how abundance and productivity changes with respect to time (seasonally) and in conjunction with environmental variables and grazing abundance (Stockner and Porter, 1988; Pernthler, et al., 1996; Adewoye and Lateef, 2004; Lopez-Archilla, et al., 2001;) are believed to regulate bacterial abundance and production.

As in both fresh water and marine waters, the biomass and size distribution of bacteria are important parameters of ecosystem function. Given the important ecological role of bacteria factors regulating the productivity (growth rate) and biomass (abundance and cell-size) of these communities are of interest. Increasing nutrient load leads to a change from dominance of small cells in nutrient poor environment to dominance of larger cells in nutrient rich environment was suggested by (Thingstad and Sakshang, 1990; Kristina, et al., 2002). The role of large bacteria in the river is important as competitors, large bacteria limit the development of small ones, and they play a buffer role in the degradation of organic matter (Gonzalez, et al., 1990; Garnier, et al., 1992; Jugnia, et al., 2000). The cell-size may have a meaningful ecological role in the planktonic food-web (Letarte and Pinel-Alloul, 1991). Further, the ups and downs in the bacterial cell-length in different season may be due to variation in food supply and in the grazing pressure from the higher trophic levels (Wright, 1988; Jugnia, et al., 2000; Hahn and Hofle, 2001). The, grazing by protests as the dominant factors controlling bacterial cell-size mortality, however in some habitats and seasons, metazoan grazing or lysis by phages may play a important role (Wommack, et al., 2000).

Generally, the food web in the aquatic ecosystem plays an important role in the consequence of high growth potential of heterotrophic bacteria (Sanders et al., 1992; Simek, et al., 2006). The ecological importance of
heterotrophic bacteria has led to much attention being paid to the abundance, biomass and activity of heterotrophic bacteria. Pace, et al., (1990) reported that on annual time scale, the abundance is remarkably stable, growth rates are sufficient to allow population turnover in much shorter time scales. Much of the primary production in aquatic ecosystems is ultimately processed by planktonic bacteria (Giorgio and Cole, 1998). Traditionally, organic carbon has been considered the main factor limiting heterotrophic bacterial growth in the aquatic environments. Many researches have suggested that substrate supply (i.e., bottom up control) could be important in regulating bacterial abundance and growth (Cole et al., 1988; White et al., 1991). The most research on the population ecology of marine bacteria continues to be carried out in rich coastal and shelf waters. Much less has been done in open ocean regions and also in lotic ecosystem like streams and rivers (Ducklow and Hill 1985; Sinsabaugh et al., 1997; Foreman, et al., 1998; Yamakanamardi and Gouider, 1999). Our lack of knowledge is due to two factors. First, bacterial respiration is simply more difficult to measure accurately than is bacterial production. Second, there has been a general belief that rates of catabolism and anabolism are tightly coupled and that maximum efficiency and economy are achieved during growth (Giorgio and Cole, 1998).

The plankton community is a heterogeneous group of microscopic organisms, which consists of two major groups of plankton i.e., phytoplankton and zooplankton. Phytoplankton, are ecologically significant as they form the basic link in the food chain of all aquatic animals, and plays a key role in maintaining proper equilibrium between abiotic and biotic components of ecosystem. (Misra et al., 2001; Harsha and Malammanavar, 2004). Heterotrophic bacteria rapidly use organic carbon of algal origin (Chen and Wangersky, 1996). Many investigators have observed a significant correlation between bacteria and Chlorophyll-a (phytoplankton) in various ecosystems, suggesting that phytoplankton productions are potentially important substrate for bacterioplankton (Wang and Priscu, 1994; Bouvey, et al., 1998b).

Micro zooplankton defined herein as heterotrophic organisms through their grazing impact they can control phytoplankton production
(Verity, et.al, 1993), and play a significant role in the fate of bacteria (Weisse and Scheffel - Moser, 1991) and in regeneration of nutrients (Goldman, et.al., 1987), and also form the vital link between autotrophs and heterotrophs in an aquatic ecosystem. Fresh water zooplankton is an important component in aquatic ecosystems whose main function is to act as primary and secondary links in the food chain (Torres et al., 1998). The role of planktonic protists such as heterotrophic nano-flagellates and ciliates in microbial food webs are to consume bacteria that are too small to serve directly as major prey items for most zooplankter (Nakano et al., 2001). Heterotrophic nano-flagellates are the most important consumers of bacteria (Nakano et al., 1998), but, while ciliates can be temporarily significant bacterial consumers (Simek et al., 1995), rotifers are usually less important (Sanders et al., 1989; Pace et al., 1990). Cladocera of the genus Daphnia are also among the most important consumers of bacteria and their impact is occasionally the greatest of all heterotrophs (Sanders et al., 1989; Pace et al., 1990). Further, many zooplankton which play important role in biological control for example, cyclopoids such as species of Microcyclops, Megacyclops and Mesocyclops attack mosquito larvae (Altaff, 2004). Finally, the heterotrophic bacteria can serve as food for protozoan and some metazoans and can ultimately support the growth of micro crustaceans (Hessen, 1998; Travnik, 1998) in aquatic ecosystems.

