HUMAN HEALTH RISK ASSESSMENT THROUGH TREATED EFFLUENT USE IN PUBLIC PARK IRRIGATION

A dissertation submitted in partial fulfillment for
the requirement to award the Degree of

MASTER OF TECHNOLOGY
IN
ENVIRONMENTAL SCIENCE & TECHNOLOGY

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CERTIFICATE

This is to certify that the report entitled **"Human Health Risk Assessment Through Treated Effluent use in Public Park Irrigation"** is a bonafide record of work done by Miss Raginee for the fulfillment of the requirement for the award of degree of **Master of Technology in Environmental Sciences and Technology** of the Thapar University Patiala, Punjab, during the academic year 2009-2011. She has fulfilled the requirement for the submission of this report, which to the best of my knowledge has reached the requisite standard.

This part of project was carried out under the guidance and co-guidance of Dr. A.K. Mittal and Mr. K.S.Babu respectively and has not been submitted elsewhere for the award of any other degree.

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ABSTRACT

The potential public health risk associated with treated effluent reuse for irrigation in public parks/lawns has been investigated in this study. The concentration of some pathogenic microorganisms in treated effluent used in park irrigation has been determined. The average concentration were found to be lie 10^5 to 10^7 MPN/100ml for total coliform, 10^5 to 10^7 MPN/100ml for fecal coliform, 10^4 to 10^5MPN/100ml for *Shigella* and 00 to 10^2 MPN/100ml for *Salmonella* and hazard concentration was found to be 1000MPN/100ml for total and fecal coliform for people exposure of the treated effluent used in park irrigation. The concentration of bacterial pathogens, namely *Shigalla, Salmonella* and coliforms (bacterial pathogen indicator) in treated effluent indicate that there is potential health risk within the study area. In general, exposure of these microorganisms through inhalation and ingestion through Sprinkler resulted in less hazard concentration compared to that through ingestion of drinking water. This study also focused on issues, such as considerations for sensitive population and concentration of microorganisms in treated effluent for assessing risks associated with exposure of microorganisms in treated effluent use in public parks irrigation.

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LIST OF ABBREVIATIONS

APHA:	American Public Health Association
BOD:	Biochemical Oxygen Demand
COD:	Chemical Oxygen Demand
CFU:	Colony Forming Unit
CPWD:	Central Public Works Department
DALY:	Disability Adjusted Life Years
DNA:	Deoxyribose Nucleic Acid
EPA:	Environmental Protection Agency
FC:	Fecal Coliform
FS:	Fecal Streptococcus
HACCP:	Hazard Analysis and Critical Point
ID:	Infectious Doses
MAC:	MacConkey Agar
MPN:	Most Probable Number
NCT:	National Capital Territory
NTU:	Nephelometric Turbidity Unit
QMRA:	Quantitative Microbial Risk Assessment
RNA:	Ribose Nucleic Acid
TC:	Total Coliform
TSI:	Triple Sugar Iron Agar
TSS:	Total Suspended Solid
UASB:	Upflow Anaerobic Sludge Blanket
WSP:	Waste Stabilization Ponds
WHO:	World Health Organization
XLD:	Xylose Lysine Desoxycholate

Introduction

1.1 General

Wastewater from the sewage treatment plants is a valuable resource in the parks of Delhi, used for irrigation at a cost estimated to be a quarter of that for potable water. The treated wastewater is used to irrigate public parks, or for municipal purposes such as fire fighting and street cleaning, or for domestic purposes such as garden watering. Most wastewater reclamation and reuse operations impose greater risk to public and workers health when exposed to pathogens and toxic substances as compared to the unpolluted water of non-sewage origin. In general, the health concern is in proportion to the degree of human contact with the water, the quality of the effluent and the reliability of the treatment processes (Crook, 1984). Microbial exposure could result from drinking or swimming in such recycled water that contains pathogens. The infection may also be caused by eating shellfish or food crops harvested from such water or through ingestion of contaminated food or by direct contact from person to person (known as secondary infection) (Hass *et al.,* 1999). According to WHO statistics, water-borne diseases cause more than 1.7 million causalities per year (WHO, 2002).

For most of the uses of reclaimed water, biological agents pose the greatest health risks. There is epidemiological evidence indicating that the reuse of municipal wastewater, particularly for the irrigation of food crops, has resulted in the transmission of diseases (Sepp, 1971). Most epidemiological studies show that direct contact increases the probability of encountering gastrointestinal and urogenital infections. Transmission of most pathogenic diseases from wastewater requires the fecal-to-oral route. There is, however, very little evidence indicating a potential health hazard in handling disinfected wastewater for irrigation on agricultural land (Burge *et al.,* 1978). The majority of documented disease outbreaks have been the result of bacterial or parasitic contamination. In all these cases, either raw sewage or infected effluent has been used for irrigation. The most commonly reported disease outbreaks were associated with the use of sewage collectively with the plant and type of effluent (Bryan, 1977).

Reuse of treated effluent for irrigation in close proximity to people (home gardens) and parks is expected to increase the human health risks. Municipal wastewater generally contains a variety of pathogenic viruses, bacteria and protozoa. The majority of these pathogens are enteric in origin, although non-enteric pathogens such as *Staphylococcus aureus*, *Legionella* sp., *Mycobacteium* sp. and *Leptospira* can also be detected in wastewaters (Fliermans 1996; Neuman *et al*., 1997). However, it is usually uneconomic to treat wastewater to the extent that complete pathogen removal is achieved prior to reuse as an irrigation source for parks and sports grounds. Although wastewater treatment can significantly reduce pathogen numbers, sustained effluent application can result in potentially high pathogen loadings on grass surface. Consequently, there remain undetermined potential public health risks from microbial pathogens, in particular through the bacterial contamination of skin wounds and abrasions. Thus, the potential longer-term risk to users of effluent irrigated parks and sports grounds will be assessed in this study.

The objective of this work is to study the effect of treated effluent reuse on public health and safety at Lodhi Garden, Chankyapuri, India Gate and Rajghat, Delhi, India, where treated effluent is being used for irrigation. To achieve the stated object the study involved evaluation of the possible increase of water borne diseases in the study area after treated effluent use, seeded with selected indicator (total and fecal coliform) and pathogenic (*Shigella* and *Salmonella*) microorganism. The concentration of each microorganism was determined under this study. Thus the objective is contemplated to achieve by the following scope.

1.2 Scope of the Work

The present work is used to investigate human health risk associated through treated effluent, used for lawn irrigation purpose at Lodhi Garden, Chankyapuri, India Gate and Rajghat, Delhi. The specific scope is as follows.

- To determine the microbial quality of the treated effluent used for irrigation.
- To determine the various physicochemical parameters of the treated effluent.

- To analyze the human health risk associated with treated effluent used for park irrigation in Delhi.

Literature Review

2.1 General

Interest in the reuse of wastewater for irrigation has increased worldwide, particularly in semi-arid countries facing water shortages. In less developed countries, sophisticated systems of wastewater treatment are not affordable, and untreated wastewater reuse is common, exposing agricultural workers or public to *Ascaris lumbricoides* and *Trichuris trichiura* infections and consumers of raw vegetables also to cholera and typhoid infections (Shuval *et al.,* 1986). The human health risk through irrigation of STPs effluent has become an important issue in the recent past. A number of common treatment methods are available and more novel methods have been introduced for the treatment of municipal wastewater / sewage. Even after the treatment, the effluents are not free from these pathogenic microorganisms. It becomes more important to investigate the microbial quality of the reclaimed water prior to reuse. The treatment method employed often depends on the final use of the reclaimed water, economical considerations, public concerns and government regulations (Asano *et al.,* 1996). Evaluation of hazards from any particular agricultural practice to the consumer, worker or neighboring community has traditionally been undertaken from sound experience and an epidemiological perspective. The epidemiology is the study of exposure factors and the occurrence of disease in human populations (Blumenthal *et al.*, 2001).The direct epidemiological evidence for excess risk resulting from wastewater use in agriculture is extremely limited (Blumenthal *et al*., 2000).

2.2 Risk Assessment

Risk assessment is a tool by which health officials can communicate with the water industry by interpreting water quality surveys and assisting in defining the adequacy of treatment adhering to EPA's recommendations of potable water quality and acceptable public health risks. This will become particularly important as states implement the Surface Water Treatment Rule, evaluate new technologies, and determine what water management practices will impact public health. The Surface Water Treatment Rule has been promulgated to address the amendments to the Safe

Drinking Water Act for controlling microorganisms in treated drinking water. This rule mandates that all surface waters be treated to achieve at least a reduction of 99.9% of microorganisms. Disinfection is required for all systems and filtration is required unless the system meets site specific criteria and has a protected watershed. The US Environmental Protection Agency (EPA) has also recommended that a treatment be provided to ensure that populations are not subject to risk of infection of greater than 1:10,000 for a yearly exposure, and that this is an acceptable level of safety for potable waters.

Risk assessment techniques were originally developed for evaluating the risk associated with exposure to pathogen and chemical hazards. Risk assessment process consists of four basic steps hazard identification, exposure identification, exposure assessment and dose response assessment (Gerba). Risk assessment provides an effective framework for determining the relative urgency of problems and the allocation of resources to reduce risks. Using the results of risk analyses, we embattle prevention, remediation, and control efforts toward areas, sources, or situations in which the greatest risk reductions can be achieved with the resources available. Risk assessment is an evaluative, multifaceted, comparative process.

2.2.1 Ecological Risk Assessment

For ecological risks, the focus is on the myriad interactions among populations, communities, and ecosystems (including food chains) at both the micro and the macro level. Ecological risks typically involve both short-term catastrophes, such as oil spills, and long term exposures to hazardous substances.

2.2.2 Microbiological Risk Assessment

In a developing urban society, the wastewater generation is usually approximately 30-70 m^3 per person per year. However, only around 10% of all wastewater in developing countries is being treated (Homsi, 2000). One tenth or more of the world's population consumes foods produced on land irrigated with wastewater (Smit and Nasr, 1992). So, it becomes essential to treat the

wastewater adequately prior to its reuse for irrigation purposes. Moreover, an appropriate health risk assessment and quality restrictions is must before implementing the reuse of wastewater. This would give regulators and the general public better information and confidence in the use of recycled waters.

Until recently, risk assessment in microbiology was almost non existence. Now it has become an important issue while preparing the guideline for water quality all over the world (Fewtrell and Bartram, 2001). Traditionally, evaluation of hazards from any particular agricultural practice to the consumer, worker or nearby community has been carried out from sound experience and an epidemiological perception. Epidemiology is the study of exposure factors and the occurrence of disease in human populations (Blumenthal *et al.,* 2001). The primary limitation of this approach is the uncertainty associated with the collection of information and the influence of confounding factor; both of which reduce the sensitivity of epidemiological methods for identifying excess risk (Petterson and Ashbolt, 2003). The traditional epidemiological methods are not sensitive enough to expose cases that might be associated with recycled water from the background incidence of these illnesses in the community (Salgot *et al*., 2006). Most outbreaks of waterborne disease are therefore not identified by epidemiological methods unless at least one percent of the population in a community becomes ill within a few months (Regli *et al*., 1991).

Quantitative Microbial Risk Assessment (QMRA) provides an alternative framework that may be used in conjunction with epidemiological methods for identifying potential excess risk (Haas, 2002; Toze, 2004). QMRA has been based on the chemical risk analysis systems used for the determination of risk from chemical pollutants in water. The chemical risk assessment approach followed by QMRA includes five steps: hazard assessment, exposure assessment, dose response analysis, risk characterization and risk management (Haas, 2002). Risk analysis system such as QMRA could also have an important role in assessing risks from microbial pathogens in recycled water (Toze, 2004). Further developments of the QMRA framework have been attempted to incorporate the unique characteristics of microorganisms into risk model (Petterson and Ashbolt, 2003). The QMRA framework has been applied to case studies in the United State (Tanaka *et al.,*

1998; Dowd *et al*., 2000), Israel (Shuval *et al.,* 1997) and Australia (Petterson *et al*., 2001) for evaluating microbial risk from wastewater reuse in agriculture.

The epidemiological framework for evaluating pathogen risk was presented by Eisenberg *et al.* 1996 considering a population perspective in the development of a mathematical model. The model made explicit the mechanistic aspects of the infectious disease process and incorporated data such as incubation period, immune status, duration of disease, and the rate of symptomatic development. Chick *et al*. (2001) have also described models with different levels of person to person spread and immunity which suggests that epidemiological models are required to complement previous approaches in microbial risk assessment to fully assess environmental pathogen pathways. Mathematical models have been established using the dose response relationship obtained experimentally to predict the probability of infection (Haas, 1983). The models developed up till now allowed risk assessment in drinking water and accordingly derivation of microbial standards (Rose and Gerba, 1991). On similar lines, the application of these models may be useful for the risk assessment in reclaimed wastewater and for setting up of standards for microbial quality of the discharged wastewater.

Previously the dose response data were analyzed using the method of maximum likelihood. Researchers have studied quantitative description of the dose response relationships of organisms to provide insight into the risk of becoming infected after the ingestion of a certain dose of organisms (Teunis *et al.,* 1996). Typically the reported dose response data have been fit to models that relate the probability of infection to the mean dose ingested. In some cases, illness as an end point was also investigated; however, the conditional modeling of illness given infection has proven to be difficult (Teunis *et al*., 1996). The most common models, used to relate an ingested dose to infection are the exponential and beta Poisson models (Haas *et al.,* 1999).

A number of microbial risk assessments have been carried out for waterborne pathogens. Haas *et al.* (1993) accounted for uncertainties in exposure assessments (lognormal distribution for

volume ingested) and the dose response relationship for viruses in drinking water by applying Monte Carlo simulation techniques. Several studies employed static risk assessment model to evaluate the potential health effects associated with rotavirus (Gerba *et al*., 1996), *adenovirus* (Crabtree *et al*., 1997), *Cryptosporidium* (Teunis *et al*., 1997; Perz *et al*., 1998), Giardia lamblia (Teunis *et al.*, 1997) and *coxsackieviruses* (Mena *et al*., 2003).

Hazard Analysis and Critical Point (HACCP) is another area of risk assessment though it is yet to be critically applied in water recycling schemes. HACCP is used widely in the food industry as a quality assurance tool (Fleet *et al.,* 2000). The World Health Organization (WHO) also supports HACCP approach which could logically be adopted for use in recycled water projects (Casani and Knrchel, 2002; Cunliffe and Stevens, 2003; Toze, 2004). Westrell *et al.* (2004) applied HACCP approach for identifying and controlling exposure to pathogenic microorganisms encountered during normal sludge and wastewater handling and applied QMRA to prioritise pathogen hazards for control purposes.

The tolerable risk also needs to be put into the framework of all exposures leading to disease. Establishing appropriate health based targets primarily involves an assessment of the risk associated with wastewater use in agriculture, using evidence from available studies of epidemiological and microbiological risk assessment studies. Individual countries may therefore set different health targets, based on their own contexts. The QMRA framework has been applied to case studies in the United States (Asano *et al.*, 1992; Tanaka *et al.*, 1998; Dowd *et al.*, 2000), Israel (Shuval *et al.*, 1997) and Australia (Gardner *et al.*, 1998; Petterson *et al.*, 2001a,b; Storey and Ashbolt, 2002) for evaluating microbial risk from wastewater reuse in agriculture.

