PARASITOLOGICAL ASSESSMENT OF WASTEWATER AND THE EFFICACY OF BIOSAND FILTER IN THE REMOVAL OF PARASITE OVA AND CYSTS

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AHMADU BELLO UNIVERSITY, ZARIA

NIGERIA

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DECLARATION

I declare that the work in the thesis entitled 'Parasitological assessment of wastewater and the efficacy of biosand filter in the removal of parasite ova and cysts'was carried out by me in the Department of Microbiology under the supervision of Dr. (Mrs) H.I. Inaboand Dr.S.E. Yakubu.

The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at any university.

Okojokwu, Ocheme Julius

_____	_____
Name of Student	Signature/Date

CERTIFICATION

This thesis entitled "Parasitological assessment of wastewater and the efficacy of biosand filter in the removal of parasite ova and cysts" by Okojokwu, Ocheme Julius meets the regulations governing the award of the degree of Master of Science of Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This thesis is dedicated to my mother, Mrs Otsanya Comfort Okojokwu,who was not sure what her son was doing yet believedin me,offered her love, support and shoulders for me to lean on when the going got tough.

To my brothers, Unogwu, Agbese and Attah, who were sparkling gold in many dark nights.

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ABSTRACT

In this study, the parasitological profile of wastewater from the Ahmadu Bello University waste stabilisation ponds (ABU-WSP) and Ahmadu Bello University Teaching Hospital sewage treatment plant (ABUTH-STP), Zaria, Nigeria were evaluated using the sucrose floatation andKinyoun modified Acid-Fast(modified Ziehl-Neelsen) staining techniques. The parasite removal efficiency of biosand filter was also tested in comparison with the waste stabilisation ponds and the sewage treatment plant. A total of 1920 L of wastewater was examined and a significant level of parasite eggs, cysts and oocysts were detected. In all, 1,648 parasites eggs, cysts and oocysts per litre were counted in sewage from ABUTH–STP. Ova of *Ascarislumbricoides* had the highest count/litre of 307 (18.63%) followed by ova of *Taenia*spp (n=287; 17.42%). The least count per litre was for cysts of *Giardia lamblia* (n=58; 3.52%). The analysis revealed that 52.61% of the parasite eggs, cysts and oocystswere removed by the sewage treatment plant while egg removal efficiency of the biosand filter was 97.45%. On the other hand, a total of 874 parasites' eggs, cysts and oocysts per litre were counted in sewage from ABU–WSP. *Ascarislumbricoides* accounted for the highest eggs/litre (n = 198; 22.65%) followed by *Taenia*spp (n = 155; 17.73%) and Hookworm (n = 123; 14.07%). Cysts of *Giardialamblia* had the least count/litre (n = 3; 0.34%). The mean egg or (oo)cyst/litre of sewage from the ABU-WSP revealed that the raw sewage had 12.38 ± 0.93 *Ascarislumbricoides* egg/litre. Further comparison of mean parasite eggs/litre in the effluents of biosand filter and the sewage treatment plant using Wilcoxon's Signed Ranks Test indicated a significant difference ($p< 0.05$) with biosand filter having lower counts per litre. One-way analysis of variance (ANOVA) of the efficiencies of the three treatment methods showed that biosand filter was significantly more efficient than the sewage treatment plant ($p <0.001$) in the removal of helminth eggs and protozoan (oo)cysts. The results obtained therefore demonstrated that the raw wastewater was laden with parasite eggs, cysts and oocysts and hence posed public health threat to the users of the effluent downstream. The biosand filter was efficient in removing oocysts of *Cryptosporidium parvum* and *Giardia lamblia*cysts and was more efficient than the sewage treatment plant; its effluent contained insufficient level of the ova, cysts and oocysts of parasites well below the less than one (<1) helminth ova/protozoa cysts as recommended by World Health Organisation (WHO).

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ABBREVIATIONS

ABU	–	Ahmadu Bello University.
ABUTH	–	Ahmadu Bello University Teaching Hospital.
ANOVA	–	Analysis of variance.
AOC	–	Assimilable organic compounds.
ATU	–	Aerobic treatment unit.
BOD	–	Biological oxygen demand.
COD	–	Chemical oxygen demand.
HDPE	–	High density polyethylene.
HSD	–	Honestly significant difference.
NTU	–	Nephlometric turbidity unit.
PAO	–	Polyphosphate accumulating organisms.
PBS	–	Phosphate buffered saline.
PFU	–	Plaque forming units.
SS	–	Suspended solids.
STP	–	Sewage treatment plant.
USEPA	–	United States Environmental Protection Agency.
WHO	–	World Health Organisation.
WSP	–	Waste stabilisation pond.

CHAPTER ONE

INTRODUCTION

Wastewater can be a vehicle of various diseases and high level of pathogens are often reported in raw sewage worldwide (USEPA, 1998).The practice of discharging wastewater into water bodies causes considerable damage to the ecosystem, waterfront inhabitants, swimmers, and fishermen (Hamdani and Assobhei, 2001; Paraskevas *et al.,* 2002). Depending on its origin, wastewater can transport many pathogens depending upon the incidence of disease in the indigenous human and animal population that discharge to the sewer (Leclerc *et al.,* 2002). Hospital wastewater is usually heavily laden with pathogens and toxic chemicals and hence requires adequate treatment to remove or appreciably reduce the available pathogens to a level that agrees with permissive ranges stipulated by regulatory authorities. The problem of contamination caused by the discharge of wastewater into water bodies is directly evident in intoxications, skin problems and intestinal parasitosis (Rodriguez *et al.,* 2004; Srikanth and Naik, 2004). This problem is compounded when these discharges are deposited in the vicinity of low-income neighbourhoods where basic hygiene practices are totally absent (Nyarango *et al.,* 2008).

Globally, millions of people suffer from parasitic infections such as Ascariasis (1.2 billion), Trichuriasis (795 million), hookworm infections (740 million) (de Silva *et al*., 2003), Amoebic dysentery (50 million) (Samuel *et al*., 2001) and Giardiasis (2.8 million) (Ali and Hill, 2003). In humans, these parasites are significantly associated with diarrhoea (Utzinger *et al.,* 1999). Faeco-oral route is important in the transmission of parasitic infections to humans vdue to poor personal hygiene, environmental conditions such as contamination of soil and water sources with human faeces (Muttalib *et al.,*1983) and poor wastewater disposal such as use of night soil for fertiliser (Mustafa *et al.,* 2001).

Cryptosporidium parvum and *Giardia lamblia* are two notorious pathogenic protozoa that produce oocysts and cysts respectively that are passed in the faeces of the hosts. These parasites are the common causes of acute gastroenteritis in humans and animals (O'donoghue, 1995; Lloyd and Harris, 2002). The size of *Giardia lamblia* cysts measures between 8 and 12 µm long and 7 – 10 µm wide and the oocysts of *Cryptosporidium parvum* is approximately 4 – 6 µm (Willey *et al*., 2008). The cell wall of oocysts and cysts is covered by a thick hyaline wall material which makes them very resistant to environmental conditions, including drinking water disinfection. In water environment, cysts and oocysts will survive and remain viable for months (Peeters*et al*., 1989; Korich*et al*., 1990; LeChevallier*et al*., 1991a; Black *et al*., 1996; Venczel*et al*., 1997; Hill, 2005; White, 2005;Lim *et al*., 2007; Sobsey, 2009).

There have been at least 325 water associated outbreaks of parasitic protozoan diseases documented worldwide. Cryptosporidiosis and giardiasis have been reported to account for a majority of these outbreaks(40.6% and 50.8% respectively) affecting hundreds of thousands of individuals (Smith and Grimason, 2003; Karanis*et al*., 2007). The evidence of potential role of sewage treatment plant contamination in Cryptosporidiosis outbreak was first highlighted in San Antonio, Texas (D'Antonio*et al*., 1986). In 1993, contaminated sewage was singled out as the culprit in the largest Milwaukee cryptosporidiosis outbreak that affected 403,000 and killed more than 100 with weakened immune systems (Sulaiman*et al*., 2004). Initially the outbreak was assumed to have been caused by run-off from upstream cattle pastures but genetic evaluation data concluded that the outbreak was due to the human type *Cryptosporidium* coming from human faecal material and human-associated wastewater (Peng*et al*., 1997;Rose*et al*., 2002).

Studies on various sewage treatment plants have been carried out throughout the world in developed and developing countries. The occurrence of *Cryptosporidium*

*parvum*and *Giardia lamblia* in sewage treatment plants varies with treatment plants. This variation is due to the differences in the standard sanitation and water treatment technology in the respective sewage treatment plants (Roach *et al*., 1993; Wallis *et al*., 1995; Amahmid*et al*., 2002; Farias*et al*., 2002; Scott *et al*., 2003). Conventional wastewater treatment processes may remove most gastrointestinal parasites of man, however, *Cryptosporidium parvum* oocysts and *Giardia lamblia* cysts are less dense and smaller in size (*Cryptosporidium* = 4 - 5 × 4 – 6 µm; *Giardia* = 8 – 12 × 7 – 10 µm) in comparison to helminthic eggs, they may penetrate through wastewater treatment systems more readily. Documented evidence indicated that oocysts and cysts can pass through conventional wastewater treatment processes with reported efficiencies of oocysts and cysts removal varying from 79 to 99% for *Cryptosporidium parvum* (Madore*et al*., 1987) and 40 to 100% for *Giardia lamblia* (Sykora*et al*., 1987) depending upon the degree of secondary treatment.

Detection of these two parasites has been reported in human and animal (i.e. cattle, goat, lamb, rodents, birds, dogs and mouse deer) populations, river, well water and raw water of drinking water treatment plants (Ahmad *et al*., 1997; Lim *et al*., 1997; Fayer*et al*., 2000; Thompson, 2000; Lim and Ahmad, 2001, 2004a and 2004b; Lim *et al*., 2005). Abstraction of contaminated river water is evident by the presence of high concentration of oocysts and cysts in raw waters of drinking water treatment plants (Lim and Ahmad, 2001). The possibility of an outbreak occurring increases when there is a treatment plant malfunction that enables these parasites to penetrate the treatment processes more easily. Sewage treatment plants have the potential to be a source of contamination to watershed if the treatment processes employed do not sufficiently treat the effluents before being discharged into nearby river or pond.

Biosand filter has been used in developed and some developing countries for the treatment of raw water meant for drinking and cooking purposes. Biosand filter is a slow sand filter that is reputed for its efficiency in the removal of pathogenic organisms such as viruses, bacteria and protozoa (Sobsey, 2009).

1.1 Statement of the problem

Cryptosporidium parvum and *Giardia lamblia* have caused several waterborne outbreaks especially in the USA and United Kingdom. In these countries a lot of research, risk assessment and risk management efforts have been directed at the incidence of *Cryptosporidium parvum* and *Giardia lamblia* in drinking water. In a study in USA, 1000 – 10,000 cysts/oocysts per litre of *Giardia lamblia*/*Cryptosporidium parvum* were counted in the primary effluent, and 10 cysts per litre and 17 oocysts per litre in the secondary effluent of sewage treatment plant(Lim *et al*., 2007).

Effluent water from sewage treatment plant is an important source for raw water contamination. Therefore, the risk of giardiasis and/or cryptosporidiosis increases with increase in the level of raw water contamination by sewage. The causative organisms of these diseases produce environmentally robust stages (oocysts and cysts for *Cryptosporidium* and *Giardia* species respectively) which are resistant to chlorination of drinking water treatment plants. The discharge of wastewater into rivers used as source of raw water abstracted for drinking water therefore poses a threat to public health.

The use of water for irrigation purposes from rivers into which wastewater is discharged is another public health threat as some vegetables (e.g. cabbage, lettuce, tomatoes, carrots, onions, etc) which are either eaten raw or partially cooked are contaminated with *Giardia lamblia* cysts and/or *Cryptosporidium parvum* oocysts. The problem is made worse

by the low infective doses (10 to 100 oocysts for *Cryptosporidium parvum* and 10 to 25 cysts for *Giardia lamblia*), and the fact that the oocysts and cysts remain viable for 2 to 6 months in a moist environment (Hill, 2005; White, 2005; Willey *et al*., 2008).

In 1993, the United States of America recorded the world's greatest outbreak of parasitic waterborne cryptosporidiosis outbreak which was traced to contamination of source water by sewage. Hence, adequate treatment of sewage will reduce the risk of parasitic protozoan waterborne infections.

A study carried out by LeChevallier*et al.* (1991b) showed that 81% and 87% of raw water samples tested had *Giardia lamblia* cysts and *Cryptosporidium parvum* oocysts. *Cryptosporidium* oocysts and *Giardia* cysts were detected in 27% and 17% respectively of drinking water samples.Giardiasis and cryptosporidiosis are life-threatening in children, AIDS patients and other immunocompromised individuals. Giardiasis can resemble other causes of traveller's diarrhoea and is often not recognised as being giardiasis (Wolfe, 1992). No chemotherapy is available for cryptosporidiosis. Supportive care with oral or intravenous rehydration is the treatment of choice for immunocompetent patients (Marshall, 1997).

1.2 Justification of the study

Oocysts of *Cryptosporidium parvum* and cysts of *Giardia lamblia* occur in aquatic environment throughout the world. They have been found in most surface waters, where their concentration is related to the quantity of sewage deposited into such water, level of faecal pollution or human use of the water (LeChevallier*et al*, 1991a). The environmentally robust oocysts are very persistent in water (DeLeon*et al.,* 1988; Chaure*et al.,* 1999; Robertson *et al*., 2000, 2006a, 2006b) and extremely resistant to the chemical agents commonly used in drinking-water treatment (Korich*et al*., 1990; Hill*,* 2005; McCuin and Clancy*,* 2005; White, 2005).These characteristics, coupled with the low numbers of oocysts and cysts required for

an infection (Rendtorff, 1954;Dupont*et al.,* 1995; Hill, 2005; White, 2005; Robertson *et al*., 2006a) placed these organisms among the most critical pathogens in the production of safe drinking-water from surface water. Well protected groundwater sources that are not mixed with surface water or otherwise contaminated are free of these and other enteropathogens. If abstraction, treatment, and distribution systems for these waters are properly designed and operated, the risk of faecal contamination is very low and there will be no waterborne transmission of parasitic protozoa. Groundwater that mixes with surface water or other sources of contamination (e.g. sewage and surface run-off) may contain high levels of oocysts and cysts of *Cryptosporidium parvum* and *Giardia lamblia* (Solo-Gabriele *et al*., 1998;Payment *et al*., 2001) which may lead to waterborne illness (Craun*et al.,* 1998).