In order to understand the regulation of micro organisms by ‘top-down’ and ‘bottom-up’ forces, it is useful to study how abundance, cell-size and SGR of heterotrophic bacteria and abundance of micro plankton changes with respect to spatial variation and in conjunction with physico-chemical (water quality) variables and grazing abundance. McDonough, et al., (1986) found that microbial communities have seasonal, spatial and between system variation in population densities and growth rates. Most studies of lotic bacteria have taken a broad, assemblage level approach, where numbers, production and biomass are measured with no idea of the underlying mechanism and diversity (Leff, 1994; Leff and Lemke, 1998). Different factors that seem likely to affect the microbial communities in lotic environments include the quantity and quality of liable DOC, inorganic nutrient concentrations and predation by higher trophic level (Meyer and
The observation that the biological availability, quantity, quality and composition of DOC changes along rivers (Leff and Meyer, 1991; Sabater et al., 1993; Sun et al., 1997) suggests that heterotrophic bacterial assemblages along a river continuum may change in a predictable manner. Recent evidences suggests that different bacterial species respond differently to changes in conditions along streams (Lemke et al., 1997) and that bacteria of different species, or in different physiological states, vary in susceptibility to predation (Gonzalez et al., 1993; del Giorgio et al., 1996; Christoffersen et al., 1997; Gunderson and Goss, 1997).

Generally, in the aquatic environment much information has been compiled about taxonomic classification and abundance of producers (photoautotroph) and their immediate consumers (micro and macro zooplankton) during the last century and even longer. Much less is known about the variables concerning decomposers (Heterotrophic bacteria). This may be due to the fact that even an accurate determination of such simple variables like abundance and biomass of these organisms was not possible until about three decades ago and only quite recently have methods been developed for the identification of non-cultivable bacteria concerning abundance and cell-number of pelagic bacteria varies considerably among different aquatic ecosystems (Gocke et al., 2004). Literature survey also shows that, enormous research literature has piled up particularly on the microbial ecology of marine waters, only; few studies are available on the lentic fresh waters, For. e.g., (Hadas and Berman, 1998; Bergstein, et al., 2000; Yannarell, et al., 2003; Kisand, et al., 2005). Very little information is available on the microbial ecology of lotic fresh waters (Carlough and Meyer, 1991; Yamakanamardi and Goulder, 1995; Mohamed, et al., 1998; Kirchner, et al., 1999; and Castillo, et al., 2004). Given the crucial and significant role of bacteria enough studies are lacking on the international scene in lotic fresh waters. Hence, there is need for a quick and reliable bio-tool to monitor the water quality. The traditional physico-chemical analysis alone does not help in determining quality of water (Hynes, 1963) quickly in the field studies, but provides an indirect indication regarding its concentration. However, bio-indicators provide direct clue and quick information of the system. Most of the organisms are now being extensively
used as indictors for monitoring of water quality and are referred to as Biological Litmus Paper (Vaas, 1991).

To the best of my knowledge, there are no reports available on the microbial ecology of lotic water ecosystems using heterotrophic bacterial abundance, cell-size and specific growth rate of heterotrophic bacteria and micro plankton abundance as indicator of pollution status of river Cauvery and its upstream tributaries like Lakshmanatheertha, Harangi, Hemavathy and Lokapavani in Karnataka state, India. Prior studies carried out on river Cauvery include the study of rotifers as biological indicators of water quality (Sampath, et.al., 1979), changes in algal flora due to pollution in river Cauvery (Paramasivam and Srinivan, 1981), distribution of diatoms (Paramasivam and Srinivan, 1982), phytoplankton as indicators of water quality (Somashekar, 1984), water quality (Somashekar, 1985a and 1985b; Suvarna and Somashekar, 1997a and 1997b) and a survey of extra aquatic fungi of river Cauvery in relation to some water quality parameters (Somashekar, 1986). Hence, this investigation was under taken. The main aims of the present research investigation were: 1] To find out whether or not there is seasonal variations in the microbial and physico-chemical (water quality) parameters in these five water courses. 2] To test the hypothesis that the mean values of microbial parameters studied in this investigation in all the four upstream tributaries are similar to each other, but markedly different in the main river Cauvery. 3] In reaction to spatial / seasonal variations in microbial and physico-chemical (water quality) variables (if any), conduct relevant experiments in order to explain and understand the underlying phenomenon for such variations. 4]. To investigate the relationship between microbial variables with physico-chemical (water quality) variables in the main river Cauvery and its four upstream tributaries like Lakshmanatheertha, Harangi, Hemavathy and Lokapavani.
PART-I

Seasonal study of Physico-chemical and Aquatic Heterotrophic Bacterial variables, Bacterial Cell-Size, Specific Growth Rate of Heterotrophic Bacteria and abundance of micro-planktons.