Table 2.1: Risk Assessment from wastewater reuse in Agriculture

Countries (Reference)	**Identified hazards**	**Exposure route**	**Risk characterization**
United States (Asano *et al.*, 1992)	Enteric viruses	1. Landscape Irrigation of Golf Course 2. Consumption of Spray Irrigated food crops 3. Swimming in recreational impoundments 4. Groundwater Recharge	Daily, Annual and Lifetime risks of infection for minimum and maximum virus concentrations
Israel (Tanaka *et al.*, 1998)	Enteric Viruses	1. Landscape Irrigation of Golf Course 2. Consumption of Spray Irrigated food crops 3. Swimming in recreational impoundments 4. Groundwater Recharge	The risk model was used to evaluate: *Reliability:* probability of meeting an acceptable risk; and *Expectation:* the average risk for many exposure events. For each exposure scenario the acceptable level of risk was the USEPA benchmark of $<10^{-4}$ infections per year
Israel (Shuval *et al.*, 1997)	Hepatitis A Rotavirus Cholera	Consumption of spray irrigated crops	Annual risk of illness compared with the USEPA benchmark of

			$< 10^{-4}$ infections per year.
Australia (Petterson, 2001)	Enteric Viruses	Consumption of spray irrigated lettuce and carrot crops	Risk estimates compared with the USEPA benchmark of $< 10^{-4}$ infections per year and used to identify critical control points, and critical limits within a HACCP framework for risk management.
Australia (Storey and Ashbolt,2002)	Enteric Viruses	Exposure to reclaimed water intended for nonpotable use. Investigation of the incorporation of viruses in distribution pipe biofilms and subsequent sloughing events.	Daily and Annual risk (only calculated for 1mL exposure) compared with the USEPA benchmark of $< 10^{-4}$ infections per year.
United States (Dowd *et al.*, 2000)	*Salmonella* Enteric Viruses	Inhalation of aerosols downwind of biosolids placement	
Australia (Gardner *et al.*, 1998)	Echovirus Rotavirus Giardia Cryptosporidium	Inhalation of wastewater aerosols by the local community	Distance between each pathogen defined as the distance at which the Calculated risk was equal to 10^{-4} per year.

2.2.2.1 Pathogen Hazards

The first step in any microbial risk assessment is to identify the pathogen hazards that are to be investigated. There are literally hundreds of different pathogenic microorganisms that may be present in human faeces collected from communities. These organisms are grouped according to common characteristics and classified as viruses, bacteria, protozoa and helminths.

For risk assessment there is no need to take the details of all potential pathogenic microorganisms. Rather, the most relevant pathogens affecting the study population must be identified and targeted.

Table 2.2: pathogen hazards: Viruses, Bacteria, Protozoa and Helminths found in excreta (Petterson & Ashbolt)

Hazard group	Reference Pathogens	Characteristics of Reference Pathogens	Environmental stage, size (μm) and shape for group
Viruses	rotavirus	Highly infectious, not as persistent as HAV, Norwalk-like viruses and some other enteric viruses	Virion (0.02-0.08) generally spherical, protein coat protecting nucleic acid (DNA or RNA)
Bacteria	*Salmonella* sp. or *E. coli*	Always present in sewage, readily inactivated by disinfection	cell or dormant cell (0.1-2) cocci-rod
Parasitic protozoa	*Cryptosporidium parvum*	Not as prevalent as *Giardia*, but highly persistent and halide resistant.	Cyst or oocyst (4-40) oval-spherical
Helminths	*Ascaris lumbricoides*	Most persistent in soil/faeces, embryo must develop prior to human exposure	Ova (egg, 30-80) variable

2.2.2.2 Exposure Route

Most of the previous Microbial Risk Assessment studies have used scenario evaluation-based approach for estimating risk using different assumptions for developing exposure (Table 2.1). Different types of exposure routes: ingestion of wastewater during recreational activity and consumption of food crops irrigated with wastewater and direct ingestion of water contaminated with wastewater. Exposure of these pathogens from wastewater through other possible exposure routes, such as dermal exposure and inhalation were assumed to risk estimates.

2.2.2.3 Dose-response analysis

The relative health risk from the contaminants depends on the type of contaminant examined. Microbial pathogens, particularly viruses and protozoa, can cause rapid infection if ingested by people in contact with recycled water in which the pathogen is present (Haas *et al.*, 1999). While laboratory measurements of infectious doses can vary between different types of pathogens (e.g., <10 viral particles ingested can cause disease while *Vibrio cholerae* may require the ingestion of more than 10^5 cells to infect a healthy individual), the reality is that an infection can be caused by only a single pathogen cell/particle/cyst if that pathogen unit can pass intact through the alimentary canal into the intestines of a host (Haas *et al.*, 1999).

Dose-response modeling is the key to microbial risk assessment as it provides a link between exposure and the probability of potential infection. The primary source of data for undertaking dose-response analysis is based on human feeding trials. Human feeding trials are undertaken using healthy volunteers who are given a known dose of a particular pathogen under controlled conditions. The response of each individual in the study is then followed to determine the numbers who become infected. Infection occurs when the pathogenic organism multiplies within the host. For this to take place three conditions need to be fulfilled: the organism must have been ingested or inhaled, the organism must have survived to reach a suitable site for colonization in the host and finally the organism needs to be infectious and therefore able to multiply. Models for infection have been developed based the ‘single hit’ theory. The assumptions of the single hit model are: that the inoculum is known but for Poisson uncertainty, that organisms act

independently, individual probabilities of success do not depend on their numbers (independence), and that any single organism can start infection (Teunis *et al.*, 2002).

The simplest form of the single-hit model is the exponential relationship ($Pinf\,(n; r) = 1- (1- r)^n$ after ingestion of *n* dose where *r* is the probability of a single hit of an organism overcoming host barriers to reach a site for infection (Haas, 1983). In this relationship, *r* is constant for the population and susceptibility is assumed to be constant. The value of *r* is however likely to vary, between pathogens and hosts. When *r* is assumed to have a beta-distributed probability, a very complicated dose-response relationship emerges containing a confluent hypogeometric function. Furumoto and Mickey, (1967) made some simplifying assumptions to this relationship, and derived a simple dose-response relationship referred to as the β-Poisson:

$$P\mathit{inf}\,(D; \alpha, \beta) = 1-(1+D/\beta)^{-\alpha}$$

Which holds when $\beta \geq 1$ and $\alpha \leq \beta$

The β-Poisson model has been fitted to Rotavirus data on infection by Ward *et al.* (1986) with maximum likelihood parameters of ($\alpha = 0.253$, $\beta = 0.422$). The performance of the β-Poisson approximation with Rotavirus data has been evaluated (Teunis and Havelaar , 2000). While this model produces a good fit for the data, it has been shown to produce misleading results during uncertainty analysis since the parameter conditions of the approximation are not met, and the β-Poisson is strictly not a single hit model (Teunis and Havelaar, 2000).

An important property of the single-hit relationship is that it has a maximum risk curve that limits the upper confidence level of the dose-response relation. This occurs when the probability that an ingested organism will pass the host's defence mechanisms and find a site suitable for colonisation is equal to 1 (r=1). This property is not retained by the β-Poisson approximation, where the upper confidence level of the dose-response relation may exceed the maximum risk curve (Teunis and Havelaar, 2000). The maximum risk curve is therefore important for

uncertainty analysis and for risk assessment of pathogens with unknown properties. An assumption of the exponential and β-Poisson dose-response models is that individuals ingest a number of microorganisms that is a random sample from a Poisson distribution. In many circumstances, however, micro-organisms have been shown to be over dispersed in environmental samples (Pipes *et al.*, 1977; Haas and Heller, 1988; Maul *et al.*, 1990; Petterson *et al.*, 2001a). In response to this conflict, (Haas, 2002) presented a conditional dose-response function that could be combined with any theoretical or empirical distribution function for the number of microorganisms ingested to determine the risk associated with exposure. Development of a dose-response relationship based on an over dispersed distribution of microorganisms led to a reduced estimated population risk for the same mean dose (Haas, 2002).

Alternative models for describing variability in *r*, particularly in relation to a covariate such as immune status, have been suggested (Teunis *et al.*, 2002). These models retain the properties of the single hit model and provide potential for QMRA as a means of accounting for variation in susceptibility to infection in the general population.

2.2.2.4 Risk Characterization

The adverse human health effects occurring as a result of a defined exposure scenario to a microbial contaminant is estimated. The exposure profile and dose-response information are combined during the process of risk characterization, and probability of infection rates are calculated for the exposed population. Single calculated values of risk are essentially meaningless unless they are interpreted within the framework of the model assumptions and the circumstances of the exposed population. In its simplest form, interpretation of risk estimates is undertaken by comparing the calculated value to some benchmark of tolerable risk. By far the most commonly applied benchmark in risk assessment has been the USEPA's 10^{-4} risk of infection per annum from drinking water (Regli *et al.*, 1991). It may however be argued that the tolerable risk of infection from a particular disease should be dependent upon the duration and severity of the symptoms. (Havelaar *et al.*, 2000) applied Disability Adjusted Life Years (DALY) measure for evaluating the total health burden of infection from *Campylobacter* spp. in

the Dutch Population. Infection with thermophilic *Campylobacter* spp. usually leads to an episode of acute gastroenteritis. Occasionally, more severe diseases may be induced notably Guillain- Barre syndrome and reactive arthritis.

2.3 Exposure analysis

Exposure analysis have been generally obtained using scenario-specific information during the hazard identification step and the flow of pathogens through the hazard pathway to the point of exposure (Petterson and Ashbolt). The removal of different pathogens from full-scale wastewater treatment plant (Table 2.3) shows that some of the pathogens are removed completely from wastewater whereas some of other pathogens are persistent. Also, most of the pathogens investigated were found to be removed more than 90% from effluent in advanced water treatment plant. These findings indicate that the effect of removal effectiveness of different plant types should be included in Quantitative microbial risk assessment. For the case of low pathogens based exposure risks from water, related pathogens based exposure risks from irrigation water would also be smaller due to effect of wastewater treatment plant in removing pathogens from water.

2.3.1 Pathogen Numbers in Human Excreta

The number of pathogens present in excreta varies as a function of the health of the host and the local environment. Communities with poor hygiene and a high proportion of children will produce excreta especially rich in enteric pathogens. Healthy individuals do not normally excrete pathogens for prolonged periods and therefore their contribution to pathogens in excreta is subject to wide fluctuations. Due to the very limited quantitative data on individual pathogens in human excreta, it may be better to estimate numbers from epidemiologic data. Estimates should consider the incidence of gastrointestinal infections, along with typical excretion times and densities to generate mean or probability density functions representing the range of pathogens expected in a population’s faeces, as described in Table 2.3. These datasets are useful for providing approximate ranges and limits of pathogen numbers, however they do not necessarily

represent the real variability that may be expected in pathogen concentration in sewage effluents (Petterson and Ashbolt).

Addition data on pathogen occurrence in sewage will not only provide better descriptions for QMRA, but may also provide a more sensitive assessment of community infection (Ranta *et al*., 2001).

2.3.2 Removal of Pathogens during Wastewater Treatment

Wastewater treatment plants are primarily designed with an objective to remove the organic matter and suspended solids. With the emphasis on the wastewater reuse in the recent past, combinations of primary, secondary and tertiary treatment unit operations are being considered. Various processes like aerobic, anaerobic, membrane and hybrid are used for the wastewater treatment. However, these treatment operations were able to achieve a considerable reduction in dissolved organics (BOD, COD) but the complete removal of microbial contamination was unachievable. Several studies observed that only 90-99% microbial reduction in wastewater could be achieved in conventional processes such as sedimentation, activated sludge or trickling filters (Koivunen *et al.,* 2003).

The removal efficiency of pathogenic and indicator microorganisms in wastewater treatment plants vary according to the treatment process type, retention time, other biological flora present in the biomass, pH, temperature and the efficiency in removing suspended solids. Most treatment processes produced treated effluents of poor microbiological quality, which represent a major source of FC in rivers and coastal waters. Conventional wastewater treatment plants have some form of primary sedimentation tank to remove solids; however various researchers reported 30-50% fecal coliforms and 29-99% *Salmonella* removal in sedimentation tank (Yaziz and Lloyd, 1979; Godfree and Farrell, 2005). Biological processes like activated sludge, trickling filter and anaerobic filters/UASB reactor are generally designed to allow short hydraulic retention times. Thus, the opportunities for bacterial removal are essentially inadequate (Curtis, 2003). Previous studies reported fecal coliforms removal between 90 and 99% in activated sludge process (Koivunen *et al.,* 2003). Curtis (2003) reported 99% removal of the microaerophilic

Camphylobacter in suspended growth systems. Removal of indicator bacteria and *Salmonella* is poor in trickling filter (Yaziz and Lloyd, 1979).

For anaerobic processes like septic tanks and anaerobic ponds, the retention times are typically of 1-5 days, while for anaerobic filters and UASB retention time may only be a matter of hours. Cullimore and Viraraghavan (1994) reported poor removal of indicator microbes in septic tanks followed by anaerobic filters. However, UASB and anaerobic ponds are being used for the domestic treatment of wastewater in number of countries. UASB effluents require a post treatment to achieve a significant removal of bacterial pathogens. As waste stabilization ponds (WSP) are frequently used as a post treatment and pathogen removal in ponds is related to the organic load, a UASB can make a substantial indirect contribution to bacterial pathogen removal (Curtis, 2003). Biological processes like activated sludge, trickling filter, extended aeration, facultative aerated lagoons could remove up to 90% of microorganisms while the waste stabilizations ponds and land treatment/irrigation can remove up to 99% microorganisms.

Typical abundance of bacterial indicators (total coliforms and fecal coliforms) in raw sewage is 10^7-10^9 and 10^6-10^8 per 100ml respectively (Rose *et al*., 1996; George et al., 2002). Generally, conventional municipal sewage treatment plants, which generally do not include disinfection process, reduce fecal microbial density by 1 to 3 logs (Miescer and Cabelli, 1982). The treatment of such wastewaters using adequate process is essential to avoid the problem of waterborne diseases to human beings. Besides, the disinfection of the treated wastewater is desired before its final discharge.

Table 2.3 Pathogen removal in various steps of wastewater treatment steps (Yates and Gerba, 1998)

	Enteric Viruses	***Salmonella***	***Giardia***	***Cryptosporidium***
Concentrations in raw Wastewater ($no.L^{-1}$)	100 000-1 000 000	5 000-80 000	9 000-200 000	1-4 000
Removal during:				
Primary treatment[a]				
% removal	50-98.3	95.5-99.8	27-64	0.7
No. remaining L^{-1}	1,700-500,000	160-3,360	72,000-146,000	
Secondary treatment[b]				
% removal	53-99.92	98.65-99.996	45-96.7	
No remaining L^{-1}	80-470,000	3-1,075	6,480-109,500	
Tertiary treatment[c]				
% removal	99.983-99.9999998	99.99-99.9999995	98.5-99.99995	2-7[d]
No. remaining L^{-1}	0.007-170	0.000004-7	0.099-2,951	

a Primary sedimentation, and disinfection

b Primary sedimentation, trickling filter/activated sludge and disinfection,

c Primary sedimentation, trickling filter/activated sludge, disinfection, coagulation, filtration, and disinfection

d Filtration only

2.4 Relationship between Pathogen and Indicator organism

The presence of indicator microorganisms in natural water indicates pollution, but their absence does not essentially warranty the quality of the water (Dutka, 1973; Morinigo *et al*., 1990). The specific pathogens may act as direct indicators of fecal contamination, although it is difficult to select and quantify them. The *Salmonella* spp., *Cryptospordium* spp. and *Giardia* spp. are the most common pathogens which are being used as standard pathogens in most of the studies (Hurst *et al.,* 2002). Moreover, it is most critical to have the information about the correlation

between fecal indicators and enteric pathogens in environmental waters besides having the records of occurrence of any pathogens (Horman *et al*., 2004).

Generally, coliform group did not show good correlation with various pathogenic microorganisms. The variation in survival and persistence of coliforms and pathogens in different environmental waters may be the basis of these poor correlations (Ottoson and Stenstrom, 2003). The coliforms may increase in aquatic environments, especially in grey waters.