Filtration of such waters is essential to the production of potable drinking-water: treatment by disinfection alone offers no protection against *Cryptosporidium parvum* and only limited protection against *Giardia lamblia* (Hill, 2005; White, 2005; Lim *et al*., 2007). Many waterborne outbreaks of giardiasis and cryptosporidiosis have been reported in industrialised countries (MacKenzie*et al.,* 1994; Craun*et al.,* 1998; Hill, 2005; White, 2005). In these outbreaks, oocysts and cysts of *Cryptosporidium parvum* and *Giardia lamblia* entered the drinking-water because of surface-water treatment failure, contamination of the water source with sewage, and leakage into the distribution system. In a significant number of these outbreaks, the drinking-water implicated as the cause complied with the WHO Guidelines for *Escherichia coli* levels and turbidity (Craun*et al.,* 1998), but deviations from normal raw water quality or treatment operations recommended in the guidelineswere identified. However, in an outbreak in Las Vegas, USA, that was traced to drinking-water, no abnormalities in treatment operations or in the quality of raw or treated water was detected (Solo-Gabriele *et al*., 1998).

A major public health problem arises from the fact that oocysts of *Cryptosporidium parvum* and *Giardia lamblia* cysts are only 4 to 6 µm and 7 to 10 µm in diameters respectively (Willey *et al*., 2008). Sewage treatment plants use rapid sand filters with effective size of 0.55 mm, whereas the sizes of the cysts and oocysts are so small that they pass through the interstices of the sand used as filter medium in the rapid sand filter of the sewage treatment plants.

Biosand filter, on the other hand, is a slow sand filter with the sand (filter medium) having effective size of between 0.10 to 0.25 mm, and interstices that are smaller than the sizes of the oocysts of *Cryptosporidium parvum* and *Giardia lamblia* cysts (CAWST, 2009). Therefore if biosand filter is found efficacious in removing the oocysts of *Cryptosporidium parvum* and *Giardia lamblia* cysts from wastewater, it could be used to replace the rapid sand filter in the wastewater treatment.

The fact that outbreaks occur in the absence of any warning signal from the routine water quality monitoring for coliforms points to a severe limitation of coliform level as an indicator for microbiological safety of drinking-water. Additional means of safeguarding drinking-water by ensuring that sewage is properly treatedbefore being channelled into source water are therefore imperative.

1.3 Scope and delimitation of the study

This study is restricted to determination of parasite ova, cysts and oocysts in raw sewage and in effluents of Ahmadu Bello University wastewater treatment plant, Zaria, Nigeria. The study involved examining wastewater to decide whether these organisms exist in the wastewater at all, and if so in what level. The second part of the work focused on the efficacy of biosand filter in the removal of *Cryptosporidium parvum* oocysts and *Giardia lamblia* cysts from raw wastewater.

1.4 Aim of the study

The aim of the research was to evaluate the parasitological status of wastewater and the efficacy of biosand filter in the elimination of *Giardia lamblia* cysts and oocystsof *Cryptosporidium parvum*.

1.5 Objectives of the study

1. To determine the prevalence of parasite ova and (oo)cysts in wastewater treatment plants.
2. To assess the efficacy/efficiency of biosand filter for the removal of *Giardia lamblia* cysts and *Cryptosporidium parvum* oocysts from influent and effluent waters.
3. To evaluate and compare the parasite eggs removal efficiency of biosand filter (slow sand filter) with the conventional rapid sand filter used in the wastewater treatment and the wastewater stabilisation pond used in wastewater treatment.

CHAPTER TWO

LITERATURE REVIEW

2.1 Origin of sewage

Sewage or wastewater is created by residential, hospital, institutional, commercial and industrial establishments and includes household waste liquid from toilets, baths, showers, kitchens, sinks and so forth that is disposed of via sewers (Campbell and Wallis, 2002).

The separation and draining of household waste into greywater and blackwater is becoming more common in the developed world, with greywater being permitted to be used for watering plants or recycled for flushing toilets. Most sewage also includes some surface water from roofs or hard-standing areas and may include stormwater runoff(Ayres and Mara, 1996).

Sewerage systems capable of handling stormwater are known as combined systems or combined sewers. Such systems are usually avoided since they complicate and thereby reduce the efficiency of sewage treatment plants owing to their seasonality. The variability in flow also leads to often larger than necessary, and subsequently more expensive, treatment facilities. In addition, heavy storms that contribute more flows than the treatment plant can handle may overwhelm the sewage treatment system, causing a spill or overflow. Modern sewered developments tend to be provided with separate storm drain systems for rainwater(Campbell and Wallis, 2002).

As rainfall travels over roofs and the ground, it may pick up various contaminants including soil particles and other sediment, heavy metals, organic compounds, animal waste, and oil and grease. Some jurisdictions require stormwater to receive some level of treatment

before being discharged directly into waterways(KumaranandShivaraman,1988). Examples of treatment processes used for stormwater include retention basins, wetlands, buried vaults with various kinds of media filters, and vortex separators (to remove coarse solids). Sanitary sewers are typically much smaller than storm sewers, and they are not designed to transport stormwater. In areas with basements, backups of raw sewage can occur if excessive stormwater is allowed into a sanitary sewer system(KumaranandShivaraman,1988).

2.2 Discharge criteria for wastewater

Pathogenic microorganisms such as bacteria, viruses, protozoa and helminths are almost always present in untreated municipal wastewater. However, the number and species of organisms present in wastewater vary from community to community depending on urbanisation, population density, sanitary habits, season of the year and rate of disease in the community (Davutluoglu, 2005).

Hence there is considerable risks of exposure to pathogens in irrigation with reclaimed wastewaters, but the health concerns is proportional to the degree of human contact with the reclaimed water and the degree and efficiency of the wastewater treatment processes (Davutluoglu, 2005). In order to minimise health risks, regulations were therefore set up to safeguard different cross sections of the population, i.e., starting with the agricultural workers down to the consumers.

Table 2.1 summarises some of the worldwide existing standards for reuse of wastewater in agriculture. Kretschmer *et al.* (2000) reported that in many countries like USA and Spain, only regional standards exist; while a very limited number of European countries have guidelines or regulations on wastewater reclamation as reuse of wastewaters is not necessary and their rivers pose sufficient dilution capacity Kretschmer *et al.* (2000).

Table 2.1: Comparison of selected water quality guidelines/standards

Parameter	California Ca/T22 (1978)	USEPA (1992)	WHO (1989)	Israel (1978)	Tunisia (1975)	Cyprus (1997)	Chile 1984/1978	France (1991)	Italy (1977)	Germany
								EU guidelines (for water use in agriculture not existent until now)		
Type	Law	Guidelines	Guidelines	Law	Law	Provisional standards	Guidelines	Guidelines	Law	Guidelines/ LAW
Minimum treatment required	Advanced	Advanced	Stabilisation ponds	Secondary	Stabilisation ponds	Tertiary		Stabilisation ponds	Secondary	Only Heavy metals are considered in the guidelines
BOD total (mg/l)		10		15	30	10				
SS (mg/l)		5		15	30	10				
Total coliform (/ml)		14	1000			50	1000	1000		
Helminths (egg/l)	-	-	1		< 1	0				
Main treatment process	Oxidation Clarification Filtration Disinfection	Filtration disinfection	Stabilisation ponds or equivalent	Long storage, disinfection	Stabilisation ponds or equivalent	Filtration; disinfection		Stabilisation ponds or equivalent		

Source: Kretschmer *et al.*(2000)

2.3 Process overview of wastewater treatment

Physical forces as well as chemical and biological processes drive the treatment of wastewater. Treatment methods that rely on physical forces are called unit operations. These include screening, sedimentation, filtration, or flotation. Treatment methods based on chemical and biological processes are called unit processes. Chemical unit processes include disinfection, adsorption, or precipitation. Biological unit processes involve microbial activity, which is responsible for organic matter degradation and removal of nutrients (Metcalf and Eddy, 1991). Sewage collection and treatment is typically subject to local, state and federal regulations and standards. Industrial sources of wastewater often require specialized treatment processes.

Conventional sewage treatment may involve four stages, called preliminary treatment, primary, secondary and tertiary treatment. The objective of preliminary treatment operation is to remove debris and coarse materials that may clog equipment in the plant (Bitton, 2005). Primary treatment consists of temporarily holding the sewage in a quiescent basin where heavy solids can settle to the bottom while oil, grease and lighter solids float to the surface. The settled and floating materials are removed and the remaining liquid may be discharged or subjected to secondary treatment (Stott *et al.,* 2003a). Secondary treatment removes dissolved and suspended biological matter. Secondary treatment is typically performed by indigenous, water-borne microorganisms in a managed habitat. Secondary treatment may require a separation process to remove the microorganisms from the treated water prior to discharge or tertiary treatment(Nelson *et al.,* 2004). Tertiary treatment is sometimes defined as anything more than primary and secondary treatment. Treated water is sometimes disinfected chemically or physically (for example by lagoons and microfiltration) prior to discharge into a stream, river, bay, lagoon or wetland, or it can be used for the irrigation of a golf course,

green way or park. If it is sufficiently clean, it can also be used for groundwater recharge or agricultural purposes (Schwartzbrod *et al.,* 2002; Nelson *et al.,* 2004).

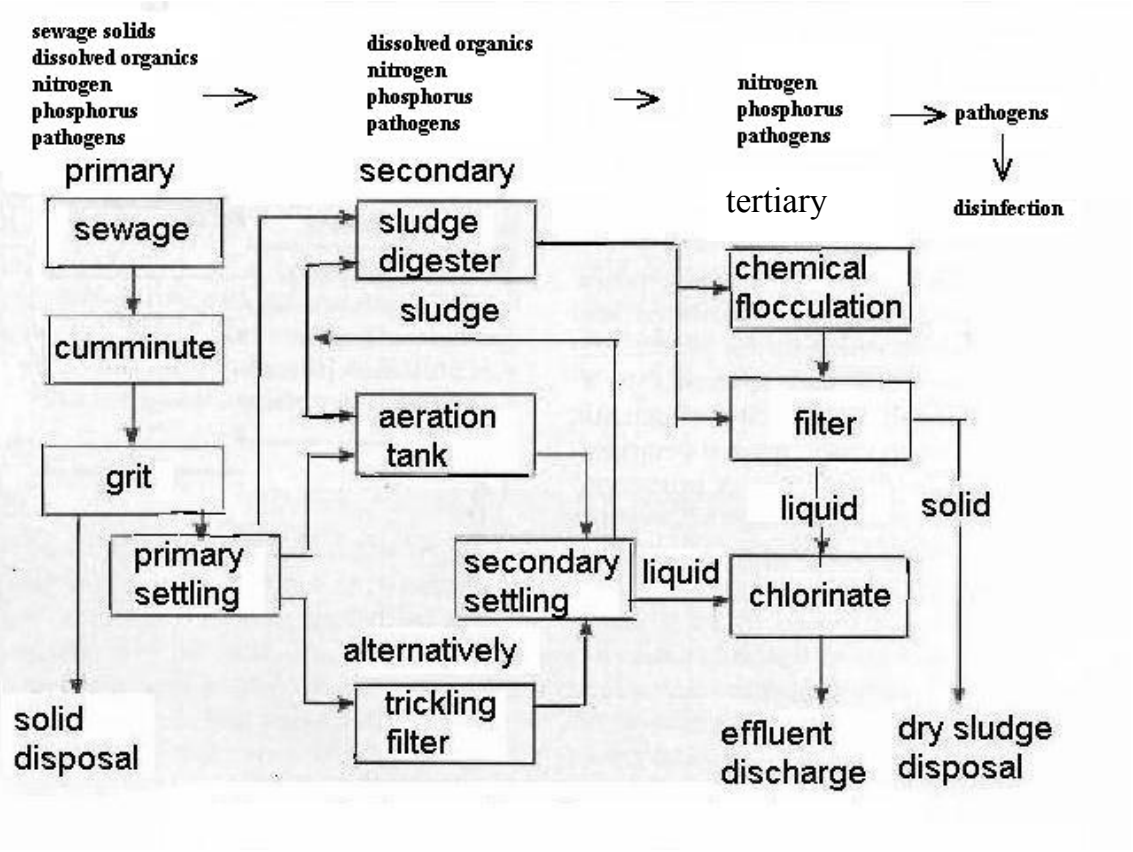


Figure 2.1:Process flow chart for a typical large-scale sewage treatment plant (Bitton, 2005)

2.3.1 Preliminary treatment

Preliminary treatment removes materials such as trash, tree limbs, leaves, etc that can be easily collected from the raw wastewater before they damage or clog the pumps and skimmers of primary treatment clarifiers (Stott *et al.,* 2003a).

2.3.1.1 Screening

The influent sewage water is screened to remove all large objects carried in the sewage stream (Khopkar, 2004). This is most commonly done with an automated

mechanically raked bar screen in modern plants serving large populations, whilst in smaller or less modern plants a manually cleaned screen may be used. The raking action of a mechanical bar screen is typically paced according to the accumulation on the bar screens and/or flow rate. The solids are collected and later disposed in a landfill or incinerated. Bar screens or mesh screens of varying sizes may be used to optimise solids removal. If gross solids are not removed they become entrained in pipes and moving parts of the treatment plant and can cause substantial damage and inefficiency in the process (Stott *et al.,* 2003a; Bitton, 2005).

2.3.1.2 Grit removal

Preliminary treatment may include a sand or grit channel or chamber where the velocity of the incoming wastewater is adjusted to allow the settlement of sand, grit, and stones. These particles are removed because they may damage pumps and other equipment. For small sanitary sewer systems, the grit chambers may not be necessary, but grit removal is desirable at larger plants (Davis and Cornwell, 1985).

2.3.1.3 Fat and grease removal

In some larger plants fat and grease is removed by passing the sewage through a small tank where skimmers collect the fat floating on the surface. Air blowers in the base of the tank may also be used to help recover the fat as froth. In most plants however, fat and grease removal takes place in the primary settlement tank using mechanical surface skimmers (Bitton, 2005).