Salmonella is one of the most common pathogenic microorganisms present in sewage causing severe gastroenteritis in humans. The reliability of the indicator based standards to predict the presence of pathogenic microorganisms, such as *Salmonella*, is still a matter of debate (Morinigo *et al.*, 1993; Lemarchand and Lebaron, 2003). There is general agreement that indicator microorganisms are present at high densities in wastewaters, which are positive with *Salmonella* as compared to wastewaters without *Salmonella* (Morinigo *et al.*, 1993; Arvanitidou et al., 1995). However, several studies of enteric survival have indicated that Salmonella spp. persist longer than FC in water (Tobias and Heinemeyer, 1994; Gabutti *et al.*, 2000).

Various authors reported that the fecal coliforms are highly sensitive to salinity and sunlight (Burton *et al*., 1987; Gabutti *et al.*, 2000), which indicates their inability to be the suitable indicators of the presence of pathogenic enteric microorganisms (such as *Salmonella* spp. or Enterovirus) in sea and in brackish water. Several authors reported the absence of correlation between *Salmonella* spp. and fecal indicators (Morinigo *et al*., 1990; Lemarchand and Lebaron, 2003). This may be explained on the basis that the concentration of pathogens is dependent, as are the indicators, upon the size of the contributing community and, unlike the indicators, upon the levels of infection within that community. Moreover, several hydrodynamic, chemical and biological factors govern the transport and behavior of the different organisms (Lemarchand and Lebaron, 2003). *Salmonella* species were absent on the beaches where the densities of fecal

indicator organisms was very low (25 CFU/100ml of total coliforms, 13 CFU/100ml of fecal coliforms and 17 CFU/100 ml of fecal streptococci) (Polo *et al.*, 1998). Therefore, it is considered that *Salmonella* spp. is likely to be present if density of fecal indicator organism is high.

Enterococcus spp. showed good correlation with pathogens than fecal coliforms, since their pers istence is related to that of possible disease causing pathogens (Kinzelman *et al.*, 2003). Various authors reported that the survival rate of Enterococcus spp. is higher as compared to coliforms in surface waters (Figueras *et al.*, 1996; Kinzelman *et al.*, 2003). The fecal enterococci showed correlation with the densities of viruses in case of greywater, which are more important due to their environmental persistence and low infection dose (Ottoson and Stenstrom, 2003). Polo *et al* (1998) observed a good correlation of *Salmonella* with FS in less contaminated sea water. However, FS explained low correlation with *Salmonella* spp. for freshwater due to their growth (Morinigo *et al*., 1990; Desmarais *et al*., 2002).

A good correlation with *Salmonella* spp. was also observed for polluted freshwaters and seawaters. The *Clostridium perfringens* showed a significant correlation with *Salmonella* spp. in less polluted seawaters (Morinigo *et al*., 1990). Moreover, during the epidemiological studies of waterborne infections in beach waters a direct correlation between the incidence of gastrointestinal infection and the count of *C. perfringens*, *Aeromonas* spp. and *Vibrio* cholera was observed (Kueh *et al*., 1995).

In marine waters, positive correlations between fecal bacteriophage indicators, enteric viruses, and other pathogens have been observed (Rozen and Belkin, 2001). Borrego *et al*. (1987) reported linear correlations of *coliphages*, a resistant microorganism in aquatic environments, with *Salmonella* for freshwaters and seawaters. Indicator bacteria concentrations are poor predictors of enterovirus concentrations in marine water (Wyer *et al*., 1995). Turner and Lewis (1995) suggested that the *enterococci* group may be more useful than F-specific bacteriophages as indicators of oxidation pond treatment efficiency. The presence or absence of correlation between fecal indicators and pathogens could reveal the sporadic presence of enteric pathogens

in environmental waters, signifying the dissimilar rates of survival and recovery of pathogens and indicators.

2.5 Human Health effects associated with wastewater irrigation

WHO guidelines, 1989 were based on a number of available epidemiological studies, many of which were reviewed (Shuval *et al.,* 1986). The evidence at that time suggested that the use of untreated wastewater in agriculture presented a high actual risk of transmitting intestinal nematodes and bacterial infections especially to produce consumers and farm workers; but that there was limited evidence and the health of people living near wastewater irrigated fields was affected. There was less evidence for the transmission of viruses and no evidence for the transmission of parasitic protozoa to farm workers, consumers or nearby communities. The review of epidemiological evidence (Shuval *et al.,* 1986) also indicated that irrigation with treated wastewater did not lead to excess intestinal nematode infections among field workers or consumers (WHO, 1989).

Blumenthal and Peasey, 2002 completed a critical review of epidemiological evidence on the health effects of wastewater and excreta use in agriculture. A sub-set of analytical epidemiological studies were selected that included the following features: well-defined exposure and disease, risk estimates calculated after allowance for confounding factors, statistical testing of associations between exposure and disease, and evidence of causality, used as a basis for estimating threshold levels below which no excess infection in the exposed population could be expected. A summary of the results of this epidemiological review are presented in Table 2.4.

Table 2.4: Health risks associated with the use of wastewater in irrigation. (Blumental *et al*., 2000a; Armon *et al.* 2002; Blumental and Peasey, 2002)

Group exposed	**Nematode infection**	**Bacterial/Viruses**	**Protozoa**
Consumers	Significant risks of Ascaris infection for both adults and children with untreated wastewater; no excess risk when wastewater treated to <1 nematode egg/l except where conditions favour survival of eggs	Cholera, typhoid and shigellosis outbreaks reported from use of untreated wastewater, sero-positive responses for Helicobacter pylort (untreated); Increase in non-specific diarrhoea when water quality exceeds 10^4FC/100ml	Evidence of patasitic protozoa found on wastewater. Irrigated vegetable surfaces but no direct evidence of disease transmission
Farm workers and their families	Significant risks of Ascaris infection for both adults and children with contact with untreated wastewater, risks remain, especially for children when wastewater treated to <1 nematode egg/l. Increased risk of hookworm infection to workers	Increased risk of diarrhoeal disease in young children with wastewater contact if water quality exceeds 10^4 FC/100ml: elevated risl of salmonella infection in children exposed to untreated water, elevated seroresponse to Norovirus in adults exposed to partially treated wastewater	Risk of *Giardia* Intestinal infection was significant for contact with both untreated and treated wastewater, Increased risk of amoebiasis observed from contact with untreated wastewater

Nearby communities	Ascaris transmission not studied for sprinkler irrigation but same as above for flood or furrow irrigation with heavy contact	Sprinkler irrigation with poor quality water 10^4 TC/100ml, and high aerosol exposure associated with increased rates of viral infection; use of partially treated water 10^4 FC/100ml or less in sprinkler irrigation not associated with increased viral infection	No data for transmission of protozoan infections during sprinkler irrigation with wastewater

Most wastewater reclamation and reuse operations impose greater risk to public or workers exposure to pathogens or toxic substances than would the use of unpolluted water of non-sewage origin. In general, the health concern is in proportion to the degree of human contact with the water, the quality of the effluent, and the reliability of the treatment processes (Crook, 1984).

For most of the uses of reclaimed water, biological agents pose the greatest health risks. There is epidemiological evidence indicating that the reuse of municipal wastewater, particularly for the irrigation of food crops, has resulted in the transmission of disease (Sepp, 1971). Most epidemiological studies show that direct contact increases the probability of encountering gastrointestinal and urogenital infections. Transmission of most pathogenic diseases from wastewater requires the fecal-to-oral route. There is, however, very little evidence indicating a potential health hazard in handling disinfected wastewater for irrigation on agricultural land (Burge and Marsh, 1978). The majority of documented disease outbreaks have been the result of bacterial or parasitic contamination. In all cases, either raw sewage or contaminated effluent was

the source of irrigation water. The most commonly reported disease outbreaks associated with the use of sewage together with the plant and types of effluent (Bryan, 1977) are shown in Table 2.5.

The role of water in the overall incidence of viral diseases may be limited. Other modes of transmission, such as personal contact, are probably responsible for the great majority of viral diseases (Bryan, 1977). However, any excreted virus capable of producing infection when ingested could be transmissible by inadequately treated wastewater. Over 100 different enteric viruses capable of producing infections or disease are excreted by humans. The most important are the enteroviruses (polio. echo and coxsackie), Rotaviruses, reoviruses, parvoviruses, adenoviruses, and hepatitis A virus (Sorber and Sagik, 1978). Hepatitis A virus is most frequently reported and documented to be transmitted by contaminated water.

There is little information concerning the occurrence of viral diseases resulting from the reuse of wastewater. This may be attributed to several factors: the present virus detection methods are not sensitive enough to accurately detect low concentrations of viruses in large volumes of water; enteric virus infections are often not apparent, thus making it difficult to establish the endemicity of such infections; the apparently mild nature of most enteric virus infections preclude reporting by the patient or the physician; the morbidity of enteroviral infections may not become obvious for several months or years (Horstmann et al. , 1973); and once introduced into a population, person to person contact would become a major mode of transmission of an enteric virus, thereby obscuring the role of water in its transmission.

Table2.5: Disease outbreak associated with plants contaminated with wastewater (Bryan, 1977).

Disease	Plant	Source of Wastewater	Year
Typhoid fever	Celery	Sewage sludge irrigation	1899
Typhoid fever	Raw vegetables, fruit	Sewage polluted water	1911
Typhoid fever	Vegetables, Blackberries	Sewage irrigation	1919

Amebiasis	Vegetables	Sewage irrigation	1934
Typhoid fever, Para typhoid fever	Vegetables	Secondary treated effluent	1942
Shigellosis	Cabbage	Primary treated effluent	1946
Ascariasis	Vegetables	Sewage spray irrigation	1947
Typhoid fever	Apples	Sewage irrigation	1953
Salmonellosis	Vegetables	Sewage irrigation	1954
Hook worm infection	Vegetables	Sewage forming	1955
Typhoid fever	Vegetables, fruits	Sewage	1957
Salmonellosis	Grass	Sewage flooding	1972
animal and human cholera	Vegetables	Sewage irrigation	1973

In general, disease organisms responsible for epidemics in the past are still present in today's sewage. Good sanitary engineering practice results in control rather than total eradication of the disease agents. The number of pathogens in sewage have markedly declined over the decade as a result of disease control with antibiotics and improved sanitary conditions and practices. During an outbreak, pathogen numbers in local sewage go up and it would be inappropriate to be

careless simply because present pathogen densities may be relatively low. Table 2.6 summarizes the major infectious agents potentially present in raw domestic Wastewater.

One of the most common pathogens is the genus Salmonella. There are three distinct forms of salmonellosis in humans: enteric fevers, septicemia and acute gastroenteritis. The most severe is the typhoid fever caused by *Salmonella typhi*. The most common form of *Salmonella* isolated from human sources is Salmonella typhimurium. Other bacteria of lesser importance have been isolated from sewage. These include *Vibrio. Mycobacterium, Clostridium, Leptospira* and Yersinia species. Although these pathogens may be present in wastewater, their concentrations are usually too low to initiate disease outbreaks. Waterborne gastroenteritis of unknown cause is frequently reported, and the suspected agent is bacterial. These include enteropathogenic Escherichia coli and certain strains of Pseudomonas. E. coli has been implicated in outbreaks of traveler's diarrhea (Gorbach et al., 1975). *Campylobacter* coli have been identified as the cause of a form of bacterial diarrhea in humans. Probably the most serious of the parasites is the protozoan, *Entamoeba histolytica*, which is responsible for amoebic dysentery and amoebic hepatitis.

Several helminthic parasites may be found in wastewater. The most important are the intestinal worm *Ascaris lumbricoides*, the tapeworm *Taenia saginata*, the whipworm *Trichuris trichiura*, the hookworms Ancylostoma duodenale and Necator americanus and the threadworm Strongyloides stercoralis. Many of the helminths have complex life cycles, including a required stage in intermediate hosts. The eggs and larvae are resistant to environmental stresses and can be expected to survive usual wastewater disinfection procedures.

Disease can be transmitted to humans either directly by contact, ingestion, or inhalation of infectious agents in reclaimed water, or indirectly by contact with objects previously contaminated by the reclaimed water. Certain conditions must be present for a person to become ill: the infectious agent must be present in the community producing the wastewater and, hence, in the wastewater from that community; the agents must survive all the wastewater treatment

processes to which they are exposed; the person must come in contact with the effluent; and the agents must be present in sufficient numbers at the time of contact to cause illness.

2.5.1 Pathogen and human health

Microbial pathogens that are present in wastewater can be divided into four groups: bacteria, viruses and the pathogenic protozoan and helminths. The enteric are the majority of pathogens because they are excreted in fecal matter and contaminate the environment.

Table 2.6: Major pathogens potentially present in raw domestic sewage (Crook, 1984).

Pathogen	Disease
Protozoa	
• *Entamoaba histolytica*	Amebiasis (amebic dysentery)
• *Giardia lamblia*	Giardiasis
• *Balantidium coli*	Balantidiasis (dysentery)
Helminths	
• *Asceris lumbricoides (Roundworm)*	Ascariasis
• *Ancylostoma duodenale (Hookworm)*	Ancylostomiasis
• *Necator americanus (Roundworm)*	Nectoriasis
• *Ancylostoma spp. (Hookworm)*	
• *Strongyloides stercoralis (Threadworm)*	Cuteneous Larva Migrans Strongyloidiasis
• *Trichuris trichiura (Whipworm)*	Trichuriasis
• *Taenia spp. (Tapworm)*	Taeniasis
• *Enterobius vermicularis (Pinworm)*	Enterobiasis
Bacteria	
• *Shigella (4spp.)*	Shigellosis (dysentery)
• *Salmonella typhi*	Typhoide fever
• *Salmonella (1700 spp.)*	Salmonelloisis
• *Vibrio cholera*	Cholera Gastroenteristis

• *E.coli (enteropathogenic)* • *Yersinia enterocolitica* • *Leptospira spp.* Viruses • Enteroviruses (71 types) • Hepatitis A virus • Adenovirus • Rotavirus • Parvovirus (2types)	Yersiniosis Leptospirosis Gastrointeritis, heart Infectious hepatitis Respiratory disease Gastrointeritis Gastrointeritis

2.5.1.1 Bacteria

Bacteria are the most common and numerous of the microbial pathogens found in recycled waters (Toze, 1999). There are a wide range of bacterial pathogens and opportunistic pathogens which can be detected in wastewaters. Many of the bacterial pathogens are enteric in origin; however, bacterial pathogens which cause non-enteric illnesses (*Legionella* spp., *Mycobacterium* spp., and *Leptospira*) have also been detected in wastewaters (Fliermans, 1996; Wilson *et al.*, 1995). Enteric pathogenic bacteria can often infect both humans and animals, e.g., *Salmonella* (Haas, 1999), and thus animals can form another contamination source for recycled water as well as being at risk from contact with poorly treated recycled water. The majority of pathogenic enteric bacteria require ingestion of a high dose of cells to be ingested to cause infection(usually $>10^6$ cells), although *Shigella dysenteriae* and *Campylobacter jejuni* have been observed to only require the ingestion of as few as 100 cells to cause infection in susceptible hosts (Teunis, *et al.*, 1996).

2.5.1.2 Viruses

Enteric viruses are the smallest of the pathogens found in water. They are all obligate intercellular parasites that are only able to replicate by forcing a host cell to produce multiple copies of the virus (Toze, 1997). Most human enteric viruses have a narrow host range, meaning

that only human faecal contamination of water need be considered as a risk for viral infection of humans (Haas, 1999). Enteric viruses are highly infectious and commonly require the ingestion of only a few viral particles to cause infection. These infectious doses (ID50) can be as few as 10 viral particles or less (Ward *et al.,* 1986). In addition, it would be expected that these viruses would have a greater potential to cause infection in susceptible sections of the population such as the elderly, very young and the immuno-compromised.