2.4 Primary treatment

In the primary sedimentation stage, sewage flows through large tanks, commonly called "primary clarifiers" or "primary sedimentation tanks." The tanks are used to settle sludge while grease and oils rise to the surface and are skimmed off. Primary settling tanks are usually equipped with mechanically driven scrapers that continually drive the collected sludge towards a hopper in the base of the tank where it is pumped to sludge treatment facilities (Stott *et al.,* 2003a; Bitton, 2005). Grease and oil from the floating material can sometimes be recovered for saponification.

Stott *et al.* (2003a) suggested that the dimensions of the tank should be designed to effect removal of a high percentage of the floatables and sludge. A typical sedimentation tank may remove from 60 to 65% of suspended solids, and from 30 to 35% of biochemical oxygen demand (BOD) from the sewage.

2.5 Secondary treatment

Secondary treatment is designed to substantially degrade the biological content of the sewage which is derived from human waste, food waste, soaps and detergent (Grimason *et al.,* 1996; Bouhoum *et al.,* 2000). The majority of municipal plants treat the settled sewage liquor using aerobic biological processes. To be effective, the biota requires both oxygen and food to live. The bacteria and protozoa consume biodegradable soluble organic contaminants (e.g. sugars, fats, organic short-chain carbon molecules, etc) and bind much of the less soluble fractions into floc (Grimason *et al.,* 1996; Bouhoum *et al.,* 2000). Secondary treatment systems are classified as fixed-film or suspended-growth systems.

- **Fixed-film** or **attached growth** systems include trickling filter and rotating biological contactors, where the biomass grows on media and the sewage passes over its surface.

- **Suspended-growth** systems include activated sludge, where the biomass is mixed with the sewage and can be operated in a smaller space than fixed-film systems that treat the same amount of water. However, fixed-film systems are more able to cope with drastic changes in the amount of biological material and can provide higher removal rates for organic material and suspended solids than suspended growth systems(Grimason *et al.,* 1996; Bouhoum *et al.,* 2000).

2.5.1 Activated sludge

In general, activated sludge plants encompass a variety of mechanisms and processes that use dissolved oxygen to promote the growth of biological floc that substantially removes organic material (Ellis and Korth, 1993).

The process traps particulate material and can, under ideal conditions, convert ammonia to nitrite and nitrate and ultimately to nitrogen gas.

2.5.1.1 Pathogen and parasite removal by activated sludge

Bitton (2005) reported that two components (aeration and sedimentation tanks) of the activated sludge process affect, to some extent, the removal/inactivation of pathogens and parasites. He stated that during the aeration phase, environmental (e.g., temperature, sunlight) and biological (e.g., inactivation by antagonistic microorganisms) factors, possibly aeration, have an impact on pathogen/parasite survival. Araki *et al.* (2001) observed that floc formation during the aeration phase is also instrumental in removing undesirable microorganisms and thatduring the sedimentation phase, certain organisms (e.g., parasites) undergo sedimentation, while floc-entrapped microbial pathogens settle readily in the tank. As compared with other biological treatment processes, activated sludge is relatively efficient in removing pathogenic microorganisms and parasites from incoming primary effluents.

2.5.1.2 Bacteria

Activated sludge is generally more efficient than trickling filters for the removal of indicator (e.g., coliforms) and pathogenic (e.g., Salmonella) bacteria. The removal efficiency may vary from 80% to more than 99%. Bacteria are removed via inactivation, grazing by ciliated protozoa (grazing is particularly effective for free-swimming bacteria), and adsorption to sludge solids or encapsulation within sludge flocs, or both, followed by sedimentation (Feachem*et al*., 1983; Omura*et al*., 1989; Yaziz and Lloyd, 1979).

2.5.1.4 ***Viruses***

The activated sludge process is the most efficient biological process for virus removal from sewage. It appears that most of the virus particles (90%) are solids-associated and are ultimately transferred to sludge (Bitton, 1980a; Rao*et al*., 1986; Omura*et al*., 1989). The ability of activated sludge to remove viruses is related to the capacity to remove solids. Thus, many of the viruses found in the effluents are solids-associated (Gerba*et al*., 1978). Viruses are also inactivated by environmental and biological factors. Attempts have been made to estimate the contribution of both association to solids and inactivation to virus removal in activated sludge. After 10 h of aeration, 25% are removed by adsorption to sludge flocs, and 75% are removed by inactivation (Glass and O'Brien, 1980). Therefore, inactivation alone is not sufficient for removing most of the viruses with a retention time varying from 6 to 12 h.

Coliphages have been used as model viruses for studying virus removal by the activated sludge process (Bitton, 2005). These studies generally show that the activated sludge process removes 90–99% of phage (Safferman and Morris, 1976). Studies on indigenous coliphage in wastewater treatment plants showed that while 12–30% of coliphage are associated with suspended solids in raw wastewater, more than 97% are solids-associated in the aeration tank (Ketratanakul and Ohgaki, 1989).

2.5.1.4 Parasitic protozoa

Protozoan cysts such as those of *Entamoeba histolytica* are not inactivated under the conditions prevailing in the aeration tank of an activated sludge process. They are, however, entrapped in sludge flocs and are thus transferred to sludge after sedimentation. Similar removals were observed for both *Entamoeba histolytica* and *Giardia* cysts (Panicker and Krishnamoorthi, 1978). More than 98% of *Giardia* cysts are removed and become concentrated in sludge. Cacció*et al*. (2003) reported that a survey of four activated sludge plants in Italy showed that *Giardia* removal following activated sludge treatment and disinfection ranged from 87.0 to 98.4%; while Villacorta-Martinez deMaturana *et al.* (1992) saidunder laboratory conditions, the activated sludge process removes 80–84% of *Cryptosporidium parvum* oocysts. In-plant reduction of *Cryptosporidium* by the activated sludge process varied from 84.6% in a California plant to 96.8% in a Canadian plant (Chaure*et al*., 1995; Mayer and Palmer, 1996). A survey of six wastewater treatment plants in the United States showed that the removal of total or infectious *Cryptosporidium* oocysts varied between 94 and 99% following biological treatment. However, 40% of the samples were still positive for infectious oocysts following biological treatment, post-filtration, and final disinfection (Gennaccaro*et al*., 2003). A study in Scotland showed that biological treatment (activated sludge or trickling filter) was superior to primary treatment as regards the removal of *Giardia* cysts and *Cryptosporidium* oocysts. The removal efficiency was higher for *Giardia* than for *Cryptosporidium*. However, the removal of protozoan parasites by biological treatment is very variable. The removal efficiency is estimated at 0–90%t for *Cryptosporidium*oocysts and 60–90% for *Giardia* cysts in a wastewater treatment plant that incorporates both primary and secondary stages. Oocysts, introduced into survival chambers that were immersed in wastewater, survived well in this environment. Thus, Robertson *et al*.

(2000) concluded that the removal observed in activated sludge was probably due to sedimentation along with entrapment inside the flocs.

2.5.1.5 Helminth eggs

Due to their size and density, eggs of helminth parasites (e.g., *Taenia* species, *Ancylostoma* species, *Necator* species) are removed by sedimentation during primary treatment of wastewater and during the activated sludge treatment, thus they are largely concentrated in sludges (Dean and Lund, 1981).

Fitzgerald (1982) reported that parasite eggs were not detected in unchlorinated sewage effluents. Some investigators, however, have reported that activated sludge does not completely eliminate parasite eggs of *Ascaris* and T*oxocara* from wastewater (Schwartzbrod*et al*., 1989).

2.5.2 Surface-aerated basins (Lagoons)

Most biological oxidation processes for treating industrial wastewaters have in common the use of oxygen (or air) and microbial action (Bitton, 2005). Surface-aerated basins achieve 80 to 90% removal of biochemical oxygen demand with retention times of 1 to 10 days. The basins may range in depth from 1.5 to 5.0 metres and use motor-driven aerators floating on the surface of the wastewater (Nelson *et al.,* 2004).

Schwartzbrod *et al.* (2002) observed that in an aerated basin system, the aerators provide two functions: they transfer air into the basins required by the biological oxidation reactions, and they provide the mixing required for dispersing the air and for contacting the reactants (that is, oxygen, wastewater and microbes). Typically, the floating surface aerators are rated to deliver the amount of air equivalent to 1.8 to 2.7 kg O_2/kW·h. However, they do not provide as good mixing as is normally achieved in activated sludge systems and therefore

aerated basins do not achieve the same performance level as activated sludge units (Schwartzbrod *et al.,* 2002).

Biological oxidation processes are sensitive to temperature and, between 0°C and 40°C, the rate of biological reactions increase with temperature. Most surface aerated vessels operate at between 4°C and 32°C (Schwartzbrod *et al.*, 2002).

2.5.2.1 Removal of protozoa cysts and helminth eggs by surface-aerated basin (lagoons)

The factors influencing cyst and oocyst removal in stabilization ponds are retention time, temperature, pH, and solar radiation. A review of the literature showed close to 100% removal of *E. histolytica, Giardia lamblia* and *Cryptosporidium parvum* reported in several countries (Schwartzbrod*et al*., 2002). The reported removal of *Giardia lamblia* cysts by waste stabilization ponds in Kenya and France was 99.1% (25 days retention time) and 99.7% (40 days retention time), respectively. However, the cysts were always found in the ponds' final effluents (Grimason*et al*., 1996). The percentage removal of *Giardia lamblia* and *Entamoeba histolytica* in an aerated lagoon varies from 67 to 100% (Panicker and Krishnamoorthi, 1981). More than 97% of *C. parvum* oocysts were inactivated when placed inside cellulose semi-permeable bags suspended in a high-rate algal pond. It was suggested that the high inactivation was due to the high pH generated by the high algal biomass, solar radiation, and ammonia (Araki *et al*., 2001). Sedimentation is the main factor responsible for the removal of helminth parasite eggs (Bouhoum*et al*., 2000). Physicochemical factors (e.g., ionic conditions) may also adversely affect the viability of helminth eggs, as shown by a study on eggs in a high-rate algal pond in semi-permeable cellulose bags (Araki *et al*., 2000). The percent removal of parasites (*Ascaris lumbricoides*, hookworm) in an aerated lagoon varied between 50 and 100% (Panicker and Krishnamoorthi, 1981). Under the arid climate of Marrakech, Morocco, 100% removal of helminth eggs (nematodes such as *Ascaris, Trichuris*;

cestodes such as *Taenia, Hymenolepis*) was achieved after raw wastewater treatment by two lagoons in series with a total detention time of 10 –50 days. Most of the eggs were transferred to the lagoon sediments (Schwartzbrod*et al*., 1989; Mandi*et al*., 1994). Parasite egg removal in a series of five ponds (anaerobic pond → facultative pond → three maturation ponds in series) in Brazil was 100% when the combined hydraulic retention time was about 25 days. Moreover, most of the eggs (99.9%) were removed after the secondary facultative pond (Stott *et al*., 2003a; 2003b). However, eggs of *Necator americanus* were not well removed in a stabilization pond system with a total retention period of 23 days (Ellis and Korth, 1993). Helminth ova can survive for years in the sludge accumulated at the bottom of the pond and may pose a potential hazard when the pond is dredged (Nelson *et al*., 2004).

2.5.3 Trickling filter beds (Oxidizing Beds)

In older plants and those receiving variable loadings, trickling filter beds are used where the settled sewage liquor is spread onto the surface of a bed made up of coke (carbonized coal), limestone chips or specially fabricated plastic media (Lewis *et al*., 2002). Such media must have large surface areas to support the biofilms that form. The liquor is typically distributed through perforated spray arms. The distributed liquor trickles through the bed and is collected in drains at the base. These drains also provide a source of air which percolates up through the bed, keeping it aerobic (Lewis *et al*., 2002). Biological films of bacteria, protozoa and fungi form on the media's surfaces and eat or otherwise reduce the organic content (Lewis *et al*., 2002). Thisbiofilm is often grazed by insect larvae, snails, and worms which help maintain an optimal thickness. Overloading of beds increases the thickness of the film leading to clogging of the filter media and ponding on the surface (Robertson *et al*., 2000).

2.5.3.1 Removal of pathogens and parasites by trickling filters

The removal of pathogens and parasites by trickling filters is generally low and erratic. Bacterial removal is inconsistent and varies from 20 to > 90%, depending on the operation of the trickling filter. Removal of *Salmonella* by trickling filters is lower than the activated sludge process and may vary from 75 to 95% (Feachem*et al*., 1983). The removal of viruses by trickling filters is also generally low and erratic. Filtration rate affects the removal of viruses, and probably other pathogenic microorganisms. Laboratory experiments showed that at medium filtration rate (10 MGD/acre), the removal of viruses, coliforms, faecal streptococci, and BOD and COD, was greater than at a higher rate (23 MGD/acre) (Clarke and Chang, 1975). At the Kerrville, Texas, wastewater treatment plant, the mean removal of total and faecal coliforms by a trickling filter was 92 and 95%, respectively. However, the removal of enteroviruses was erratic and varied from 59 to 95% (Moore *et al*., 1981). Similarly, removal of bacterial phage is inconsistent and varies between 40 and 90%, depending on the season of the year (Kott*et al*., 1974). In New Zealand, two trickling filter systems removed between 0 and 20% of viruses despite a significant reduction of faecal coliforms (> 90% removal) (Lewis *et al*., 1986; 2002). Similar observations were made in Japan; the removal efficiency of trickling filters is lower for viruses than for indicator bacteria. The mechanism of removal of viruses by trickling filters is poorly understood. Some investigators have suggested that viruses are removed by adsorption to the biofilm material (Omura*et al*., 1989).

Schwartzbrod *et al*. (2002) stated that cysts and oocysts removal by trickling filters is generally low and erratic, with removals varying from 10 to 99%. Trickling filter plants in India removed from 74 to 91% of *Entamoeba histolytica*, and the removal of *Giardia* cysts is similar to that of *Entamoeba* (Panicker and Krishnamoorthi, 1978). The removal efficiency of trickling filters is generally lower than that of activated sludge as cysts are frequently

detected in effluents (Cassonet *al*., 1990). Therefore, Robertson *et al*. (2000) concluded that no significant difference was found between activated sludge and trickling filters as regards the removal of *Giardia* cysts and *Cryptosporidium* oocysts.