Some viruses that have been detected in wastewaters include adenoviruses, rotaviruses, reoviruses, astroviruses, and caliciviruses such as Norwalk virus and other small round structured viruses. These viruses can cause a range of diseases such as: acute gastroenteritis, diarrhoea, pneumonia. The most infectious of all enteric viruses are the rotaviruses and if present in wastewater are considered to be a high health risk. Small children are the most sensitive group of population and have the highest infection rate from these viruses. This group of population is more at risk, may be because of a possibility for developing the more rare forms of diseases caused by these viruses. Viruses and other pathogens that exist in wastewater used for irrigation do not get into the fruits or vegetables unless their skin is broken.

2.5.1.3 Protozoa

Enteric protozoan pathogens are unicellular eucaryotes which are obligate parasites. Outside of an infected host they persist as dormant stages known as cysts or oocysts. There are several protozoan pathogens that have been isolated from wastewater and recycled water sources (Gennaccaro, *et al.,* 2003). The most common detected are *Entamoeba histolytica*, *Giardia intestinalis* and *Cryptosporidium parvum* (Toze, 1997). Infection from all three of these protozoan pathogens can occur after consumption of food or water contaminated with the oocysts or through person to person contact (Carey, *et al*., 2004). The main reservoir for *C. parvum* and *G. intestinalis* is man, but several domestic and wild animals have been shown to be potential reservoirs for these parasites (e.g., cattle can become infected with *C parvum* and then cause infection in humans due to contact with infected bovine faeces) (Feachem, *et al.,* 1983; Haas, 1999). Like the enteric viruses, all human protozoan pathogens are significantly more

infectious than most enteric bacterial pathogens. *Cryptosporidium*, *Giardia*, and *Entamoeba* have all been observed to have the potential to cause infection with less than 10 oocysts (Dillingham *et al*., 2002; Rose *et al*., 1991).

There are a number of them that have been isolated from wastewater sources. The most important of them is the protozoan *Entamoeba histolytica*, which is responsible for amoebic dysentery and amoebic hepatitis, *Giardia intestinalis*, and *Cryptosporidium parvum*. The three of them are all enteric pathogens and have been detected in wastewater contaminated with faecal material. Infection from the three can occur after consumption of food or water which has been contaminated with the cysts or oocysts *Entamoeba histolytica* can be detected in all parts of the world, although it is more prevalent in tropical regions (Feachem *et al.*, 1983). *Cryptosporidium parvum* is connected with a number of outbreaks involving drinking water. The most serious of these outbreaks was in Milwaukee, Wiskonsin, where it was estimated that at least 400,000 people became infected (MacKenzie *et al*., 1994).

2.5.1.4 Helminths

Helminths (nematodes and tape worms) are common intestinal parasites which can be transmitted by the faecal–oral route (Toze, 1997). Helminth parasites that are a significant health risk in reclaimed waters include the round worm (*Ascaris lumbricoides*) (Khuroo, 1996), the hook worm (*Ancylostoma duodenale* or *Necator americanus*), and the whip worm (*Trichuris trichiura*) (Blumenthol et al., 1996). These helminths have a simple life-cycle with no intermediate hosts and are capable of causing infection via the faecal-oral route (Toze, 1997).

It has been estimated that 25% of the world's population have been infected with the round worm nematode *Ascaris lumbricoides* (Toze, 2004). A number of other helminthes are developed in certain regions of the world. For example *Strongloides stercoralis*, a soil transmitted parasitic nematode, is endemic in northern Australia (Toze, 2004). *Strongloides* infections are rare in the southern regions of the continent. If a wastewater reuse plant is considered in such a region this

parasite must be considered. Helminth infections are a problem for infants and that chronic infection begins at a young age. Chronic Helminth infections affect the physical and mental development of children (Khurro, 1996). Helminth eggs need a period of five to ten days before they are able to cause infection. In contaminated soil the eggs can remain infectious for up to ten years (Toze, 2004). This means that soils which have been in contact with recycled waters contaminated with faecal material could be considered as long- term sources of these parasites (Ellis *et al*., 1993; WHO, 1989). Helminths can be removed by sedimentation, filtration, or stabilization ponds.

Occurrence of illness depends on a series of complex interrelationships between the host and the infectious agents. These include the numbers of the invading microorganism, the numbers of organisms necessary to initiate infection, the ability of microorganisms to cause disease and the relative susceptibility of the host.

2.6 Survival of pathogens on non- food crops

Salmonella and other enteric bacteria can survive for several weeks on grass if sufficient organic matter and moisture is available. Helminth eggs such as *Ascaris* are believed that they can survive for 30 to 60 days, although they may survive for many months in the soil (Feachem *et al*., 1983). Overall pathogens can be ranked in the following descending order of risk (Shuval *et al*., 1986). Table 2.7 shows the pathogen survival times in various environments.

• High – Helminths (the intestinal nematodes – ascaris, trichuris, hookworm, and taeniasis)

• Lower – Bacterial Infections (cholera, typhoid, and shigellosis) and Protozoan infections (amebiasis, giardiases)

• Least – Viral Infections (viral gastroenteritis and infectious hepatitis).

Table 2.7: Typical Pathogen Survival Times at 20-30 ^{0}C (Feacham *et al.*, 1983)

pathogen	Fresh water & sewage	crop	soil
Virus			
Enteroviruses[b]	<120 but usually<50	<60 but usually<15	<100 but usually<20
Bacteria			
Fecal coliforms[a,c]	<60 but usually<30	<30 but usually<15	<70 but usually<20
Salmonella spp[a]	<60 but usually<30	<30 but usually<15	<70 but usually<20
Shigella spp[a]	<30 but usually<10	<10 but usually<5	……
Vibrio cholerae[d]	<30 but usually<10	<5 but usually<2	<20 but usually<10
Protozoa			
Entamoeba histolytica cysts	<30 but usually<15	<10 but usually<2	<20 but usually<10
Helminths			
Ascaris lumbricoides eggs	Many months	<60 but usually<30	Many months

a In seawater, viral survival is less and bacterial survival is very much less,than in fresh water.

b Includes polio,echo and coxsackieviruses

c Faecal coliform is not a pathogen but is often used as an indicator organism

d *V.cholerae* survival in aqueous environments is a subject of current uncertainty

2.7 Microbial Ecology of Wastewater

The exposure of humans to pathogens is the major health risk associated with the disposal and reuse of wastewater. Pathogens of concern in wastewater in general include bacteria such as *Escherichia coli, Salmonella, Shigella, Vibrio cholerae, Campylobacter* and *Legionella*; viruses such as enterovirus, Hepatitis A, rotavirus and Norwalk virus; protozoa such as Giardia lambial and *Cryptosporidium parvum*; and helminthes such as *Ascaris lumbricoides* and *Trichuris trichiura*. The number and range of pathogens found in wastewater are of significant consideration. Information of the range of pathogens also enlightens the ecology of the

organisms to be removed. The wastewater of any major community can be assumed to have nearly all the pathogens normally found in excreta at any one time.

The enteric pathogens are usually present in high number in treated sewage effluent. Even then rarely, the pathogens are directly enumerated from the wastewater, apparently due to the expenses involved, and the risk of exposure to the investigator. Instead, various indicator microorganisms are identified. These indicator microorganisms are non pathogenic, easy to enumerate and their presence may infer the presence of pathogens. Commonly used indicators are total coliform (TC), fecal coliform (FC), fecal streptococci (FS) and *E. coli*. These microorganisms are of fecal origin from higher mammals and birds, and their presence in water may be indicating fecal pollution and possible association with enteric pathogens. Total coliform are not an accurate indicator of fecal contamination as these are not solely enteric bacteria rather they can be found naturally in water, plant and soil samples. The presence of fecal coliforms in water indicates that there is contamination with fecal matter and the enteric pathogens may be present. Recentaly, fecal streptococci are also used as indicators of enteric pathogens. *Enterococci* have been useful indicators of fecal contamination in marine and recreational waters due to their high salt tolerance (Roesner *et al.*, 2006). Determining the relationship between different indicator microorganisms and pathogens, may provide some information about the confidence degree of indicators (Morinigo *et al.*, 1986). Table 2.8 shows the water recycling guidelines adopted in United States and other countries.

Table 2.8 Summary of Water Recycling Guidelines and Mandatory Standards in the United States and Other Countries

Country/Region	Fecal Coliforms (CFU/100ml)	Total coliforms (cfu/100 ml)	Helminth eggs (#/L)	BOD_5 (ppm)	Turbidity (NTU)	TSS (ppm)	DO (%of Sat)	pH	Chlorine residual (ppm)
Australia (New South Wales)	<1	<2/50	--	>20	<2	--	--	--	--
Arizona	<1	--	--	--	1	--	--	4.5-9	--
California	--	2.2	--	--	2	--	--	--	--
Cyprus	50	--	--	10	--	10	--	--	--
EC bathing water	100 (g) 2,000 (m)	500 (g) 10,000 (m)	--	--	2 (g) 1 (m)	--	80-120	6-9	--
France	<1000	--	<1	--	--	--	--	--	--
Florida (m)	25 for any sample for 75%	--	--	20	--	5	--	--	1
Germany (g)	100(g)	500 (g)	--	20 (g)	1-2 (m)	30	80-120	6-9	--
Japan (m)	10	10	--	10	5	--	--	6-9	--
Israel	--	2.2 (50%) 12(80%)	--	15	--	15	0.5	--	0.5
Italy	--	--	--	--	--	--	--	--	--
Kuwait Crops not eaten raw	--	10,000	--	10	--	10	--	--	1
Kuwait Crops eaten raw		100		10		10			1
Oman 11A	<200	--	--	15	--	15	--	6-9	--
Oman 11B	<1000			20		30		6-9	
South Africa	0 (g)	--	--	--	--	--	--	--	--
Spain (Canary islands)	--	2.2	--	10	2	3	--	6.5-8.4	1
Texas (m)	75(m)	--	--	5	3	--	--	--	--
Tunisia	--	--	<1	30		30	7	6.5-8.5	--
UAE	--	<100	--	<10	--	<10	--	--	--
United Kingdom Bathing Water Criteria	100 (g) 2000 (m)	500 (g) 10000 (m)	--	--	2 (g) 1 (m)	--	80-120	6-9	--
US EPA (g)	14 for any sample, 0 for 90 %	--	--	10	2	--	--	6-9	1
WHO (lawn irrigation)	200 (g) 1000 (m)	--	--	--	--	--	--	--	--

Note: (g) signifies that the standard is a guideline and (m) signifies that the standard is a mandatory regulation

Source: EPA, Guidelines for Water Reuse, September 2004, EPA/625/R-04/108; page 251.

Material and Methods

3.1 General

Present study is aimed to analyze human health risk associated with treated effluent, used for parks/lawns irrigation in Delhi, India. The indicator microorganisms (total coliform and fecal coliform) and pathogenic microorganisms (*Salmonella* and *Shigella*) were monitored in the treated effluent. Other parameters like Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD_5), Turbidity, TSS and PH were also analyzed.

3.2 Experimental Methodology

3.2.1 Description of field sites

Lodhi Garden, Chankyapuri, India Gate and Rajghat were selected for the present study. The city of Delhi is located at latitude 28.61^0N, longitude 77.23^0E and lies in northern part of India, on the banks of the river Yamuna, approximately 200 km southwest of the Himalayan mountain front. The National Capital Territory of Delhi (NCT) spreads over a total area of 1483 km^2, of which more than 60% is now urban. Extreme temperatures range from −0.6 °C (30.9 °F) to 46.7°C (116.1 °F) and the annual mean temperature is 25°C (77 ^{0}F) in Delhi. The average annual rainfall is approximately 714 mm (28.1 inches), most of which is during the monsoon in July and August. Noting a fall in groundwater levels, the fact that all major parks in Delhi are facing water shortage and that the treated effluent used for horticulture purposes leaves behind a foul smell. The NDMC gets 120 million liters water per day from the Delhi Jal Board. According to officials, the quantity is not sufficient to meet the daily needs of a population of at least three lakh. To supplement supply, the civic body has 100 tube wells in its area, apart from an alternative supply of treated effluent for horticulture purposes, provided by the Central Public Works Department (CPWD). Tube wells provide water for horticulture, but we have realized that the major parks are dry due to over exploitation of groundwater. Also, it has been noted that the treated effluent used for horticulture has a bad smell and pathogenic microorganism. These

sites were selected for study because these are popular picnic destination and attract large crowd, exposed through the treated effluent used for parks/lawns irrigation.

3.2.1.1 Lodhi Garden

Geographical location of Lodhi garden is 28^0 38’ 41.49” N and 77^0 13’ 11.39” E elevation 219 m. Lodhi garden is a park in Delhi, India, spread over 90 acres (360000 m^2) of area. It is a popular picnic destination and attracts large crowd, around 1500 people visit it per day. The park is irrigated with treated effluent collected from STP located at Okhla, leaves behind a foul smell. In a first, the civic body is planning to introduce additional filtration and aeration treatment of the effluent by setting up a plant at the Lodhi Garden.

3.2.1.2 Chankyapuri

Geographical location of Chankyapuri (Hasanpur tank) is 28^0 35’ 11.74’’N and 77^0 11’ 27.83’’E elevation 232m. It is a water storage tank in Delhi, India. The water comes in the tank through STP located at Okhla and Rajghat pumping station then supplied in parks and house lawn for irrigation purpose in Chankyapuri industrial area, Sarogani Nagar, Lakshmibai Nagar, RK Puram, Kidwai Nagar areas. The parks of these locations are spread over large area, attract large crowd.

3.2.1.3 India Gate

India Gate is geographically located at latitude 28^0 36’ 46.15’’N, longitude 77^0 13’ 45.47’’E and elevation 215m in Delhi, India. The lawn of India Gate is spread over a large area. It is a very popular tourist spot and attracts large crowd. The lawn of India Gate is irrigated with water collected from the river Yamuna, directly, without any treatment.

3.2.1.4 Rajghat

Rajghat is geographically located at latitude 28^0 38' 41.49''N, longitude 77^0 14' 57.08''E and elevation 213m in Delhi, India. Rajghat is very popular tourist smudge and attract large crowd. It is irrigated with water collected from the river Yamuna, directly, without any treatment.

3.2.2 Collection of samples

Wastewater samples were collected at four different sites (Lodhi Garen, Chankyapuri, India Gate, Rajghat). Wastewater samples were collected in sampling bottles from these locations at an interval of 4 weeks for a period of 6 months. Samples were stored in an ice-packed box, and were immediately transported to the laboratory for analysis. In laboratory, samples were stored at less than 4^0C in a deep freezer. The microbiological analysis of the fresh samples was started within 4 hours of sample collection, and the microbiological enumeration was carried out for total coliforms, fecal coliforms, *Salmonella* and *Shigella*.

3.2.3 Reagent preparation

All reagents for microbiological and physicochemical analysis were prepared according to the procedures outlined in the Standard Method for Examination of water and wastewater (APHA 1998).

3.2.4 Microbiological assay of field samples

The total coliforms, fecal coliforms, *Salmonella* and *Shigella* were enumerated using multiple tube fermentation technique to obtain most probable number (MPN). For the enumeration of total coliforms, fecal coliforms and *Shigella* sample were suitably diluted using sterile deionized water before inoculation in appropriate medium in decimal dilutions (0.01, 0.1 and 1 ml). The *Salmonella* was enumerated by direct inoculation into enrichment medium in decimal dilutions (0.1, 1 and 10 ml) (APHA, 1998).

3.2.4.1 Total coliforms and Fecal coliforms

3.2.4.1.1 Presumptive procedure

Lauryl tryptose broth was used as a presumptive medium for Total coliforms and Fecal coliforms. The samples were diluted with sterile deionized water using serial dilution technique. The 0.1 ml, 1ml and 10 ml samples were inoculated into 5 tubes each containing presumptive media for Total coliforms and Fecal coliforms. All tubes were then incubated at 35±0.5^{0}C. After 24±2 hours swirl each tube gently and examined it for growth, gas, and acidic reaction (shades of yellow color) and, if no gas or acidic reaction was evident, reincubate and reexamined at the end of 48 ± 3 hours. Record presence or absence of growth, gas, and acid produced. If the inner vial was omitted, growth with acidity signified a positive presumptive reaction.