2.5.4 Rotating biological contactors

Rotating biological contactors are mechanical secondary treatment systems, which are robust and capable of withstanding surges in organic load(Sagy and Kott, 1990). Rotating biological contactors were first installed in Germany in 1960 and have since been developed and refined into a reliable operating unit. The rotating disks support the growth of bacteria and microorganisms present in the sewage, which break down and stabilise organic pollutants(Sagy and Kott, 1990). To be successful, microorganisms need both oxygen to live and food to grow. Oxygen is obtained from the atmosphere as the disks rotate. As the microorganisms grow, they build up on the media until they are sloughed off due to shear forces provided by the rotating discs in the sewage. Effluent from the rotating biological contractor is then passed through final clarifiers where the microorganisms in suspension settle as sludge. The sludge is withdrawn from the clarifier for further treatment(Tawfik *et al*., 2002; 2004).

A functionally similar biological filtering system has become popular as part of home aquarium filtration and purification. The aquarium water is drawn up out of the tank and then cascaded over a freely spinning corrugated fibre-mesh wheel before passing through a media filter and back into the aquarium(Tawfik *et al*., 2002). The spinning mesh wheel develops a biofilm coating of microorganisms that feed on the suspended wastes in the aquarium water and are also exposed to the atmosphere as the wheel rotates. This is especially good at removing waste urea and ammonia urinated into the aquarium water by the fish and other animals(Tawfik *et al*., 2002; 2004).

2.5.4.1 Pathogen removal in rotating biological contactors

Little is known concerning the removal of indicator and pathogenic microorganisms in rotating biological contactors. They appear to be fairly efficient in removing indicator bacteria. A total of 1 log or more of faecal coliforms is removed by this process (Sagy and Kott, 1990). It was found that adsorption to the biofilm and the grazing activity of protozoa and nematodes (in the second and third stage of the RBC system) were the most important factors controlling *E. coli* removal (Tawfik*et al*., 2002; 2004). Between 79 and 99% of *Campylobacters* were removed by this treatment process (Jones, 2001).

2.6 Tertiary treatment

2.6.1 Filtration

Filtration is defined as the passage of fluids through porous media to remove turbidity (suspended solids, such as clays, silt particles, microbial cells) and flocculated particles. This process depends on the filter medium, concentration and type of solids to be filtered out, and the operation of the filter (Bitton, 2005).

Filtration is one of the oldest processes used for water treatment. In 1685, an Italian physician, Luc Antonio Porzio, conceived a filtration system for protecting soldiers' health in military installations. This process has been in use since and has contributed greatly to the reduction of waterborne diseases such as typhoid fever and cholera (Slezak and Sims, 1984). An examination of waterborne outbreaks around the world clearly shows that filtration has been historically instrumental as a barrier against pathogenic microorganisms and has largely contributed to the reduction of waterborne diseases. The purpose of tertiary treatment is to provide a final treatment stage to raise the effluent quality before it is discharged to the receiving environment (sea, river, lake, ground, etc.). More than one tertiary treatment

process may be used at any treatment plant. If disinfection is practiced, it is always the final process. It is also called "effluent polishing."

2.6.1.1 Slow sand filtration

Although more popular in Europe than in the United States, slow sand filters serve mostly communities of less than 10,000 people because capital and operating costs are lower than for rapid sand filters (Slezak and Sims, 1984). In the United States, the first slow sand filter was installed in Lawrence, Massachusetts, to remove *Salmonella typhi* from water.

Slow sand filters contain a layer of sand (60–120 cm depth) supported by a graded gravel layer (30–50 cm depth). The sand grain size varies between 0.15 and 0.35 mm, and the hydraulic loading range is between 0.04 and 0.4 m/h (Bellamy *et al*., 1985b).

Biological growth inside the filter comprises a wide variety of organisms, including bacteria, algae, protozoa, rotifers, microtubellaria (flatworms), nematodes (round worms), annelids (segmented worms), and arthropods (Duncan, 1988). The build-up of a biologically active layer, the schmutzdecke, occurs during the normal operation of a slow sand filter. The top layer is composed of biological growth and filtered particulate matter. This leads to a head loss across the filter, a problem corrected by removing or scraping the top layer of sand. The length of time between scrapings depends on the turbidity of the raw water and varies between 1 to 2 weeks and several months (Logsdon and Hoff, 1986). Scraping is followed by replenishing of the filter bed with clean sand, an operation called resanding. There is sometimes a deterioration of water quality for some days after scraping, but it later improves during the ripening period (Cullen and Letterman, 1985). However, some investigators (Logsdon and Lippy, 1982) have not observed deterioration after scraping. Bacterial activity in the schmutzdecke helps remove assimilable organic compounds (AOC), some of which are precursors of chlorinated organics such as trihalomethanes (Fox *et al*., 1984; Collins *et al*., 1992; Eighmy*et al*., 1992).

The removal efficiency for coliform bacteria is influenced by temperature, sand size, and filter depth (Bellamy *et al*., 1985a, 1985b). Removal of *Giardia* and total coliforms exceed 99%, even at the highest loading rate of 0.4 m/h. Pilot plant studies showed that slow sand filtration achieves 4 to 5–log reductions of coliforms (Fox *et al*., 1984). A survey of slow sand filters in the United States showed that most of the plants reported coliform levels of 1 per 100 ml or less (Slezak and Sims, 1984). In water treatment plants in Paris, France, slow sand filters were found to be more efficient in mycobacteria removal than rapid sand filters (Le Dantec*et al*., 2002). No *Cryptosporidium* species oocysts were detected in treated water from two water treatment plants using slow sand filters, despite the frequent isolation of this parasite in the raw waters entering the plants (Chaure*et al*., 1995). The microbial community developing on the sand filter greatly influences the removal of bacteria, protozoan cysts, and turbidity by slow sand filters (Huisman and Wood, 1974; Cleasby*et al*., 1984; Bellamy *et al*., 1985b).

With regard to viruses, a removal exceeding 99.999% was observed in a slow sand filter operated at 118°C at a rate of 0.2 m/h (Poynter and Slade, 1977). An established filter appears to perform better than a new filter with regard to virus removal (Wheeler *et al*., 1988). The factors controlling virus removal by slow sand filters include filter depth, flow rate, temperature, and the presence of a well-developed biofilm on the filter.

2.6.1.2 Rapid sand filtration

A rapid sand filter consists of a layer of sand supported by a layer of anthracite, gravel, or calcite (Bitton, 1980a). The rapid sand effluent is collected by an under-drain system. Rapid sand filters are operated at filtration rates of 5– 24 $m^3/h/m^2$ as compared with 0.1–1 $m^3/h/m^2$ for slow sand filters (Huisman and Wood, 1974; Logsdon and Hoff, 1986). These filters are periodically cleaned by backwashing (i.e., reversing the flow) at a sufficient flow rate to allow a thorough cleaning of the sand.

This process appears to be less effective for the removal of bacteria, viruses, and protozoan cysts unless it is preceded by coagulation–flocculation. The removal of *Salmonella* and *Shigella* by filtration and coagulation is similar to that of coliforms. *Giardia* cysts pass through water treatment plants that use direct filtration without any chemical pretreatment. The breakthrough is more pronounced during winter months when temperature is below 58°C (Hibler and Hancock, 1990). Significant changes in flow rates may result in deterioration of water quality due to the release of retained particles, including protozoan cysts.

The combination of filtration with coagulation–flocculation is particularly important in respect to *Giardia* cysts and *Cryptosporidium* oocysts removal from water (Logsdon *et al*., 1981). For low-turbidity water, proper chemical coagulation of the water before rapid sand filtration is necessary to achieve a good removal of turbidity and protozoan cysts and oocysts. In a plant in Japan, coagulation–flocculation–sedimentation followed by rapid sand filtration removed approximately 2.5–$\log_{10}$ of *Cryptosporidium* and *Giardia* (Hashimoto *et al*., 2002). It was suggested that the removal of turbidity could serve as a surrogate indicator of *Giardia* cysts removal in water with low turbidity (Al-Ani*et al*., 1986). However, *Giardia* and *Cryptosporidium* monitoring in filtered drinking water samples indicated that turbidity removal was a good predictor of *Cryptosporidium* oocysts removal but not *Giardia* cysts (LeChevallier*et al*., 1991b). Similarly, oocyst-sized polystyrene microspheres appear to be reliable surrogates for *C. parvum*oocyst removal by filtration (Emelko and Huck, 2004). Filtration, without the prior addition of coagulants, removes approximately 90% of *Cryptosporidium* oocysts. Surface coating of sand particles with a hydrous iron aluminium oxide was found to improve the removal of *Cryptosporidium* (Shaw *et al*., 2000). However, the relatively long-term effect of the coating on oocyst removal is not known at the present time. If the source water is surface water, then a dual barrier is necessary to prevent

contamination with *Giardia lamblia* cysts. This dual barrier consists of coagulation–sand filtration to retain cysts and turbidity, and disinfection (AWWA, 1987).

Entamoeba histolytica cysts are also effectively removed when rapid sand filtration ispreceded by coagulation (Bitton, 2005);because sand particles are essentially poor adsorbents toward viruses, pathogen removal by sand filtration is variable and often low. However, coagulation prior to sand filtration removes more than 99% of viruses (Bitton, 1980a). Coagulation, settling, and filtration remove more than one–log of hepatitis virus, simian rotavirus (SA-11), and poliovirus (Rao*et al*., 1988). A pilot plant, using the Seine River water, which contained 190– 1420 PFU/1000 L, removed 1 to 2–logs of viruses after coagulation–flocculation followed by sand filtration (Joret*et al*., 1986). A preozonation step (0.8 ppm ozone level) greatly improved virus removal, which was increased to 2 to 3–logs. Helminths form relatively large eggs, which are effectively removed by sedimentation, coagulation, and filtration.

Thus, optimum coagulation followed by filtration in water treatment plants is essentialfor parasites and pathogens control. Other parameters that adversely affect filter operation are sudden changes in water flow rates, interruptions in chemical feed, inadequate filter backwashing, and the use of clean sand, which was not allowed to undergo a ripening period (Logsdon and Hoff, 1986; Ongerth, 1990).

Because of their limited capacity to retain solids, rapid sand filters must be backwashed to remove the trapped solids from the filter matrix. The water treatment plant finished water is generally used as backwash water. However, the spent filter backwash water must be adequately treated prior to reuse within the plant or disposal into a receiving stream (Arora and LeChevallier, 2002). The recycling of spent filter backwash water, mostly by treatment plants that use surface water as the source water, is regulated by the U.S. EPA, due to the increased levels of biological (protozoan cysts, bacterial, and viral pathogens) and

chemical (disinfection by-products, metals) contaminants in spent filter backwash water. The spent filter backwash water must be treated to reduce the risk of breakthrough of pathogens and parasites into finished water. The treatments include sedimentation (preferably in the presence of coagulants), dissolved air flotation, filtration through granular media or membrane filters, and disinfection, preferably after clarification.

Dual-stage filtration is an alternative to rapid sand filtration for small water treatment plants. This process consists of chemical coagulation followed by a filter assembly consisting of two tanks, a depth clarifier and a depth filter. At a flow rate of 10 gpm/ft^2, this treatment removed more than 99% of *Giardia* cysts from water with an effluent turbidity less than 1 NTU (Horn *et al*., 1988).

2.6.2 Lagooning

Lagooning provides settlement and further biological improvement through storage in large man-made ponds or lagoons. These lagoons are highly aerobic and colonization by native macrophytes, especially reeds, is often encouraged. Small filter feeding invertebrates such as Daphnia and species of *Rotifera* greatly assist in treatment by removing fine particulates.

2.6.3 Constructed wetlands

Constructed wetlands include engineered reedbeds and a range of similar methodologies, all of which provide a high degree of aerobic biological improvement and can often be used instead of secondary treatment for small communities (Nemade *et al.,* 2009).

2.6.4 Nutrient removal

Wastewater may contain high levels of the nutrients nitrogen and phosphorus. Excessive release to the environment can lead to a build-up of nutrients, called eutrophication, which can in turn encourage the overgrowth of weeds, algae, and cyanobacteria (blue-green algae). This may cause an algal bloom, a rapid growth in the population of algae. The algae numbers are unsustainable and eventually most of them die. The decomposition of the algae by bacteria uses up so much of oxygen in the water that most or all of the animals die, which creates more organic matter for the bacteria to decompose. In addition to causing deoxygenation, some algal species produce toxins that contaminate drinking water supplies. Different treatment processes are required to remove nitrogen and phosphorus (Nemade *et al.,* 2009).

2.6.4.1 Nitrogen removal

The removal of nitrogen is effected through the biological oxidation of nitrogen from ammonia (nitrification) to nitrate, followed by denitrification, the reduction of nitrate to nitrogen gas. Nitrogen gas is released to the atmosphere and thus removed from the water (Mandi *et al.,* 1994).

Nitrification itself is a two-step aerobic process, each step facilitated by a different type of bacteria. The oxidation of ammonia (NH_3) to nitrite (NO_2^-) is most often facilitated by *Nitrosomonas* spp. (nitroso referring to the formation of a nitroso functional group). Nitrite oxidation to nitrate (NO_3^-), though traditionally believed to be facilitated by *Nitrobacter* spp. (nitro referring the formation of a nitro functional group), is now known to be facilitated in the environment almost exclusively by *Nitrospira* spp(Mandi *et al.,* 1994; Bouhoum *et al.,* 2000).

Denitrification requires anoxic conditions to encourage the appropriate biological communities to form. It is facilitated by a wide diversity of bacteria. Sand filters, lagooning and reed beds can all be used to reduce nitrogen, but the activated sludge process (if designed well) can do the job the most easily (Bouhoum *et al.,* 2000). Since denitrification is the reduction of nitrate to dinitrogen gas, an electron donor is needed. This can be, depending on the wastewater, organic matter (from faeces), sulphide, or an added donor like methanol. Sometimes the conversion of toxic ammonia to nitrate alone is referred to as tertiary treatment.

2.6.4.2 Phosphorus removal

Phosphorus removal is important as it is a limiting nutrient for algae growth in many fresh water systems. It is also particularly important for water reuse systems where high phosphorus concentrations may lead to fouling of downstream equipment such as reverse osmosis(Bitton, 2005).