3.2.4.1.2 Confirmatory procedure

Brilliant green bile broth and EC broth were used as a confirmatory medium for Total coliforms and Fecal coliforms respectively. After presumptive phase, a loopful of each positive presumptive tube was inoculated on tubes of confirmatory medium Brilliant green bile broth and EC broth individually for the enumeration of Total coliforms and Fecal coliforms respectively. All Brilliant green bile tubes were incubated at 35± 0.5^{0}C for 24 hours in incubator and all EC broth tubes were incubated at 44.5 ± 0.2°C for 24 hours in water bath. Formation of
gas in any amount in the inverted vial of the brilliant green lactose bile broth tubes and EC broth tubes within 48 hours constitutes a positive confirmed phase. Calculated the MPN value from the number of positive tubes and EC tubes for Total coliforms and Fecal coliforms individually from MPN calculator.

3.2.4.2 *Salmonella* and *Shigella*

3.2.4.2.1 Enrichment procedure

Tetrathionate broth and Nutrient broth were used as an enrichment medium for *Salmonella* and *Shigella* respectively. The 0.1 ml, 1 ml and 10 ml samples were inoculated into 5 tubes each containing respective selective enrichment broth for Salmonella. The 0.1 ml and 1 ml samples were inoculated into 10 ml of single strength respective enrichment broth and 10 ml samples were inoculated into 10 ml of double strength respective enrichment broth. Enumeration of Shigella was carried out by direct inoculation technique. The samples were diluted with sterile deionized water using serial dilution technique. The 0.01 ml, 0.1 ml and 1 ml samples were inoculated into 5 tubes each containing respective selective enrichment broth for *Shigella.* All tubes were then incubated at 35 ± 0.5^0C for 24 hours.

3.2.4.2.2 Plating procedure

After enrichment, a loopful of each tube was streaked on plates of culture media XLD and MAC individually for the isolation of *Salmonella* and *Shigella* respectively. All of these plates were incubated at 35 ± 0.5^0C for 24 hours. After incubation, two or more suspected colonies from each plate were inoculated into triple sugar iron agar (TSI) butt slants and further incubated for 24 hours at 35 ± 0.5^0C. *Salmonella* and *Shigella* isolates were identified by biochemical API stripes. Calculate the MPN value from the number of positive TSI slants for *Salmonella* and *Shigella* from MPN calculator.

3.2.5 Physicochemical analysis

The physicochemical parameters, viz., biochemical oxygen demand (BOD_5), chemical oxygen demand (COD), total suspended solid (TSS), Turbidity and pH were analyzed according to the procedures outlined in the procedures of (APHA 1998). Turbidity of sample was measured by using 2100 P Turbidity meter and pH was measured by using Ino lab pH 720 in the laboratory.

Results and Discussion

4.1 Outcome of studies

Parks irrigated with treated effluent were found to be exposed to higher average (mean) densities of fecal coliforms, total coliforms, *Shigella* and *Salmonella*. Figure 4.3, 4.6, 4.8 and 4.11 represent the monthly coliforms (total and fecal cpliform) and pathogens (*Shigella* and *Salmonella*) occurrence in irrigation water at four different locations at Delhi. To analyze the variation in coliforms and pathogen occurrence, data from Jan to Jun were compiled. The highest Total coliform count was seen during the month of Feb and April at India Gate and in March Lodhi Garden, highest Fecal coliform count was seen during the month of April at India Gate and during the month of March at Lodhi Garden. The highest *Shigella* count was seen during the month of April and May at India Gate, Rajghat and Chnkyapuri and in Feb Lodhi Garden and Chankyapuri, highest *Salmonella* count was seen at Lodhi Garden and India Gate, negligible or very less number was seen at Chankyapuri. The significance of these results is that the microbes loading in irrigation water was varying in these locations. The other parameters which were studied for same samples are COD, TSS, pH and Turbidity and are given in Table 4.1.

Table: 4.1 Range of physicochemical parameters at different sampling locations

Location	COD mg/L	TSS mg/L	pH	Turbidity NTU
Lodhi Garden	79-152	101-145	7.7-8.3	56-244
Chankyapuri	69-135	25-95	7.5-8.3	19-50
India Gate	104-162	94-122	7.6-7.9	64-107
Rajghat	56-127	41-107	7.6-7.7	39-99

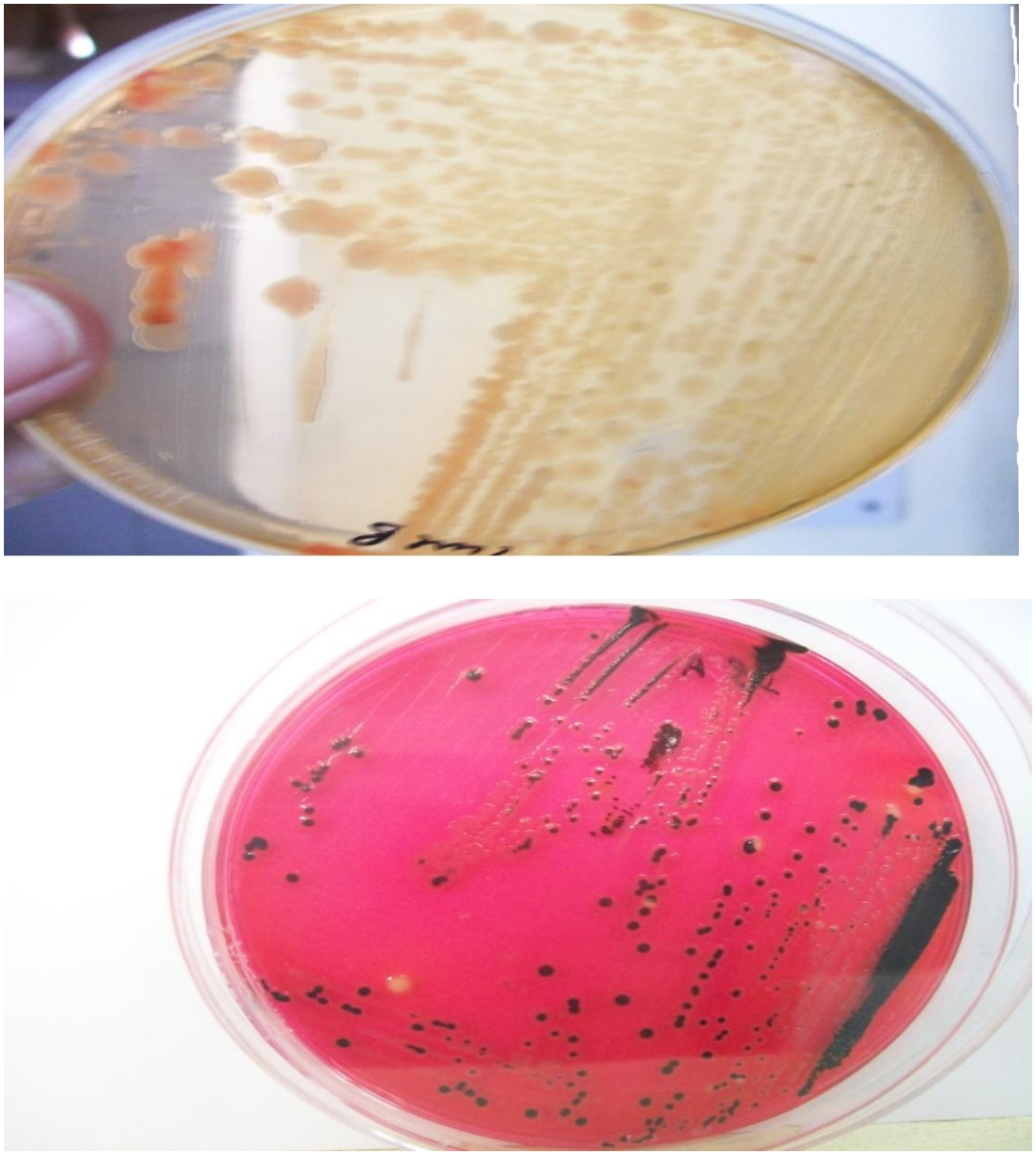


Figure 4.1 (a) *Shigella* in MacConkey agar plat (b) *Salmonella* in XLD plate

4.2 Concentration of coliforms and pathogens in irrigation water

To investigate the microorganism concentration in the irrigation water, total coliform, fecal coliform, *Shigella* and *Salmonella* were enumerated. The average concentration of total coliform at Lodhi Garden is 8.03×10^6 MPN/100ml, fecal coliform is 6.9×10^6 MPN/100ml, *Shigella* is 1.72×10^5 MPN/100ml and *Salmonella* is 2.04×10^2 MPN/100ml. The concentration of total coliform and fecal coliform at this site is always higher than from the standard limit, indicating that the irrigation water having disease causing microorganism are present at Lodhi Garden. In Chankyapuri the average concentration of total coliform is 4.59×10^5 MPN/100ml, fecal coliform is 4.59×10^5 MPN/100ml, *Shigella* is 9.64×10^4 MPN/100ml and *Salmonella* is 1.6 MPN/100ml. The concentration of coliforms and pathogens are slightly less than the Lodhi Garden irrigation water, the concentration of *Salmonella* is very less or negligible in this water, indicate that this irrigation water is less harmful comparatively with Lodhi Garden irrigation water.

Table: 4.2 Monthly total coliforms and fecal coliforms concentration in the different sites (value are MPN/100ml)

Site		**Month**				
		Jan	Feb	March	April	May
Lodhi Garden	TC	4.5×10^6	3.25×10^5	2.95×10^7	4.1×10^6	1.7×10^6
	FC	4.5×10^6	2.0×10^5	2.4×10^7	4.1×10^6	1.7×10^6
Chankyapuri	TC	4.5×10^5	4.0×10^5	4.4×10^5	4.4×10^5	5.65×10^5
	FC	4.5×10^5	4.0×10^5	4.4×10^5	4.4×10^5	5.65×10^5
India Gate	TC	4.0×10^6	1.3×10^7	7.8×10^6	1.35×10^7	9.4×10^6
	FC	4.0×10^6	7.8×10^6	5.65×10^6	1.35×10^7	5.65×10^6
Rajghat	TC	1.8×10^6	4.0×10^5	4.25×10^5	6.4×10^6	2.3×10^6
	FC	1.8×10^6	1.8×10^5	4.25×10^5	7.9×10^6	2.3×10^6

The average concentration of total coliform at India Gate is 9.54×10^6 MPN/100ml, fecal coliform is 7.32×10^6 MPN/100ml, *Shigella* is 1.22×10^5 MPN/100ml and *Salmonella* is 2.96×10^2

MPN/100ml. In Rajghat the average concentration of total coliform is 2.27×10^6 MPN/100ml, fecal coliform is 2.52×10^6 MPN/100ml, *Shigella* is 8.72×10^4 MPN/100ml and *Salmonella* is 1.21×10^2 MPN/100ml. The average concentration of coliforms and pathogens in both sites are nearly same. The concentration of coliforms and pathogens at these sites are always higher than the standard limit, the result indicates that the irrigation water has disease causing microorganism.

Table: 4.3 *Shigella* and *Salmonella* in different sampling sites (value are in MPN/100ml)

Site	**Month**				
	Jan	Feb	March	April	May
Lodhi Garden *Shigella*	4.0×10^6	2.0×10^5	6.1×10^4	2.4×10^5	6.8×10^4
Salmonella	1.3×10^2	2.4×10^2	3.5×10^2	1.7×10^2	1.3×10^2
Chankyapuri *Shigella*	4.5×10^4	2.12×10^5	4.5×10^4	4.0×10^4	1.4×10^5
Salmonella	00	00	4	00	4
India Gate *Shigella*	4.0×10^4	9.2×10^4	2.0×10^4	1.4×10^5	3.2×10^5
Salmonella	2.7×10^2	3.5×10^2	2.4×10^2	2.7×10^2	3.5×10^2
Rajghat *Shigella*	4.0×10^4	6.8×10^4	6.8×10^4	1.2×10^5	1.4×10^5
Salmonella	6.8×10^1	9.2×10^1	1.7×10^2	2.4×10^2	3.3×10^1

Figure 4.2 TSI slant of *E.coli, Shigella, Salmonella* and control

Table 4.4 Concentration of selected microorganisms in irrigation water in different sites (value are in MPN/100ml)

Microbes	Min	Median	Average	Max	Standard deviation

Lodhi Garden					
TC	3.25×10^{5}	4.1×10^{6}	8.03×10^{6}	2.95×10^{7}	1.21×10^{7}
FC	2.0×10^{5}	4.1×10^{6}	6.9×10^{6}	2.4×10^{7}	9.72×10^{6}
Shigella	4.0×10^{4}	6.8×10^{4}	1.72×10^{5}	4.5×10^{5}	1.75×10^{5}
Salmonella	1.3×10^{2}	1.7×10^{2}	2.04×10^{2}	3.5×10^{2}	9.32×10^{1}
Chankyapuri					
TC	4.0×10^{5}	4.4×10^{5}	4.59×10^{5}	5.65×10^{5}	6.23×10^{4}
FC	4.0×10^{5}	4.4×10^{5}	4.59×10^{5}	5.65×10^{5}	6.23×10^{4}
Shigella	4.0×10^{4}	4.5×10^{4}	9.64×10^{4}	2.12×10^{5}	7.7×10^{4}
Salmonella	00	00	1.6	4	2.19
India Gate					
TC	4.0×10^{6}	9.4×10^{6}	9.54×10^{6}	1.3×10^{7}	3.92×10^{6}
FC	4.0×10^{6}	5.65×10^{6}	7.32×10^{6}	1.35×10^{7}	3.71×10^{6}
Shigella	2.0×10^{4}	9.2×10^{4}	1.22×10^{5}	3.2×10^{5}	1.2×10^{5}
Salmonella	2.4×10^{2}	2.7×10^{2}	2.96×10^{2}	3.5×10^{2}	5.08×10^{1}
Rajghat					
TC	4.0×10^{5}	1.8×10^{6}	2.27×10^{6}	6.4×10^{6}	2.46×10^{6}
FC	1.8×10^{5}	1.8×10^{6}	2.52×10^{6}	7.9×10^{6}	3.14×10^{6}
Shigella	4.0×10^{4}	6.8×10^{4}	8.72×10^{4}	1.4×10^{5}	4.13×10^{4}
Salmonella	3.3×10^{1}	9.2×10^{1}	1.21×10^{2}	2.4×10^{2}	8.36×10^{1}

4.3 Lodhi Garden

The data presented in Table 4.4 show that the average concentration of TC, FC, *Shigella* and *Salmonella* were 8.03×10^{6} MPN/100ml, 6.9×10^{6} MPN/100ml , 1.72×10^{5} MPN/100ml and 2.04×10^{2} MPN/100ml respectively. The concentration of TC and FC are exceeding the allowable limits 1000FC/100ml according to EPA guideline (2004) for lawns/parks irrigation. Microbial water quality guideline value for irrigation water was established by (WHO, 1973) 1000 fecal coliforms per 100 ml, based on the findings of epidemiological studies of wastewater irrigation. High *E.coli* counts recorded are mainly due to the absence of disinfection in the treatment units that would not remove bacteria. In the previous study (Fattal *et al.*, 1986b) found a twofold excess risk of clinical enteric disease in young children (0-4 years) living within 600-1000m from sprinkler irrigated fields, but this was in the summer irrigation months only, with no excess illness found on an annual basis.