Phosphorus can be removed biologically in a process called enhanced biological phosphorus removal. In this process, specific bacteria, called polyphosphate accumulating organisms (PAOs), are selectively enriched and accumulate large quantities of phosphorus within their cells (up to 20% of their mass). When the biomass enriched in these bacteria is separated from the treated water, these biosolids have a high fertilizer value(Mandi *et al.,* 1994; Bitton, 2005).

Phosphorus removal can also be achieved by chemical precipitation, usually with salts of iron (e.g. ferric chloride), aluminium (e.g. alum), or lime. This may lead to excessive sludge productions as hydroxides precipitates and the added chemicals can be expensive (Davutluoglu, 2005). Chemical phosphorus removal requires significantly smaller equipment

footprint than biological removal, is easier to operate and is often more reliable than biological phosphorus removal. Another method for phosphorus removal is to use granular laterite.Once removed, phosphorus, in the form of a phosphate rich sludge, may be stored in a land fill or resold for use in fertilizer (Davutluoglu, 2005).

2.7 Disinfection

The purpose of disinfection in the treatment of wastewater is to substantially reduce the number of microorganisms in the effluent to be discharged into the environment. The effectiveness of disinfection depends on the quality of the wastewater being treated (e.g., cloudiness, pH, etc.), the type of disinfection being used, the disinfectant dosage (concentration and time), and other environmental variables. Cloudy wastewater will be treated less successfully since solid matter can shield organisms, especially from ultraviolet light or if contact times are low. Generally, short contact times, low doses and high flows all militate against effective disinfection. Common methods of disinfection include ozone, chlorine, ultraviolet light, or sodium hypochlorite (Bitton, 2005). Chloramine, which is used for drinking water, is not used in wastewater treatment because of its persistence.

2.7.1 Chlorination

Chlorination remains the most common form of wastewater disinfection in the world due to its low cost and long-term history of effectiveness. Being and oxidiser, chlorine typically destroys the outer membrane of the bacterial cell and renders it non-infectious (Davutluoglu, 2005). One disadvantage is that chlorination of residual organic material can generate chlorinated-organic compounds that may be carcinogenic or harmful to the environment. Residual chlorine or chloramines may also be capable of chlorinating organic material in the natural aquatic environment. Further, because residual chlorine is toxic to

aquatic species, the treated effluent must also be chemically dechlorinated, adding to the complexity and cost of treatment.

2.7.1.1 Inactivation of microorganisms by chlorine

Three chlorine compounds (HOCl, OCl^-, and NH_2Cl) are used for disinfection, out of whichhypochlorous acid is the most effective for the inactivation of microorganisms in water and wastewater. The presence of interfering substances in wastewater reduces the disinfection efficacy of chlorine, and relatively high concentrations of chlorine (20 – 40 ppm) are required for adequate reduction of viruses. In wastewater effluents, no free chlorine species are available after a few seconds of contact (Bitton, 2005).

Chlorine, specifically HOCl, is generally quite efficient in inactivating pathogenic and indicator bacteria. Water treatment with ≤ 1 mg/L for about 30 min is generally efficient in significantly reducing bacterial numbers. For example, *Campylobacter jejuni* displays more than 99% inactivation in the presence of 0.1 mg/L free chlorine (contact time = 5 min) (Blaser*et al*., 1986). Although there is wide variation in the resistance of enteric viruses to chlorine, these pathogens are generally more resistant to this disinfectant than are vegetative bacteria. That explains why viruses are frequently detected in chlorinated secondarily treated effluents. Chloramines are much less efficient than free residual chlorine (about 50 times less efficient) as regards viral inactivation. Protozoan cysts (e.g., *Giardia lamblia*, *Entamoeba histolytica, Naegleriagruberi*) are more resistant to chlorine than are both bacteria and viruses. In the presence of HOCl at pH 6, the *Ct* for *E. coli* is 0.04, compared with a *Ct* value of 1.05 for poliovirus type 1 and 80 for *G. lamblia* (Logsdon and Hoff, 1986).

Cryptosporidium species oocysts are extremely resistant to disinfection but can be inactivated by ammonia at concentrations found in natural environments (Jenkins *et al*., 1998). A chlorine or monochloramine concentration of 80 mg/L is necessary to cause 90% inactivation after 90 min contact time (Korich*et al*., 1990). This parasite is not completely

inactivated in a 3% solution of sodium hypochlorite (Campbell *et al*., 1982), and the oocysts can remain viable for 3 – 4 months in 2.5% potassium dichromate solution (Current, 1988). The *Ct* value for *Cryptosporidium* species is in the 1000s (Driedger*et al*., 2000). This parasite is thus extremely resistant to disinfection as carried out in water and wastewater treatment plants (Korich*et al*., 1989).

2.7.2 Ultraviolet radiation

Ultraviolet (UV) light can be used instead of chlorine, iodine, or other chemicals. Because no chemicals are used, the treated water has no adverse effect on organisms that later consume it, as may be the case with other methods (Das, 2001). UV radiation causes damage to the genetic structure of bacteria, viruses, and other pathogens, making them incapable of reproduction (Das, 2001). The key disadvantages of UV disinfection are the need for frequent lamp maintenance and replacement and the need for a highly treated effluent to ensure that the target microorganisms are not shielded from the UV radiation (i.e., any solids present in the treated effluent may protect microorganisms from the UV light). In the United Kingdom, UV light is becoming the most common means of disinfection because of the concerns about the impacts of chlorine in chlorinating residual organics in the wastewater and in chlorinating organics in the receiving water (Chang *et al*., 1985; Mofidi *et al.,* 2001).

2.7.2.1 Inactivation of pathogens and parasites by ultraviolet (UV)radiation

In general, the resistance of microorganisms to UV follows the same pattern as with chemical disinfectants and is as follows (Chang *et al*., 1985).

Protozoan cysts > Bacterial spores > Viruses > Vegetative bacteria

As regards protozoan parasites, a 3.9–$\log_{10}$inactivation was obtained for *Cryptosporidium* at a dose of 19 mJ/cm^2, using a medium pressure UV lamp and animal infectivity assays (Bukhari*et al*., 1999). A UV dose of 11 mJ/cm^{2}led to a 2–log inactivation of *C. parvum* (genotype 2 Iowa isolate) using pulsed UV light or medium-pressure UV lamps

and cell culture infectivity assays (Mofidi*et al*., 2001). *Cryptosporidium parvum* oocysts were found to be sensitive to both low-pressure and medium-pressure UV radiation, displaying a 3–log_{10}inactivation at a UV dose of 3 mJ/cm^2(Clancy *et al*., 2000; Shin *et al*., 2001). Similar results were obtained for *Giardia lamblia* cysts (Mofidi*et al*., 2002). In another study with *Giardia lamblia*, it was found that 10 and 20 mJ/cm^2were required to obtain 2–$log_{10}$and 3–log_{10}inactivation, respectively (Campbell and Wallis, 2002). A comparison of the sensitivity of five strains of *C. parvum*oocysts (Iowa, Moredun, Texas A and M strain, Maine, and Glasgow) showed that a UV dose of 10 mJ/$cm^2$was sufficient to cause at least 4–log_{10}inactivation in all strains (Clancy *et al*., 2004).

2.7.3 Ozonation

Ozone (O_3) is generated by passing oxygen (O_2) through a high voltage potential resulting in a third oxygen atom becoming attached and forming O_3. Ozone is very unstable and reactive and oxidizes most organic materials it comes in contact with, thereby destroying many pathogenic microorganisms. Ozone is considered to be safer than chlorine because, unlike chlorine which has to be stored on site (highly poisonous in the event of an accidental release), ozone is generated onsite as needed. Ozonation also produces fewer disinfection by-products than chlorination. A disadvantage of ozone disinfection is the high cost of the ozone generation equipment and the requirements for special operators(CloeteandBrozel,2002).

2.7.3.1 Effect of ozone on indicator and pathogenic microorganisms

Ozone is a much more powerful oxidant than chlorine. The standard oxidation potential of ozone is 2.07 eV as compared with a potential of 1.36 eV for chlorine or 0.95 eV for chlorine dioxide (Acher*et al*., 1997). The threshold ozone concentration above which bacterial inactivation is very rapid is only 0.1 mg/L. The *Ct* values for 99% inactivation are

very low and range between 0.001 and 0.2 for *E. coli* and from 0.04 to 0.42 for enteric viruses (Engelbrecht, 1983; Hall and Sobsey, 1993).

Ozone, at a concentration of 1.1 mg/L, totally inactivates *Cryptosporidium parvum* oocysts in 6 min at levels of 10^4oocysts/ml (Peeters*et al*., 1989). Cysts of *Giardia lamblia* and *G. muris* are also effectively inactivated by ozone (Wickramanayake*et al*., 1985). At pH 7, at 58°C, more than 2–log reduction of the viability of *G. lamblia* cysts is achieved in a few minutes with, < 0.5 mg ozone/l.

Temperature greatly affects the effectiveness of ozonation. The resistance of *G. lamblia* cysts to zone increased when the temperature was lowered from 25 to 58°C (Wickramanayake*et al*., 1985). A similar phenomenon was observed for *Cryptosporidium* oocysts (Joret*et al*., 1992; Renneckert*et al*., 2000) and for *Bacillus subtilis* spores (Driedger*et al*., 2001). Ozone is one of the few chemical oxidants that have been shown to be capable of inactivating *Cryptosporidium* spp. under normal water treatment conditions as demonstrated by Korich *et al.* (1990). They found that ozone was able to inactivate *C. parvum* oocysts. In that study, an inactivation of greater than two orders of magnitude (greater than 90 % inactivation) was observed after 5 minutes of exposure to 1 mg/l of ozone (Ct value of 5 mg/l). These authors concluded that with the possible exception of ozone, the use of disinfectants alone should not be expected to inactivate *C. parvum* in drinking water (Korich *et al.,* 1990).

2.8 Sludge treatment and disposal

The sludges accumulated in a wastewater treatment process must be treated and disposed of in a safe and effective manner. The purpose of digestion is to reduce the amount of organic matter and the number of disease-causing microorganisms present in the solids.

The most common treatment options include anaerobic digestion, aerobic digestion, and composting;incineration is also used *albeit* to a much lesser degree (Kadam *et al.,* 2008).

Sludge treatment depends on the amount of solids generated and other site-specific conditions. Composting is most often applied to small-scale plants with aerobic digestion for mid-sized operations, and anaerobic digestion for the larger-scale operations (Kadam *et al.,* 2008).

2.8.1 Anaerobic digestion

Anaerobic digestion is a bacterial process that is carried out in the absence of oxygen. The process can either be *thermophilic* digestion, in which sludge is fermented in tanks at a temperature of 55°C, or *mesophilic*, at a temperature of around 36°C. Though allowing shorter retention time (and thus smaller tanks), thermophilic digestion is more expensive in terms of energy consumption for heating the sludge.

Anaerobic digestion is the most common (mesophilic) treatment of domestic sewage in septic tanks, which normally retain the sewage from one day to two days, reducing the BOD by about 35 to 40%. This reduction can be increased with a combination of anaerobic and aerobic treatment by installing *Aerobic Treatment Units* (ATUs) in the septic tank(REF).

One major feature of anaerobic digestion is the production of biogas (with the most useful component being methane), which can be used in generators for electricity production and/or in boilers for heating purposes.

2.8.2 Aerobic digestion

Aerobic digestion is a bacterial process occurring in the presence of oxygen. Under aerobic conditions, bacteria rapidly consume organic matter and convert it into carbon

dioxide. The operating costs used to be characteristically much greater for aerobic digestion because of the energy used by the blowers, pumps and motors needed to add oxygen to the process.Aerobic digestion can also be achieved by using diffuser systems or jet aerators to oxidize the sludge.

2.8.3 Composting

Composting is also an aerobic process that involves mixing the sludge with sources of carbon such as sawdust, straw or wood chips. In the presence of oxygen, bacteria digest both the wastewater solids and the added carbon source and, in doing so, produce a large amount of heat (Kadam *et al.,* 2008).

2.8.4 Incineration

Incineration of sludge is less common because of air emissions concerns and the supplemental fuel (typically natural gas or fuel oil) required to burn the low calorific value sludge and vaporize residual water. Stepped multiple hearth incinerators with high residence time and fluidized bed incinerators are the most common systems used to combust wastewater sludge. Co-firing in municipal waste-to-energy plants is occasionally done, this option being less expensive assuming the facilities already exist for solid waste and there is no need for auxiliary fuel (Kadam *et al.,* 2008).

2.8.5 Sludge disposal

When a liquid sludge is produced, further treatment may be required to make it suitable for final disposal. Kadam *et al.* (2008) reported thattypically, sludges are thickened (dewatered) to reduce the volumes transported off-site for disposal and thatthere is no process which completely eliminates the need to dispose of biosolids. They however, observed that an additional step is taken in some cities to superheat sludge and convert it into small pelletized granules that are high in nitrogen and other organic materials. In New York City,

for example, several sewage treatment plants have dewatering facilities that use large centrifuges along with the addition of chemicals such as polymer to further remove liquid from the sludge. The removed fluid, called concentrate, is typically reintroduced into the wastewater process. The product which is left is called "cake" and that is picked up by companies which turn it into fertilizer pellets. This product is then sold to local farmers and turf farms as a soil amendment or fertilizer, reducing the amount of space required to dispose of sludge in landfills (Kadam *et al.,* 2008).

2.9 Sewage treatment in developing countries

Few reliable figures on the share of the wastewater collected in sewers that is being treated in the world exist (Tajrishy and Abrishamchi, 2005). In many developing countries, the bulk of domestic and industrial wastewater is discharged without any treatment or after primary treatment only (Tajrishy and Abrishamchi, 2005). In Latin America about 15% of collected wastewater passes through treatment plants (with varying levels of actual treatment). In Venezuela, a below average country in South America with respect to wastewater treatment, 97% of the country's sewage is discharged raw into the environment.[19] In a relatively developed Middle Eastern country such as Iran, Tehran's majority of population has totally untreated sewage injected to the city's groundwater (Tajrishy and Abrishamchi, 2005).