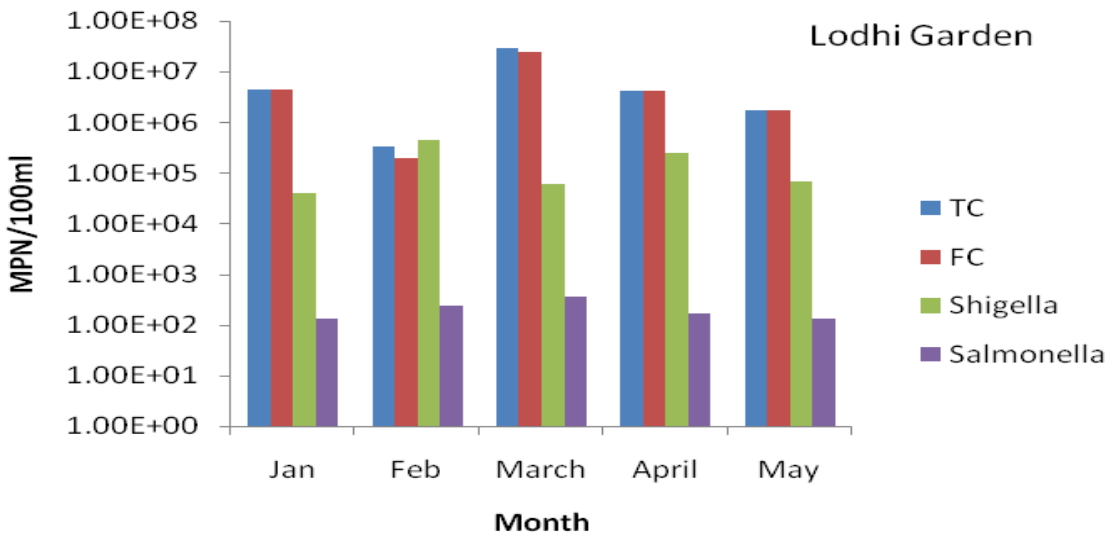
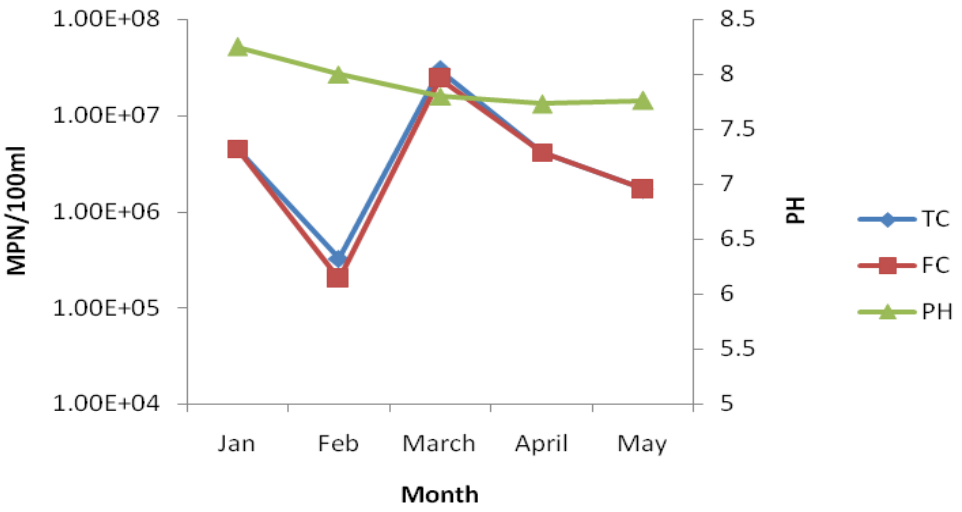


Figure 4.3 Concentrations of different microbes at Lodhi Garden irrigation water

Figure 4.3 shows the concentration of microorganisms in irrigation water at Lodhi Garden. Throughout the 6 month of investigation, it was observed that the number of pathogens (*Shigella* and *Salmonella*) was less than the coliforms (total and fecal coliform). The concentration of coliforms and *Salmonella* were high in the month of March while the concentration of *Shigella* was high in the month of Feb. The concentration of bacterial pathogens, namely *Shigalla,* Salmonella and coliforms in treated effluent indicate that there is potential health risk within the

area. In general, exposure of these microorganisms through inhalation and ingestion through sprinkler irrigation resulted in less hazard concentration compared to that through ingestion of drinking water. The continuous use of this treated effluent at Lodhi Garden irrigation exposes the people to a variety of microorganisms like coliforms, *Shigella* and *Salmonella*. Bacteria in the raw sewage, after being deposited in the soil are transported by air and spread illnesses among the people. High exposure to bacterial endotoxin cause health effects including fever, cough and dyspnoea. Inhalation of gram negative bacterial cell fragments produced both toxic and hypersensitivity effects. Chronic inhalation of bacterial endotoxin causes chronic bronchitis, emphysema and may be associated with airway hyper responsiveness.



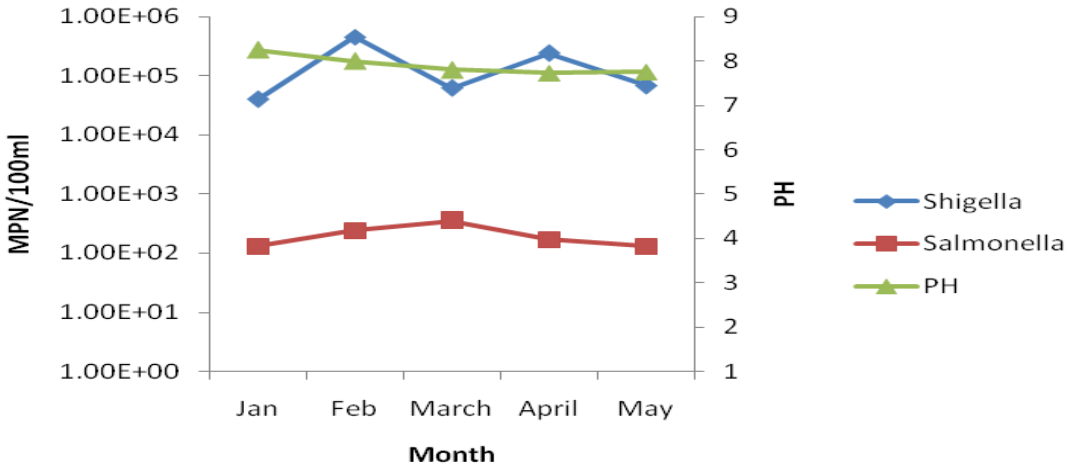


Figure 4.4(a) Effect of pH on total coliform and fecal coliform

(b) Effect of pH on *Shigella* and *Salmonella* at Lodhi Garden irrigation water

Figure 4.4 shows the effect of pH on coliforms and pathogens, not much variation was found in the pH in irrigation water at Lodhi Garden throughout the study time period. It was observed that the survival of microorganisms in irrigation water were not affected by the pH. The range of pH (7.7-8.3) in irrigation water at Lodhi Garden was within acceptable limit 6-9 as per the EPA guideline (2004) for lawns/parks irrigation.

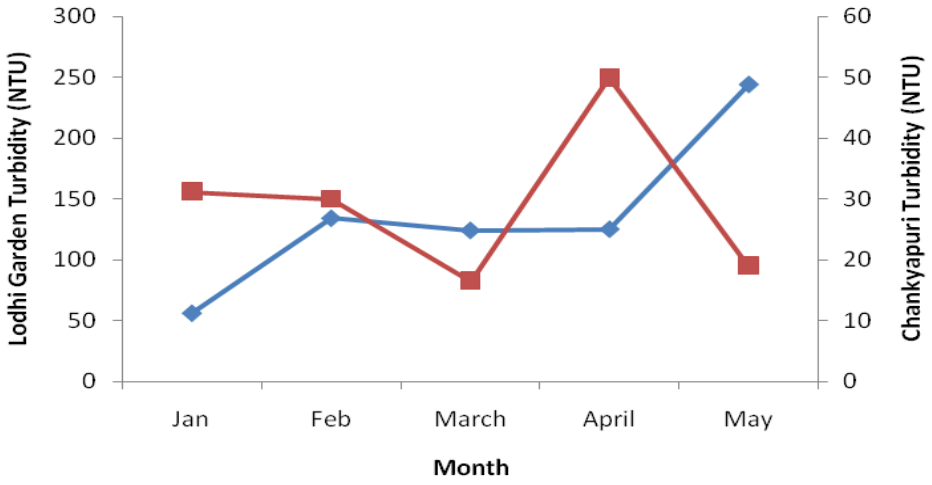


Figure 4.5: Turbidity of irrigation water at Lodhi Garden and Chankyapuri

4.4 Chankyapuri

The data presented in Table 4.4 shows that the average concentration of TC, FC, *Shigella* and *Salmonella* were 4.59×10^6 MPN/100ml, 4.59×10^6 MPN/100ml, 9.64×10^4 MPN/100ml and 1.6 MPN/100ml respectively. The concentration of TC and FC are exceeding the allowable limits 1000FC/100ml according to EPA guideline (2004) for lawns/parks irrigation. Microbial water quality guideline value for irrigation water established by (WHO, 1973) was 1000 fecal coliforms per 100 ml, based on the findings of epidemiological studies of wastewater irrigation. High E.coli counts recorded are mainly due to the absence of disinfection in the treatment units that would not remove bacteria. In the previous study (Fattal *et al.*, 1986b) found a twofold excess risk of clinical enteric disease in young children (0-4 years) living within 600-1000m from sprinkler irrigated fields, but this was in the summer months irrigation only, with no excess illness found on an annual basis.

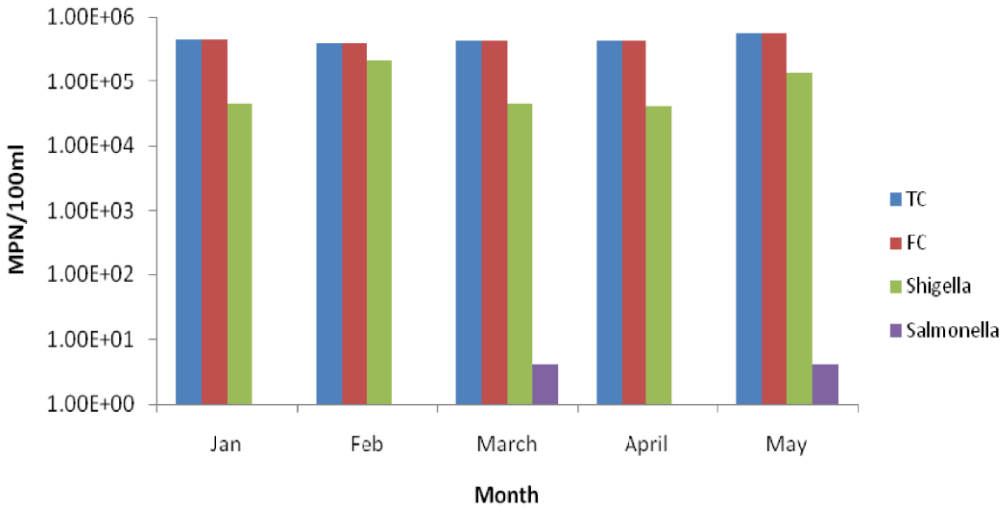


Figure 4.6 Concentration of different microbes at Chankyapuri irrigation water

Figure 4.6 shows the concentration of microorganisms in irrigation water at Chankyapuri. Throughout the 6 month of investigation, it was observed that the number of pathogens (*Shigella* and *Salmonella*) was less than the coliforms (total and fecal coliform). No variation was found in the concentration of coliforms throughout the study while slightly variation was shown in the concentration of *Shigella*, more in number during the month of Feb. The concentration of

Salmonella was very less or zero, only in the month of March and May few number of *Salmonella* was found in the water.

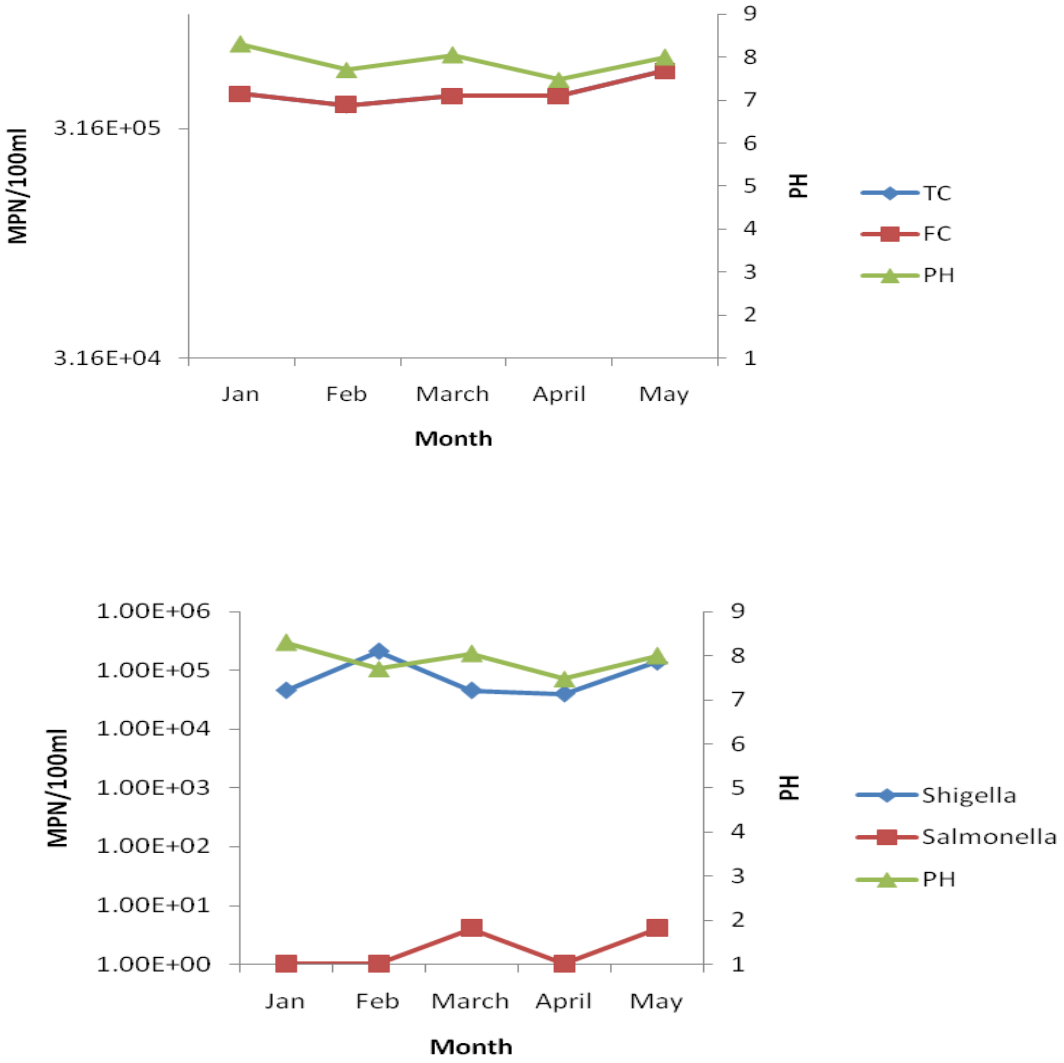


Figure 4.7(a) Effect of pH on total coliform and fecal coliform

(b) Effect of pH on *Shigella* and *Salmonella* at Chankyapuri irrigation water

Figure 4.7 shows the effect of pH on coliforms and pathogens, no more variation was found in the pH in irrigation water at Chankyapuri throughout the study time period. It was observed that the survival of microorganisms in irrigation water were not affected by the pH. The range of pH (7.5-8.3) in irrigation water at Chankyapuri was within acceptable limit 6-9 according to the EPA guideline (2004) for lawns/parks irrigation.

4.5 India Gate

The data presented in Table 4.4 show that the average concentration of TC, FC, *Shigella* and *Salmonella* were 9.54×10^{6} MPN/100ml, 7.32×10^{6} MPN/100ml , 1.22×10^{5} MPN/100ml and 2.96×10^{2} MPN/100ml respectively. The concentration of TC and FC exceed the allowable limits 1000FC/100ml according to EPA guideline (2004) for lawns/parks irrigation. Microbial water quality guidelines for irrigation water were established (WHO, 1973) was 1000 fecal coliforms per 100 ml, based on the findings of epidemiological studies of wastewater irrigation. High *E.coli* counts recorded are mainly due to the absence of disinfection in the treatment units that would not remove bacteria. In the previous study (Fattal *et al.*, 1986b) found a twofold excess risk of clinical enteric disease in young children (0-4 years) living within 600-1000m from sprinkler irrigated fields, but this was in the summer irrigation months only, with no excess illness found on an annual basis.

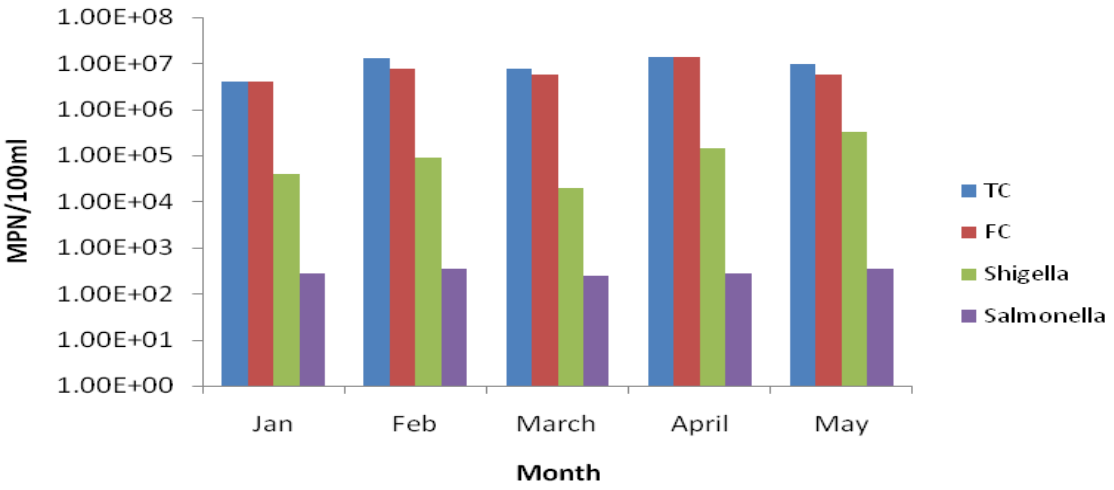
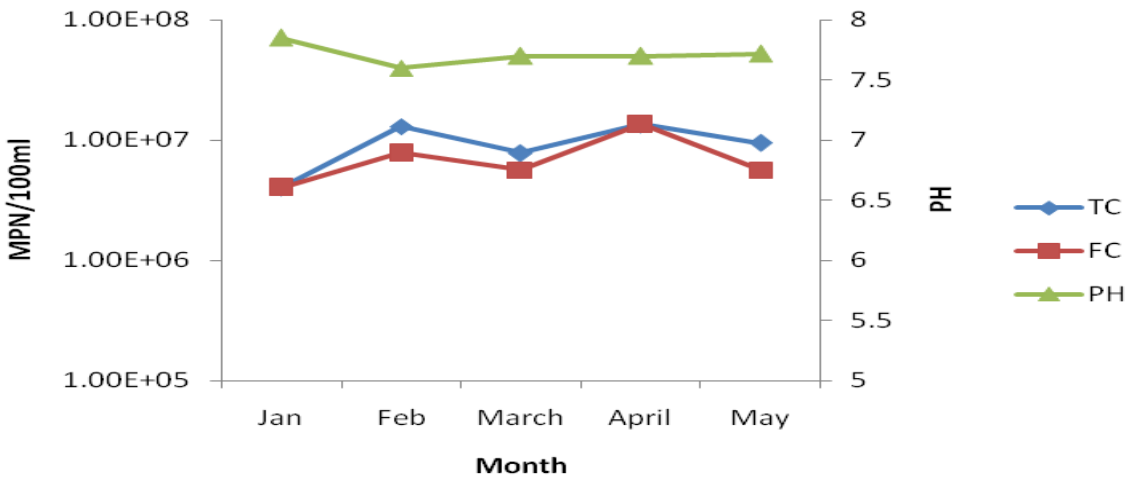


Figure 4.8 Concentration of different microbes at India Gate

Figure 4.8 shows that the concentration of microorganisms in irrigation water at India Gate. Throughout the 6 month of investigation, it was observed that not much variation was found in the concentration of coliforms and pathogens, the number of pathogens (*Shigella* and

Salmonella) was less than the coliforms (total and fecal coliform). The concentration of bacterial pathogens, namely *Shigalla*, Salmonella and coliforms in treated effluent indicate that there is potential health risk at Indian Gate irrigation water. The continuous use of this treated effluent in India Gate irrigation exposes the people through dermal inhalation and ingestion to a variety of microorganisms like coliforms, *Shigella* and *Salmonella*. Bacteria in the treated effluent, after being deposited in the soil are transported by air and spread illnesses among the people. High exposure to bacterial endotoxin cause health effects including fever, cough and dyspnoea. Inhalation of gram negative bacterial cell fragments produced both toxic and hypersensitivity effects. Chronic inhalation of bacterial endotoxin causes chronic bronchitis, emphysema and may be associated with airway hyper responsiveness.



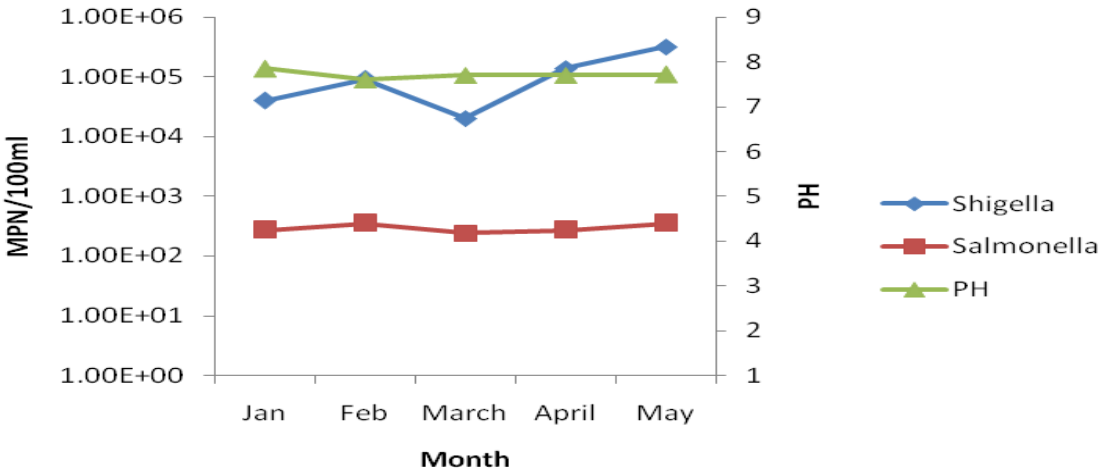


Figure 4.9(a) Effect of pH on total coliform and fecal coliform

(b) Effect of pH on *Shigella* and *Salmonella* at India Gate irrigation water

Figure 4.9 shows the effect of pH on coliforms and pathogens, no more variation was found in the pH in irrigation water at India Gate throughout the study time period. It was observed that the survival of microorganisms in irrigation water were not affected by the pH. The range of pH (7.6-7.9) in irrigation water at India Gate was within acceptable limit 6-9 according to EPA guideline (2004) for lawns/parks irrigation.

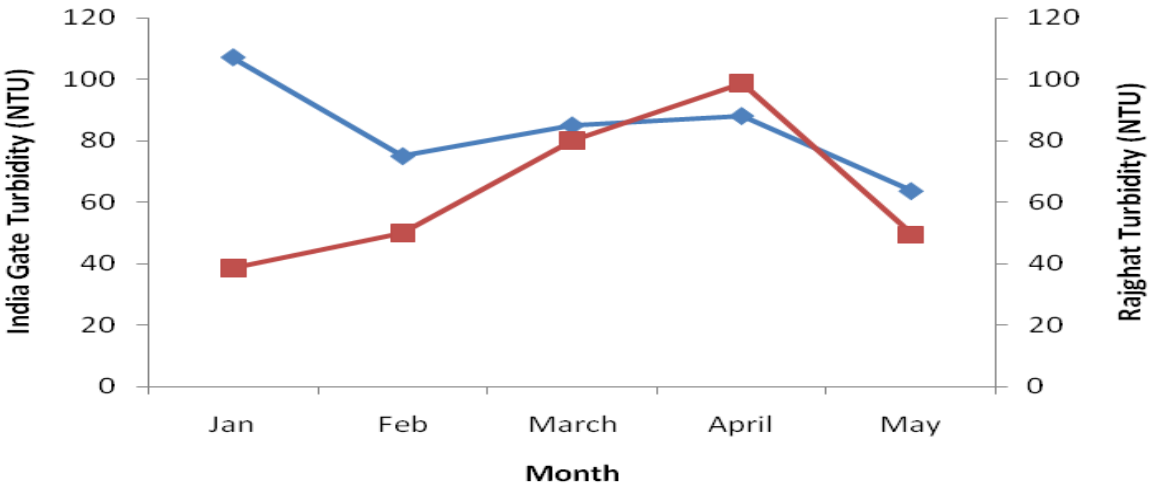


Figure 4.10 Turbidity of irrigation water at India Gate and Rajghat

4.6 Rajghat

The data presented in Table 4.4 show that the average concentration of TC, FC, *Shigella* and *Salmonella* were 2.27×10^{6} MPN/100ml, 2.52×10^{6} MPN/100ml and 8.72×10^{4} MPN/100ml, 1.2×10^{2} MPN/100ml respectively. The concentration of TC and FC exceed the allowable limits 1000FC/100ml according to EPA guideline (2004) for lawns/parks irrigation. Microbial water quality guidelines for irrigation water were established (WHO, 1973) was 1000 fecal coliforms per 100 ml, based on the findings of epidemiological studies of wastewater irrigation. High E.coli counts recorded are mainly due to the absence of disinfection in the treatment units that would not remove bacteria. In the previous study (Fattal *et al.*, 1986b) found a twofold excess risk of clinical enteric disease in young children (0-4 years) living within 600-1000m from sprinkler irrigated fields, but this was in the summer months irrigation only, with no excess illness found on an annual basis.

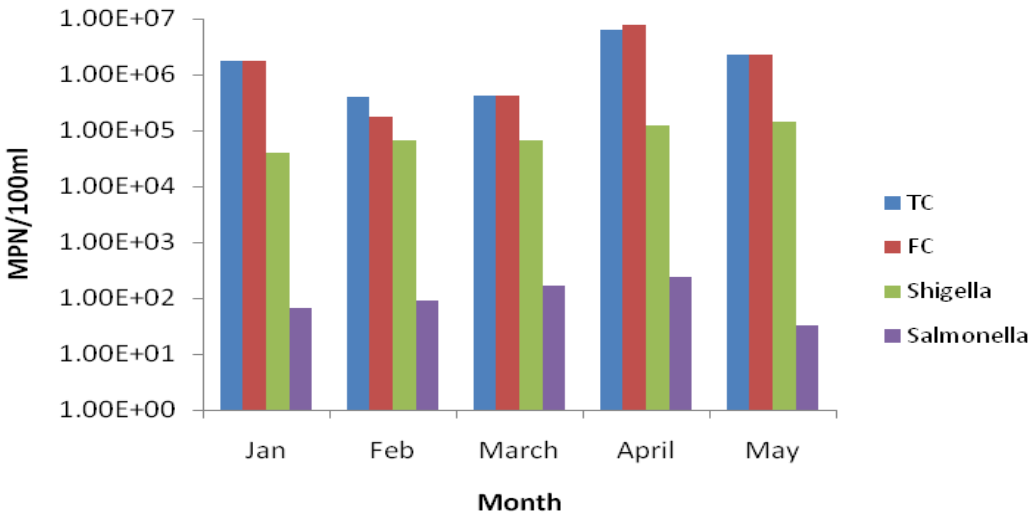


Figure 4.11 Concentration of different microbes at Rajghat irrigation water

Figure 4.11 shows the concentration of microorganisms in irrigation water at Rajghat lawn. Throughout the 6 month of investigation, it was observed that not more variation was found in the concentration of coliforms and pathogens, the number of pathogens (*Shigella* and *Salmonella*) was less than the coliforms (total and fecal coliform). The concentration of bacterial pathogens, namely *Shigella, Salmonella* and coliforms in irrigation water indicate that there is potential health risk at Rajghat. The continuous use of this water in Rajghat lawn irrigation

exposes the people through dermal inhalation and ingestion to a variety of microorganisms like coliforms, *Shigella* and *Salmonella*. High exposure to bacterial endotoxin cause health effects including fever, cough and dyspnoea. Inhalation of gram negative bacterial cell fragments produced both toxic and hypersensitivity effects. Chronic inhalation of bacterial endotoxin causes chronic bronchitis, emphysema and may be associated with airway hyper responsiveness.

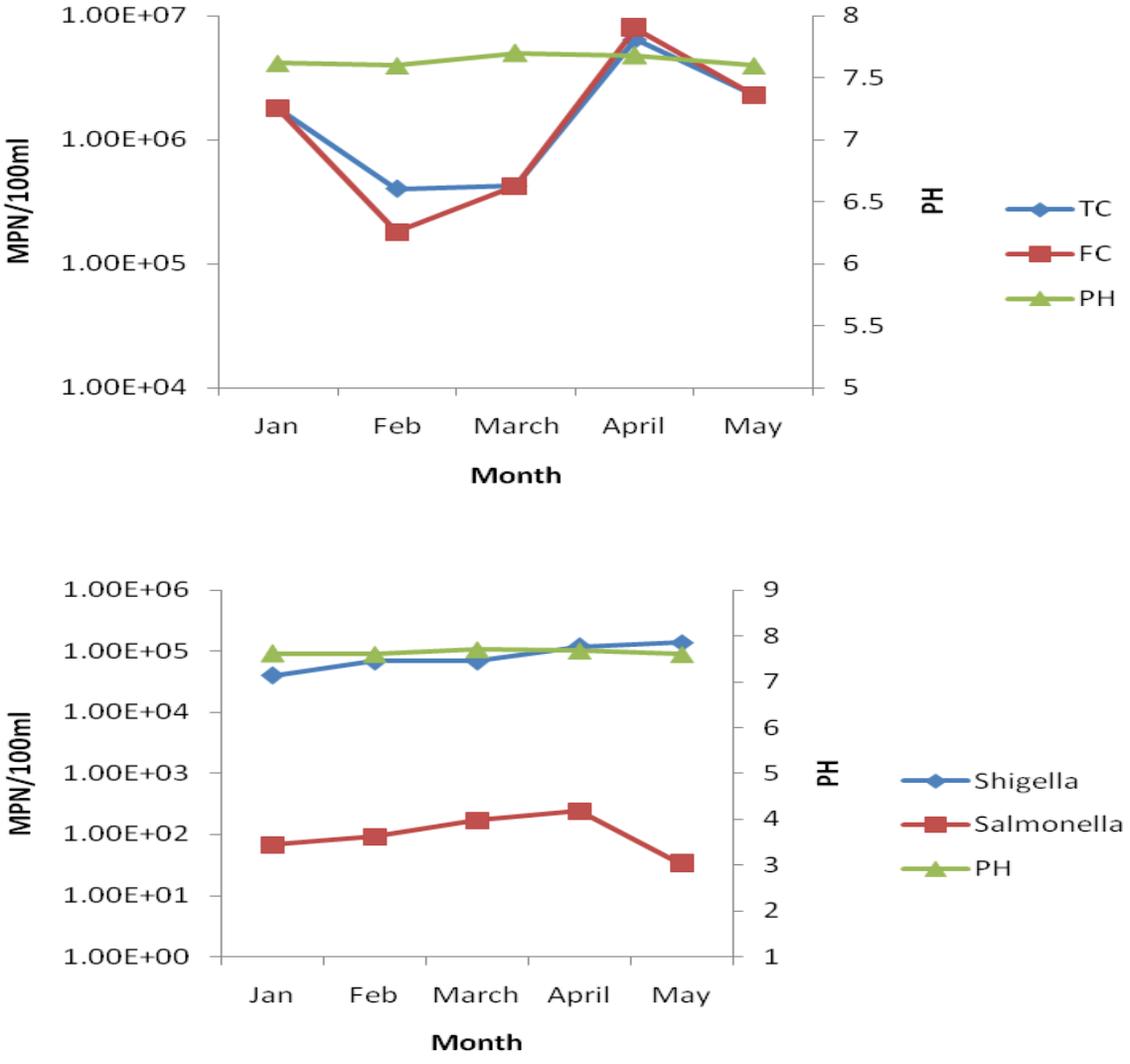


Figure 4.12 (a) Effect of pH on total coliform and fecal coliform

(b) Effect of pH on *Shigella* and *Salmonella* at Rajghat irrigation water

Figure 4.12 shows the effect of pH on coliforms and pathogens, not more variation was found in the pH in irrigation water at Rajghat throughout the study time period. It was observed that the

survival of microorganisms in irrigation water were not affected by the pH. The range of pH (7.6-7.7) in irrigation water at Rajghat was within limit 6-9 according to EPA guideline (2004) for lawns/parks irrigation.