2.10 Biosand (slow sand) filter

2.10.1 Physical characteristics of biosand filter

Slow sand filtration is a water purification process in which water is passed through a porous sand bed as filter medium. Slow sand filters are typically characterized by certain design components: the supernatant (water above the filter sand that provides hydraulic head for the process), filter sand varying in depth, the underdrain medium (usually consisting of graded gravel), and a set of control devices (Sims). In a mature sand bed, a thin upper sand

layer called a *Schmutzedecke* forms. The *Schmutzedecke*(German, meaning "dirty blanket") consists of biologically active microorganisms that break down organic matter while suspended inorganic matter is removed by straining (Van Duk and Oomer, 1978; Palmateer *et al.,* 1999). Slow sand filters are distinguished from rapid sand filters by the biologically active sand medium (including the *Schmutzedecke*), and slow detention times. Rapid sand filters utilize primarily a physical removal process, are periodically backwashed for cleaning, and operate with long detention times. Slow sand filters are cleaned by periodically scraping the existing *Schmutzedecke* (Van Duk and Oomer, 1978). Figure 2.2 is a schematic of a common transverse section of a slow sand filter.

The physical characteristics of a sand bed are important in maintaining the slow sand filter's efficiency. The effective size is the size opening that will pass ten percent by weight of the filter material (Haarhoff and John, 1991). Effective sizes in the range of 0.15 mm to 0.35 mm are used (Van Duk and Oomer, 1978). The uniformity coefficient is the ratio of the size openings that pass sixty percent of filter material to the size openings that pass ten percent of filter material, e.g. the effective size (Haarhoff and John, 1991). Uniformity coefficients range between two and five; most facilities maintain uniformity coefficients less than three (Haarhoff and John, 1991). The filter medium itself should consist of inert and durable grains; sand should be washed so that it is free of clays, loams, and organic matter. Depth of a filter bed ranges between 1.0 and 1.4 meters (Van Duk and Oomer, 1978).

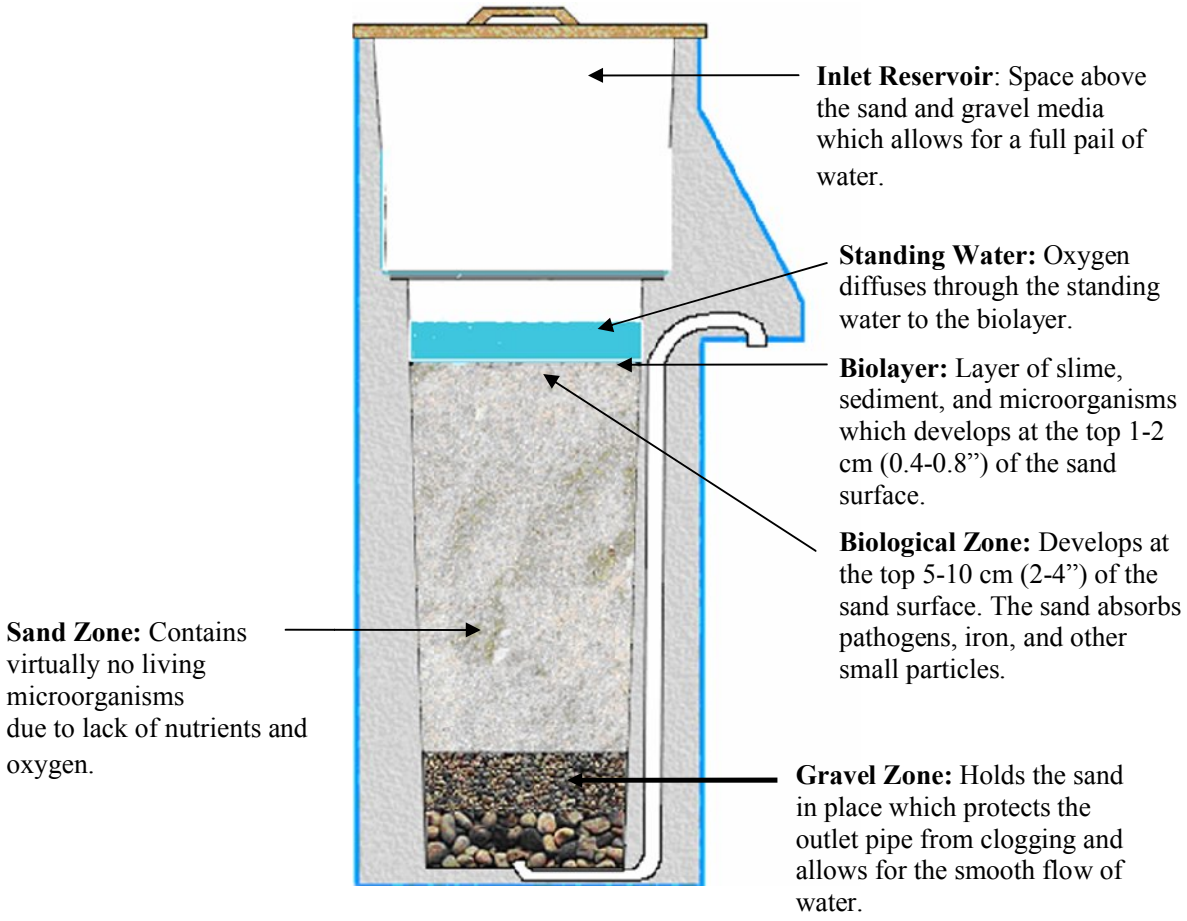


Figure 2.2: Transverse section of a biosand filter showing the component parts (Sobsey, 2009).

2.10.2 Biological and physical mechanismsof biosand filter

Biological activity in the sand bed is not well understood. Scientists have a vague idea of the processes involved, but specific interactions are still unknown. Suggested biological removal mechanisms are predation, scavenging, natural death and inactivation, and metabolic breakdown (Haarhoff and John, 1991). In the *Schmutzedecke*, algae, plankton, diatoms, and bacteria break down organic matter through biological activity. It has been hypothesized that as the raw water passes through the bed, it constantly changes direction. Thus, the sand grains develop a uniform sticky layer of organic material that adsorbs to the particles by various attachment mechanisms. The sticky layer around the sand grains is biologically active (bacteria, protozoa, bacteriophages) and the organic impurities are biologically converted to water, carbon dioxide and harmless salts. According to a study by Collins, the bacterial concentrations in the *Schmutzedecke* were a function of the elapsed time and potential for cell growth rather than the filtration of free-living bacteria from the source water (Collins*et al.,* 1992). The biologically active section of the entire filter bed extends 0.4-0.5 m downward from the surface of the *Schmutzedecke* (Van Duk and Oomer, 1978).

Physical processes are also inherent to slow sand filter mechanisms. As the biological activity of the filter bed decreases, the physical processes of adsorption and chemical oxidation are the primary mechanisms (Van Duk and Oomer, 1978). Adsorption accounts for removals that were traditionally thought to be purely biological. For example, the removal of chlorinated organics and the distribution of viruses are thought to follow adsorption isotherms (Haarhoff and John, 1991). Furthermore, suspended inorganic matter may be removed by the physical process of straining (Van Duk and Oomer, 1978).

2.10.3 Removal of *Giardia*species and *Cryptosporidiumspecies*

In the past decade the protozoan parasite *Cryptosporidium parvum* has been recognized as a significant threat to public water supplies. The resistant stage of *Cryptosporidia* is called an oocyst; this stage is relatively untouched by a chlorination disinfection process. Slow sand filtration has been looked at in numerous studies to determine the viability of this treatment process for the removal of *Cryptosporidia*. A study by Timmis*et al.*(1994) found reductions of oocysts greater than 99.97%; the oocysts were found in the filter media above 2.5 cm. Another study in British Columbia by Fogel*et al.* (1993) contradicts the aforementioned study. Fogel*et al.* (1993) found removal efficiencies of 48%; this figure is significantly different than the 100% removals Fogel*et al.* (1993) cited from previous literature. However, a point to note concerning the British Columbia filters is that they were operating well out of the range of the recommended design limits for the uniformity coefficient at 3.5 (Fogel*et al.,* 1993). Furthermore, temperature can adversely affect the performance of a slow sand filter; the British Columbia filters were operating at extremely low temperatures of less than 1°C (Fogel*et al.,* 1993). Overall, the literature supports data that strongly suggests slow sand filtration is a viable alternative for oocysts removal of*Cryptosporidium*.

Slow sand filters have also been proven highly efficient in removing *Giardia lamblia*, a frequently identified pathogenic intestinal protozoon. The same study by Fogel*et al.* (1993) found that despite the uniformity coefficient parameter and the low temperatures, *Giardia* removals were complete. This data was further supported by literature cited by Fogel*et al.* (1993). Furthermore, faecal and total coliform counts were below the detection limit, and the removal rates were similar to *Giardia* removals (Fogel*et al.,* 1993).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

The study was carried out in Ahmadu Bello University (A.B.U.) Zaria. Liquid wastes from the Ahmadu Bello University Teaching Hospital, Shika, Nigeria are treated in the sewage treatment plant and discharged into a stream while sewage from halls of residence and staff quarters are collected in a sewer and channelled to the waste stabilisation pond (WSP).There the sewage is treated biologically, before being discharged into the Kubanni dam (reservoir) which is abstracted for the water needs of the entire university community.The Kubanni dam is also known as Ahmadu Bello University dam. The dam's (reservoir's) catchment area is 57km^2 and its width is 6 meters. It is located within latitude 11°11□ N and longitude 7°38□ E in Samaru Zaria, Kaduna State(Adakole *et al.,* 2003).

The Ahmadu Bello University Teaching Hospital (ABUTH) sewage treatment plant treats sewage generated by the hospital community in ABUTH, Shika, Nigeria. The sewage is collected in an underground reservoir equipped with three pumping machines that intermittently pump the collected sewage to an above ground sedimentation tank. The pumps in the reservoir are fitted with strainers that prevent lifting of solid wastes to the sedimentation tank thereby leaving behind solid wastes such as polyethylene sachets, metal tins and cans in the reservoir.In the sedimentation tank, parasites and other pathogens settle to the bottom of the tank due to the influence of gravity. An efficient tank is able to remove 60 – 70% of the suspended solids. The retention time in primary sedimentation tank is 1.5 hours and the depth of the tank is about 4 m. Wastewater from the sedimentation tank then flows to oxidation ponds from where they are finally discharged into the stream which is used by farmers as source of irrigation water downstream.

The Ahmadu Bello University waste stabilisation ponds comprise of two sets of anaerobic ponds, facultative ponds and maturation ponds running parallel to one another.

Prior to treatment in the waste stabilisation ponds, the wastewater is first subjected to preliminary treatment – screening and grit removal – to remove large and heavy solids. The design of this preliminary treatment stage is the same as that used for conventional electromechanical sewage treatment plant, but for waste stabilisation ponds the simplest systems are generally used (that is, manually raked screens and manually cleaned constant-velocity grit channels) (Lloyd *et al.,* 2003).

The wastewater treatment processes occur in anaerobic, facultative and maturation ponds. Basically, primary treatment is carried out in anaerobic ponds, secondary treatment in facultative ponds, and tertiary treatment in maturation pons. Anaerobic and facultative ponds remove organic matter (normally expressed ad "biochemical oxygen demand" of BOD), *Vibrio cholera* and helminth eggs; and maturation ponds remove faecal viruses (especially rotavirus, astrovirus and norovirus), faecal bacteria (for example, *Salmonella* spp., *Shigella* spp., *Campylobacter* spp. and pathogenic strains of *Escherichia coli*), and nutrients (nitrogen and phosphorus) (Mara, 2004).

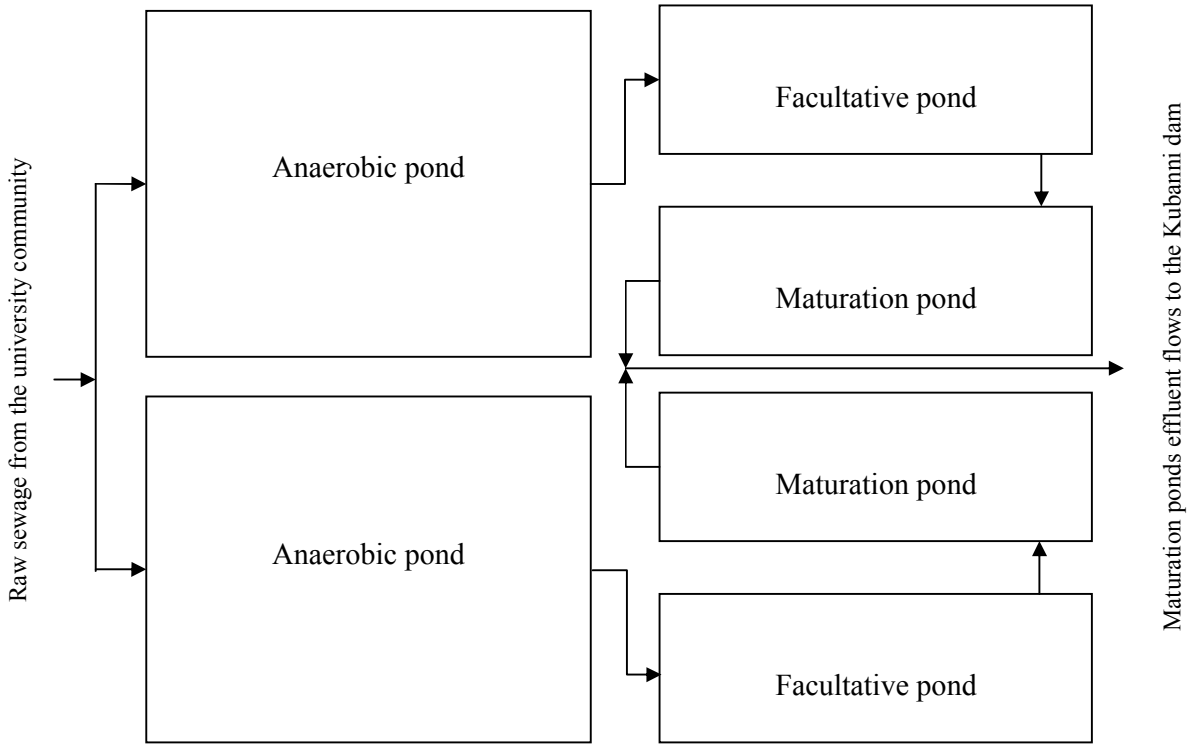


Figure 3.1: Schematic diagram of the Ahmadu Bello University waste stabilisation ponds.

3.2 Construction of biosand filter

Concrete filter body: To prepare the concrete filter body, 4 parts of Portland cement (approximately 100 kg (220 lbs), 6 parts of clean pea size gravel and 2 parts clean sand (freshly crushed from the quarry) were mixed and clean water was added to make concrete slurry. The slurry was then poured into an already constructed metal mould and allowed to set.

Fine sand filter media: Clean, freshly crushed rock (sand dust) from quarry was used. The sand dust was screened through metal mesh screen into three different sizes using mesh screen of 0.5 mm, 1 mm, and 12 mm pore sizes.

Diffuser: One hundred (100) holes, no larger than 3/16” in diameter, were drilled or punched in a plastic (HDPE) material on a 1" x 1" grid and then used as the diffuser.

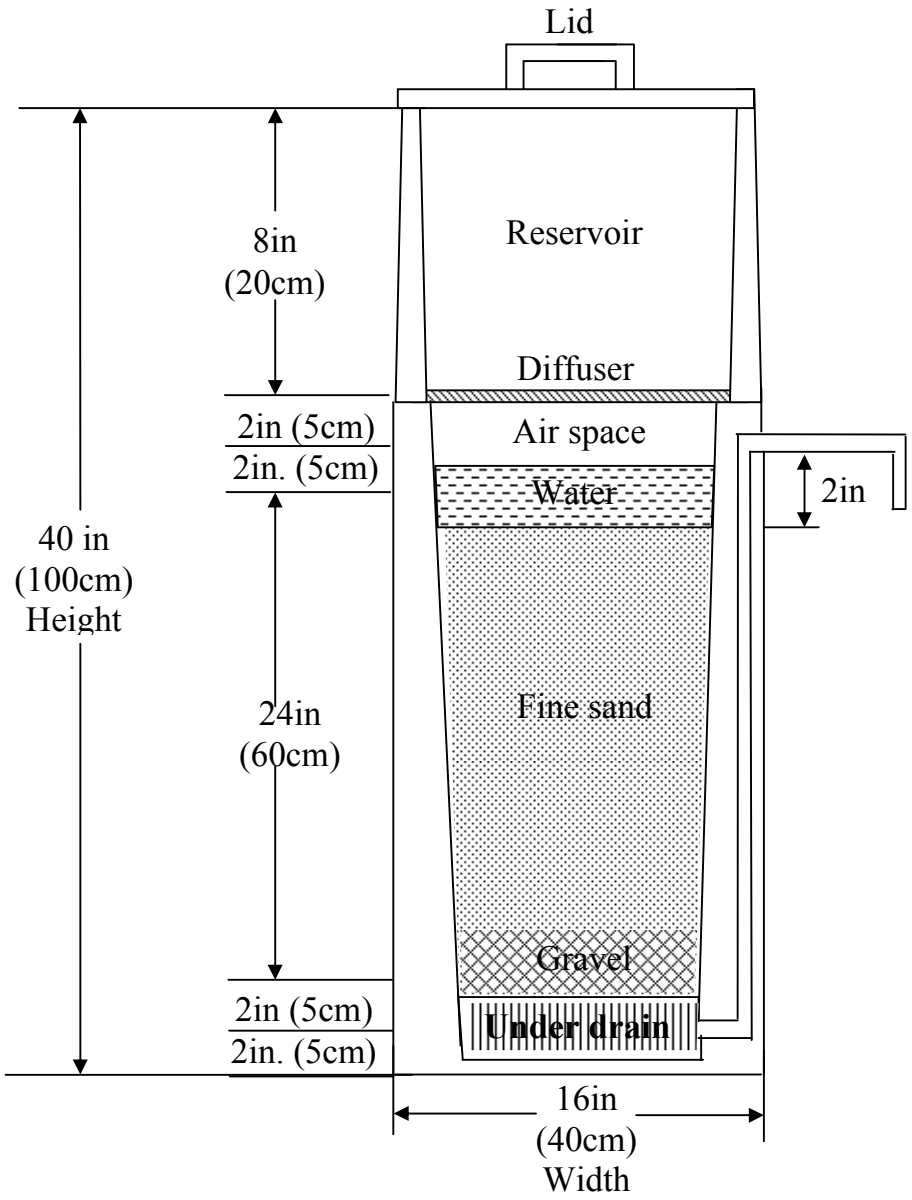


Figure 3.2: A 2-dimensional cross-sectional drawing of the constructed biosand filter showing the various sand layers.

3.3 Sample collection and processing

3.3.1 Sample collection

3.3.1.1 Sample collection from sewage treatment plant

Twenty litres (20 L) each of raw sewage, primary effluent and secondary effluent were collected weekly from the Ahmadu Bello University Teaching Hospital (ABUTH) sewage treatment plant outlet in Shika, Zaria, Kaduna State. Samples of wastewater were collected from upstream (raw wastewater of influent) and downstream (effluent) of the Ahmadu Bello University Teaching Hospital sewage treatment plant. A total of 960 L of wastewater was collected and analysed. Each sample was in volume of 20 L. Out of the 960 L collected, 320 L was filtered through the biosand filter.The filter effluent (filtrate) was collected and analysed for parasite ova and cysts.. Another 320L of raw wastewater was analysed for parasite ova, cysts and oocysts without filtering through the biosand filter. The third phase of sample collection involves 320 L of effluent from the sewage treatment plant.

3.3.1.2 Sample collection fromwaste stabilisation pond

Raw wastewater and effluents from anaerobic, facultative and maturation ponds of the waste stabilisation pond of the Ahmadu Bello University, Zaria were sampled. A total of 960 L of sample was collected consisting of 240 L each of raw wastewater, effluents from anaerobic, facultative and maturation ponds.

The method of concentration which seems the most successful in the parasitological analysis of the samples was selected based on previous studies. The method of USEPA (2005) as modified by Lim *et al.* (2007) was adopted with slight modification.

3.3.2 Sample processing

3.3.2.1 Biosand filtration

Twenty (20 L) litres of the wastewater sample was filtered through the biosand filter. The effluent (filtrate) was collected in clean 20 L plastic jerry can and then tested as outlined in section 3.3.2.2.

3.3.2.2 Sample concentration using cold sucrose floatation technique

Sewage from treatment plant: Each 20 L sample was concentrated by repeated centrifugation at 1500 × g for 10 minutes in 250 ml conical centrifuge tubes before being concentrated to 10 ml. Sample was slowly under-layered with 10 ml of cold sucrose solution (specific gravity of 1.18) and centrifuged at 1000 × g for 5 minutes. Without disturbing the pellet, the entire supernatant including the interface was gently decanted into a clean centrifuge tube. Residual sucrosewas removed by washing the pellet three times in pH 7.2 phosphate buffered saline (PBS) (Oxoid, Hampshire, UK). The final concentrated sample was then reduced to a final volume of 1 to 5 ml depending on the turbidity of the sample. Using a plastic Pasteur pipette, the well of McMaster slide was gently filled with the concentrate and observed microscopically for helminth ova.

Effluent from biosand filter and waste stabilisation ponds: filtrates (effluents) from both biosand filter and waste stabilisation ponds were concentrated as above for sewage from treatment plant.

3.3.2.3Kinyoun Modified Acid-Fast (Ziehl-Neelsen) staining for Cryptosporidium species

A smear was prepared from each sediment in 3.3.2.2 above, air-dried and fixed in methanol for 3 minutes. The methanol-fixed smear was immersed in cold strong carbol-fuchsin and stained for 15 minutes. It was then thoroughly rinsed in distilled water and decolourised in 1% acid-methanol for 10–15 seconds. The slide was rinsed in distilled water

and counterstained with 0.4% malachite green for 30 seconds, after which it was rinsed in distilled water. Duplicate slides were made from each sample.

3.4 Calibration of the ocular micrometer using graticule

The size of microorganisms is generally expressed in micrometer (μm) and is determined by the use of a microscope equipped with an ocular micrometer. An ocular micrometeris a small glass disk on which uniformly spaced lines of unknown distance, ranging from 0 to 100, are etched. The ocular micrometer is inserted into the eyepiece of the microscope and then calibrated against a stage micrometer,which has uniformly spaced lines of known distance etched on it. The stage micrometer is usually divided into 0.01 millimetre and 0.1 millimetre graduations.

The ocular micrometre was inserted into the eyepiece while the stage micrometre was placed on the stage where microslides are placed for microscopic examination.When the two micrometers were in place, the ocular in the body tube was turned until the lines of the ocular micrometrewere parallel with those of the stage micrometer. The lines at the left edges of the two micrometers were matched by moving the stage micrometre.The actual distance in millimetres between the lines of the ocular micrometer was calculated by observing how many spaces of the stage micrometer were included within a given number of spaces on the ocular micrometer.

The smallest space on the stage micrometer equals 0.01 millimetre or 10 μm, the calibration of the ocular micrometer was done as follows:

10 spaces on the ocular micrometer = *Y* spaces on the stage micrometer.

Since the smallest space on a stage micrometer = 0.01 mm,

then 10 spaces on the ocular micrometer = *Y* spaces on the stage micrometer × 0.01 mm,

and 1 space on the ocular micrometer = *Y* spaces on the stage micrometre × $\frac{\cdot}{\quad}$mm.

For example, if 10 spaces on the ocular micrometre = 6 spaces on the stage micrometre, then

1 ocular space = $\frac{\times \quad .}{\quad\quad}$mm

1 ocular space = 0.06 mm or 6.0 µm

This numerical value holds only for the specific objective-ocular lens combination used and may vary with different microscopes. Therefore, each objectives of the microscope was calibrated and recorded.

3.5 Microscopic examination and enumeration of oocysts of *Cryptosporidium parvum* and *Giardia lamblia* cysts

The smeared slides were air-dried and examined for oocysts of *Cryptosporidium parvum* and cysts of *Giardia lamblia* using the ×40 objective lens of a bright-field microscope (Olympus Microscope XSZ – 107BN No. 001677, Japan). The presence of oocysts and cysts were confirmed under the oil immersion objective lens. The shapes of the red-stained bodies were noted and their sizes were measured using micrometre mounted on the eyepiece of the microscope.

The ova, cysts and oocysts were enumerated according to the method outlined by Mahvi and Kia (2006):

No. of (oo)cysts per litre = $\frac{\times}{\times}$

N = number of (oo)cysts observed on the slide

A = volume of the McMaster slide = 0.15 ml orvolume smeared on microslide (ml) for modified Ziehl-Neelsen staining

C = concentrated volume (ml)

F = original sample volume (l)

It was assumed that a drop of sample was placed on the slide so the volume analysed "A" was 0.05 ml (Davutluoglu, 2005).

3.6 Statistical analysis

The percentage removal of the parasites was calculated according to the formula used by Falabi *et al.* (2002):

$$\% \text{ removal} = (N_{influent} - N_{effluent}) \times 100 / N_{influent}$$

Where $N_{influent}$ = number of parasites eggs in the influent wastewater,

$N_{effluent}$ = number of parasites eggs in the effluent wastewater.

Percentage removal of helminth ova, cysts and oocysts was logarithmically transformed using $\log_{10}(y + 1)$, where y = number of parasites eggs, cysts and oocysts.

The computer statistical software, Statistical Programme for Social Science version 17.0(SPSS v 17.0) for Windows®(SPSS Inc., Chicago, IL, USA), was used for the statistical analysis of the data. Wilcoxon's Signed Ranks Test was used to compare any two wastewater treatment types while one-way analysis of variance (ANOVA) was used to check the influence of wastewater treatment type in the removal of parasites. Assumptions for ANOVA test were checked and subsequently, pair-wise comparisons were performed using Tukey HSD post hoc tests (HSD, honestly significant difference). The confidence interval in 95% was used to calculate and compare the mean of parasite egg, cyst or oocyst concentration in the samples.

CHAPTER FOUR

RESULTS

A total of three hundred and twenty litres (320 L) of raw sewage was collected from the Ahmadu Bello University sewage treatment plant (ABUTH-STP) and analysed. In all, 1,648 eggs, cysts and oocysts of parasites per litre were counted during the period of the study. *Ascarislumbricoides* had the highest number of eggs per litre (n = 307; 18.63%), this was closely followed by *Taenia* spp (n=287; 17.42%) and *Schistosoma* spp (n=253; 15.35%) while cysts of*Giardialamblia* had the least count per litre (n=58; 3.52%) (Table 4.1).*Trichuristrichiura* and *Hymenolepis* spp had equal number of eggs per litre (n=92; 5.58%) with mean eggs/litre of 5.69 ± 0.44 and 5.75 ± 1.09 respectively.

On the other hand, 240 L of raw sewage (influent) was collected from the Ahmadu Bello University waste stabilisation ponds (ABU-WSP) on the Main Campus and analysed for parasite eggs, cysts and oocysts. Table 4.1 shows that a total of eight hundred and seventy-four (874) eggs, cysts and oocysts per litre were counted. *Ascarislumbricoides* accounted for the highest number of eggs/litre (n = 198; 22.65%) followed by *Taenia* spp (n = 155; 17.73%) and Hookworm (n = 123; 14.07%). Cysts of *Giardialamblia* had the least count/litre (n = 3; 0.34%). The mean egg or (oo)cyst/litre of sewage from the ABU-WSP revealed that the raw sewage had 12.38 ± 0.93 *Ascarislumbricoides* eggs/L (Table 4.3) while influent of ABUTH-STP had 19.25 ± 0.95*Ascarislumbricoides*egg/L (Table 4.2).