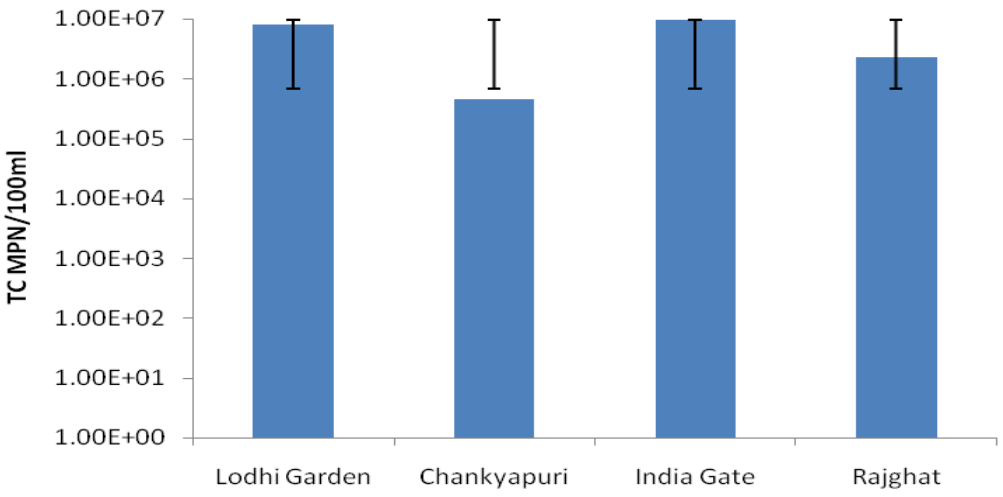


Figure 4.13 Total coliform concentration in different sites

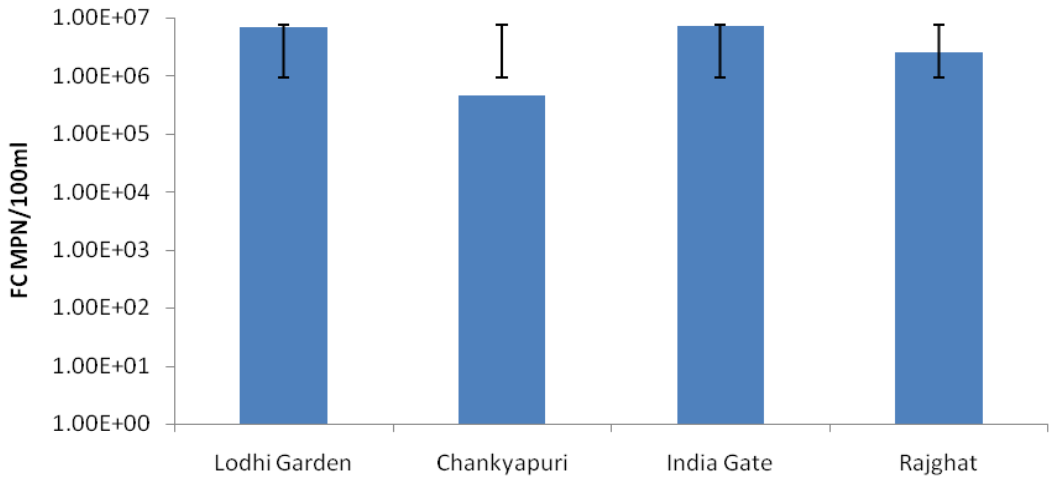


Figure 4.14 Fecal coliform concentrations in different sites

Figures 4.13 and 4.14 represent the average concentration of total coliform and fecal coliform in irrigation water for different field sites. Throughout the 6 month study, it was observed that the irrigation water used at India Gate has more number of total and fecal coliform and less number

at Chankyapuri. The concentration of total coliform and fecal coliform exceed the allowable limits 1000FC/100 ml set by EPA guideline (2004) for lawns/parks irrigation. The high coliform count obtained in the samples indicates that the water sources are faecally contaminated (EPA, 2003; Osuinde and Enuezie, 1999). The high coliform count indicates that the irrigation water contaminated with pathogenic bacteria such as *Shigella* and *Salmonella.*

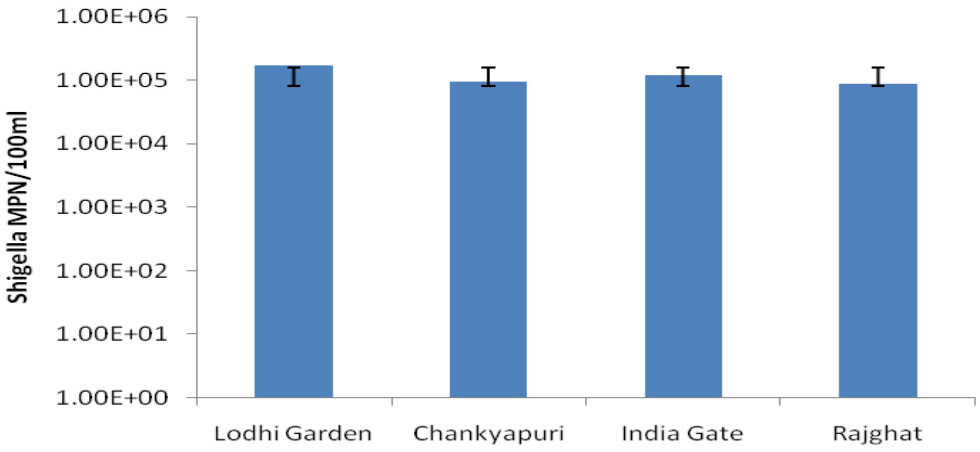


Figure 4.15 *Shigella* concentrations in different sites

Figure 4.15 shows the average concentration of *Shigella* in irrigation water for different field sites. Throughout the 6 month study of investigation, it was observed that the irrigation water use at Lodhi Garden has more number of *Shigella* and at Chankyapuri has less number of *Shigella* comparatively. There is no guideline for *Shigella* for parks/lawns irrigation water while for recreational water the infective dose of *Shigella* is very low, from 10^1 to 10^4 organisms (Rowe and Gross, 1984). *Shigella* causes acute diseases of large and small intestines, diarrhoea, fever, nausea and sometimes toxemia. Virulent *Shigella* spp. organisms cause bacillary dysentery (shigellosis), which may lead to death in some cases. Most deaths would occur in children under 10 years of age especially during the weaning period.

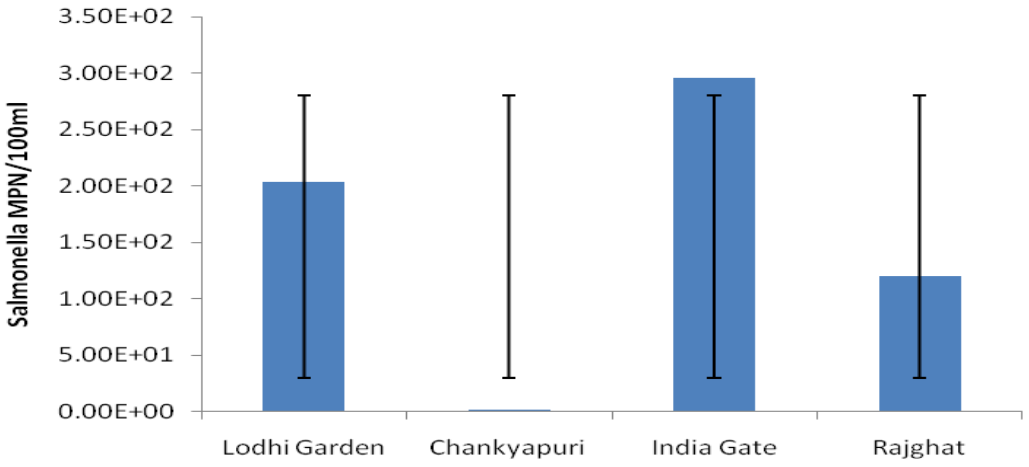


Figure 4.16 *Salmonella* concentration in different sites

Figure 4.16 shows the average concentration of *Salmonella* in irrigation water for different field sites. Throughout the 6 month of study, it was observed that the irrigation water use at India Gate has more number of Salmonella and less number at Chankyapuri, although for some time the concentration of *Salmonella* was zero. *Salmonella* is another pathogenic bacteria isolated from the irrigation water use in parks during this study. The species, *Salmonella* typhi is known to cause typhoid fever, enteric fever, anorexia and enlargement of the spleen. The detection of the organisms, *E. coli*, *Shigella*, *Salmonella*, thus indicate the potential risks and hazards faced by the nearby residents.

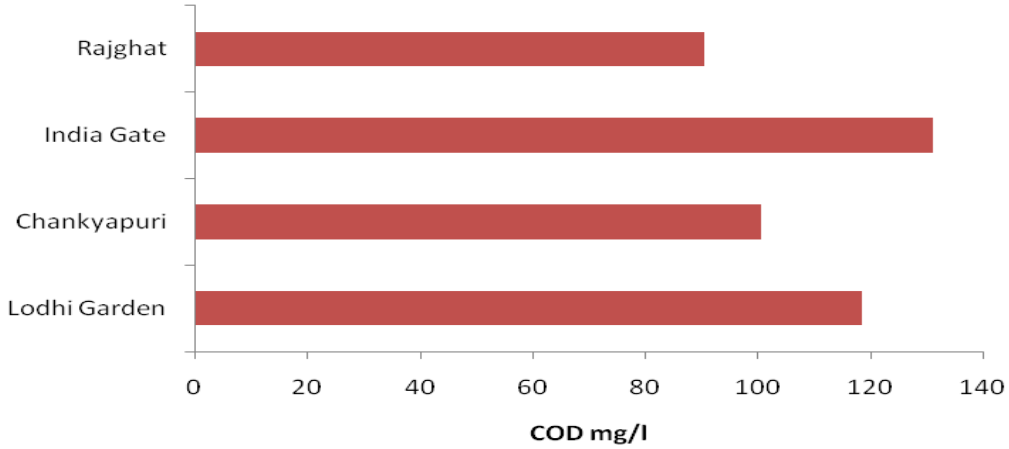


Figure 4.17 COD of irrigation water at different field site

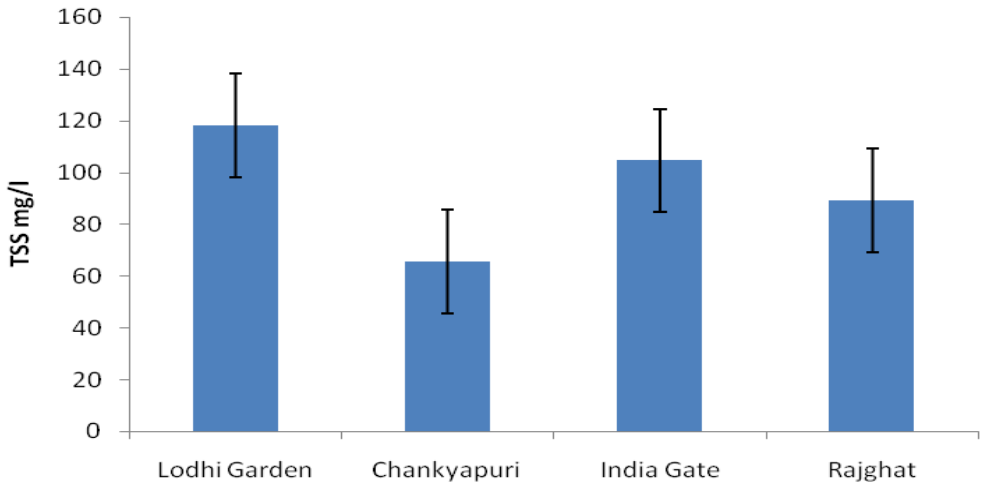


Figure 4.18 TSS of irrigation water at different field site

The results from the COD analysis (figure 4.17) for different sites indicate that the irrigation water use at India Gate have high average COD or high organic load. The TSS result for different sites indicate that the irrigation water use at Lodhi Garden has high average TSS and at Chankyapuri the average TSS concentration was low. These TSS concentrations of irrigation water are exceeding the allowable limits 30NTU according to EPA guideline (2004) for lawns/parks irrigation. These COD and TSS value indicate that the water use for irrigation are not properly treated and possibly cause health related problems.

Conclusion

This study presents the microbiological risk assessment for parks/lawns irrigated by using treated effluent. Analysis of total coliform, fecal coliform, *Shigella* and *Salmonella* was carried out for exposure of human being if using such irrigated facilities. The average concentrations were found to lie between 10^5 to 10^7 MPN/100ml for total coliform, 10^5 to 10^7 MPN/100ml for fecal coliform, 10^4 to 10^5MPN/100ml for *Shigella* and 00 to 10^2 MPN/100ml for *Salmonella* and hazard concentration were found to be lie 1000MPN/100ml for total and fecal coliform for people exposed to treated effluent used in park irrigation. The concentration of bacterial pathogens, namely *Shigella, Salmonella* and coliforms (bacterial pathogen indicator) in treated effluent indicate that there is potential health risk within the study area. In general, exposure of these microbes through inhalation and ingestion through Sprinkler resulted in less hazard concentration compared to that through ingestion drinking water. The continuous use of this treated effluent in parks irrigation exposes the people to a variety of microorganisms like coliforms, *Shigella* and *Salmonella.* Bacteria in the raw sewage, after being deposited in the soil are transported by air and spread illnesses among the people. High exposure to bacterial endotoxin may cause health effects including fever, cough and dyspnoea. Inhalation of gram negative bacterial cell fragments produce both toxic and hypersensitivity effects. Chronic inhalation of bacterial endotoxin causes chronic bronchitis, emphysema and may be associated with airway hyper responsiveness. Finally, the use of treated effluent for public park irrigation without undue hazard to health, provided that bacteria density levels are kept below those coming through analysis of treated effluent.

However, on the basis of comprehensive evaluation of the available data, it is obvious that to reduce the potential health hazard associated with treated effluent quality should be improved and disinfection should be added to the treatment process, subsequent treatment steps may be supported and simplified leading to decrease water treatment costs. Risk assessment has proven to be a very useful tool to reveal the true meaning and relevance of the concentrations of microbes found in public parks irrigated with treated effluent. By jointly considering hazard and

level of exposure, risk assessment allows the identification of the pathogen and pathways of most concern in a situation of public parks exposure.

This study provides assessing risk associated with exposure of microbes from treated effluent used in public park irrigation. This study focused on issues, such as considerations for sensitive population and concentration of microbes in treated effluent for assessing risks associated with exposure of microbes in treated effluent.

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