Table 4.1: Mean of ova or (oo)cysts/litre of parasites in raw sewage (influents) and effluents

Parasite	Size (µm)	Total number of eggs or (oo)cysts/1920 litre of Influent(ABUTH-STP)	Percentage	Total number of eggs or (oo)cysts/1920 litre of Influent(ABU-WSP)	Percentage
Cryptosporidiumparvum	4 – 6	130	7.89	80	9.15
Schistosoma spp	83 – 187 x 45 – 70	253	15.35	5	0.57
Taenia spp	30 – 43 x 29 – 38	287	17.42	155	17.73
Hookworm	60 – 75 x 36 – 40	135	8.19	123	14.07
Ascarislumbricoides	55 – 75 x 35 – 50	307	18.63	198	22.65
Toxocara spp	65 – 70 x 75 – 90	176	10.68	111	12.70
Trichuristrichiura	50 – 55 x 22 – 24	92	5.58	67	7.67
Hymenolepis spp	44 – 62 x 30 – 32	92	5.58	42	4.81
Enterobiusvermicularis	50 – 60 x 20 – 32	118	7.16	90	10.30
Giardialamblia	8 – 12 x 7 – 10	58	3.52	3	0.34
Total		**1648**	**100.00**	**874**	**100.00**

Table 4.2: Mean of ova or (oo)cysts/litre of parasites in sewage (influents and effluents) from ABUTH sewage treatment plant

Location	Parasites	Total number of eggs or (oo)cysts/1920 litre of Influent	Mean ± SEM		
			Raw Sewage (Influent)	STP Effluent	Biosand Filter Effluent
ABUTH	*Cryptosporidiumparvum*	130	8.13 ± 0.90	4.19 ± 0.34	0.13 ± 0.09
	Schistosoma spp	253	15.81 ± 1.33	6.31 ± 0.98	0.50 ± 0.22
	Taenia spp	287	17.75 ± 1.38	9.56 ± 0.98	0.38 ± 0.15
	Hookworm	135	8.56 ± 0.89	5.25 ± 0.69	0.19 ± 0.10
	Ascarislumbricoides	307	19.25 ± 0.95	7.81 ± 0.73	0.63 ± 0.18
	Toxocara spp	176	10.94 ± 1.08	6.00 ± 0.53	0.50 ± 0.16
	Trichuristrichiura	92	5.69 ± 0.44	3.44 ± 0.42	0.06 ± 0.06
	Hymenolepis spp	92	5.75 ± 1.09	2.38 ± 0.50	0.06 ± 0.06
	Enterobiusvermicularis	118	7.38 ± 0.36	3.13 ± 0.45	0.13 ± 0.09
	Giardialamblia	58	3.75 ± 0.48	1.44 ± 0.29	0.06 ± 0.06
	Total	**1648**			

SEM = Standard error of mean; STP = Sewage treatment plant; ABUTH = Ahmadu Bello University Teaching Hospital, Shika, Nigeria.

Table 4.3: Mean of ova or (oo)cysts/litre of parasites in sewage (influent and effluents) from ABU waste stabilisation ponds

Location	Parasites	Total number of eggs or (oo)cysts/1920 litre of Influent	Mean ± SEM		
			Raw Sewage (WSP Influent)	WSP Effluent	Biosand Filter Effluent
ABU main campus	*Cryptosporidiumparvum*	80	5.00 ± 0.41	0.38 ± 0.16	0.00 ± 0.00
	Schistosoma spp	5	0.31 ± 0.12	0.00 ± 0.00	0.00 ± 0.00
	Taenia spp	155	9.69 ± 0.65	0.00 ± 0.00	0.00 ± 0.00
	Hookworm	123	7.69 ± 0.30	0.19 ± 0.10	0.00 ± 0.00
	Ascarislumbricoides	198	12.38 ± 0.93	0.19 ± 0.10	0.00 ± 0.00
	Toxocara spp	111	6.94 ± 0.34	0.00 ± 0.00	0.00 ± 0.00
	Trichuristrichiura	67	4.12 ± 0.53	0.31 ± 0.51	0.12 ± 0.06
	Hymenolepis spp	42	2.62 ± 0.46	0.06 ± 0.06	0.00 ± 0.00
	Enterobiusvermicularis	90	5.62 ± 0.36	0.38 ± 0.13	0.12 ± 0.06
	Giardialamblia	3	0.19 ± 0.14	0.00 ± 0.00	0.00 ± 0.00
	Total	**874**			

SEM = Standard error of mean; STP = Sewage treatment plant; WSP = waste stabilisation pond; ABU= Ahmadu Bello University, Zaria, Nigeria.

Graphical comparison of the mean eggs and/or (oo)cysts of the parasites per litre of raw sewage (influent) from Ahmadu Bello University Teaching Hospital sewage treatment plant, effluent from sewage treatment plant and biosand filter effluent showed that biosand filter effluent had the least eggs and/or (oo)cysts per litre followed by effluent of the sewage treatment plant (Appendix I). Effluent of the biosand filter showed that the samples had < 1 egg or (oo)cyst/litre; sewage treatment plant effluent had 1.44 *Giardialamblia* cysts/litre; 2.38 egg/litre of *Hymenolepis* spp and 9.56 egg/litre of *Taenia* spp.

In Table 4.4, the Ahmadu Bello University Teaching Hospitalsewage treatment plant showed a percentage reduction of 52.61% in contrast with 97.45% reduction in total parasite eggs per litre exhibited by the biosand filter. A perusal of the percentage reduction in the individual parasite eggs showed that *Hymenolepis* spp was the most removed by the sewage treatment plant with percentage removal of 61.67% while Hookworm (37.78%) was the least removed. On the other hand, the highest percentage removal of parasite eggs by the biosand filter was observed for ova of *Hymenolepis* spp (98.91%) followed by *Trichuristrichiura* (98.90%).

A cursory look at Table 4.5 reveals that biosand filter was able to remove 98.89% of *Enterobiusvermicularis* and 98.51% of *Trichuris trichiura* as opposed to the 93.33% and 92.54% removal by waste stabilisation pond respectively. There was 100.00% removal or eggs and cysts of *Schistosoma, Taenia*, *Toxocara* and *Giardia* species by both biosand filter and waste stabilisation pond.While there was 100.00% removal of oocysts and eggs of *Cryptosporidium parvum, Hookworm, Ascaris lumbricoides, Trichuris trichiura*and *Enterobius vermicularis* by biosand filter, waste stabilisation pond removed 92.50%, 97.56%, 98.48%, 92.54% and 93.33% respectively.Appendix II compares the removal efficiency of biosand filter, waste stabilisation pond and sewage treatment plant in the removal of parasite eggs and (oo)cysts.The sewage treatment plant consistently exhibited the least efficiencies in

the removal of all the parasites examined. While sewage treatment plant was 37.78% efficient in removing *Hookworm* eggs, biosand filter and waste stabilisation ponds were 100% and 97.56% efficient respectively. The sewage treatment plant showed its highest efficiency in the removal of eggs of *Hymenolepis* species whereas waste stabilisation pond was 97.62% efficient and biosand filter was 100% efficient.

A comparison of the mean parasite eggs/litre obtained from the Ahmadu Bello University biosand filter treated effluent and the effluent of the Ahmadu Bello University sewage treatment plant using Wilcoxon's Signed Ranks Test at significant level of 0.05 revealed that there was statistically significant difference ($p < 0.05$) between the means concentration of the parasite eggs, cysts or oocysts/litre obtained from effluent of biosand filter (Table 4.6). Similarly, comparison of the means of parasite eggs/litre obtained from the Ahmadu Bello University biosand filter treated effluent and Ahmadu Bello University waste stabilisation ponds effluent indicated that both treatment methods removed 100.00% of *Schistosoma, Taenia, Toxocara*species and *Giardialamblia* (Table 4.7). Though biosand filter was ranked higher in efficiency than Ahmadu Bello University waste stabilisation ponds in the removal of *Cryptosporidium parvum* oocysts and ova of*Hookworm, Ascaris lumbricoides, Trichuris trichiura, Hymenolepis*species*,* and *Enterobius vermicularis* from raw sewage, only the removal of *Cryptosporidiumparvum* was statistically significant ($z = –2.121$; $p = 0.034$).

Table 4.4: Average of reduction of eggs or (oo)cysts/litre of influent and effluents sewage from Ahmadu Bello University Teaching Hospital sewage treatment plant.

Parasites	Average Number of eggs or (oo)cysts/litre of raw sewage (Influent)	STP Effluent		Biosand Filter Effluent	
		Number of eggs or (oo)cysts/1920 litre	Percentage reduction (%)	Number of eggs or (oo)cysts/1920 litre	Percentage reduction (%)
Cryptosporidiumparvum	130	67	48.46	2	98.46
Schistosoma spp	253	101	60.08	8	96.84
Taenia spp	284	143	49.65	6	97.89
Hookworm	135	84	37.78	3	97.78
Ascarislumbricoides	308	125	59.42	10	96.75
Toxocara spp	175	96	45.14	8	95.43
Trichuristrichiura	91	55	39.56	1	98.90
Hymenolepis spp	92	37	61.67	1	98.91
Enterobiusvermicularis	118	50	51.63	2	98.31
Giardialamblia	60	23	60.34	1	98.33
Total	**1648**	**781**	**52.61**	**42**	**97.45**

STP = Sewage treatment plant

Table 4.5: Average of reduction of eggs or (oo)cysts/litre of influent and effluents sewage from Ahmadu Bello University waste stabilisation pond.

Parasite group	No. of eggs or (oo)cysts/1920 litre of influent	Biosand filter Effluent		WSP Effluent	
		Number of eggs or (oo)cysts/1920 litre	Percentage reduction (%)	Number of eggs or (oo)cysts/1920 litre	Percentage reduction (%)
Cryptosporidiumparvum	80	0	100.00	6	92.5
Schistosoma spp	5	0	100.00	0	100.00
Taenia spp	155	0	100.00	0	100.00
Hookworm	123	0	100.00	3	97.56
Ascarislumbricoides	198	0	100.00	3	98.48
Toxocara spp	111	0	100.00	0	100.00
Trichuristrichiura	67	1	98.51	5	92.54
Hymenolepis spp	42	0	100.00	1	97.62
Enterobiusvermicularis	90	1	98.89	6	93.33
Giardialamblia	3	0	100.00	0	100.00
Total	**874**	**2**	**99.77**	**24**	**97.25**

WSP = waste stabilisation pond

Table 4.6: Wilcoxon's Signed Ranks Test of mean differences between effluents of biosand filter and sewage treatment plants.

Parasite	**BSF (n_1)**	**STP (n_2)**	***z***	***p***	**Decision**

Cryptosporidiumparvum	2	67	– 3.434	0.001*	BSF < STP
Schistosoma spp	8	101	– 3.533	<0.001*	BSF < STP
Taenia spp	6	143	– 3.524	<0.001*	BSF < STP
Hookworm	3	84	– 3.304	<0.001*	BSF < STP
Ascarislumbricoides	10	125	– 3.531	<0.001*	BSF < STP
Toxocara spp	8	96	– 3.523	<0.001*	BSF < STP
Trichuristrichiura	1	55	– 3.534	<0.001*	BSF < STP
Hymenolepis spp	1	37	– 3.081	0.002*	BSF < STP
Enterobiusvermicularis	2	50	– 3.534	<0.001*	BSF < STP
Giardialamblia	1	23	– 2.952	0.003*	BSF < STP

BSF = Biosand filter; STP = sewage treatment plant
BSF < STP = the number of eggs or (oo)cysts in effluent of BSF is less than that in STP i.e. BSF is more efficient than STP at *p* –value indicated.
* = statistically significant difference exists between the means of BSF and STP at the *p*-values indicated
Where *p* = level of significance.
z = Wilconxon's Signed Ranks value of the mean difference.
n_1 = number of parasite ova and (oo)cysts in biosand filter effluent and
n_2 = number of parasite ova and (oo)cysts in sewage treatment plant effluent.

Table 4.7: Wilcoxon's Signed Ranks Test of mean differences between effluents of biosand filter and waste stabilisation pond.

Parasite	**BSF (n_1)**	**WSP (n_2)**	***z***	***p***	**Decision**

Cryptosporidiumparvum	0	6	– 2.121	0.034*	BSF < WSP
Schistosoma spp	0	0	0.000	1.000	BSF = WSP
Taenia spp	0	0	0.000	1.000	BSF = WSP
Hookworm	0	3	– 1.732	0.083	BSF < WSP
Ascarislumbricoides	0	3	– 1.732	0.083	BSF < WSP
Toxocara spp	0	0	0.000	1.000	BSF = WSP
Trichuristrichiura	1	5	– 1.414	0.157	BSF < WSP
Hymenolepis spp	0	1	– 1.000	0.317	BSF < WSP
Enterobiusvermicularis	1	6	– 1.633	0.102	BSF < WSP
Giardialamblia	0	0	0.000	1.000	BSF = WSP

BSF = Biosand filter; WSP = waste stabilisation pond
BSF <WSP = the number of eggs or (oo)cysts in effluent of BSF is less than that in WSP i.e. BSF is more efficient than WSP at $p< 0$.
BSF = WSP means both treatment types exhibited equal efficiency in egg removal at the p –values indicated.
* = statistically significant difference exists between the means of BSF and STP at the p-values indicated.
Where p = level of significance.
z = Wilconxon's Signed Ranks value of the mean difference.
n_1 = number of parasite ova and (oo)cysts in biosand filter effluent and
n_2 = number of parasite ova and (oo)cysts in waste stabilisation pond effluent.

Comparison of the parasite removal efficiencies of the three wastewater treatment methods [biosand filter (BSF), waste stabilisation pond (WSP) and sewage treatment plant

(STP)] using one-way analysis of variance (ANOVA) indicated that there were statistically significant differences in the removal of all the parasites except in the removal of *Giardialamblia*cysts where the difference in the removal was not significant ($F = 1.901$, $df = 2$, $p = 0.161$) (Appendix II).

The removal efficiencies of the treatment methods were probed further to identify which method had the highest number of eggs in its effluent (least efficient) and which one had the lowest number of eggs in its effluent (most efficient) using Tukey HSD Test. The test showed that the difference in mean egg and/or (oo)cysts per litre in effluent of BSF and STP is statistically significant except in the removal of *Giardialamblia* cysts ($p = 0.221$) (Appendix III). Though there were differences between mean parasite eggs and/or (oo)cysts in effluents of biosand filter and waste stabilisation ponds but the differences were not significant. However, when the mean parasites in the effluents of biosand filter and waste stabilisation ponds were compared, it was found that the effluent of waste stabilisation ponds had lower mean values than the effluent of sewage treatment plant in the removal of all the parasites except *Giardialamblia* cysts; the differences between the two mean was not statistically significant ($p = 0.221$) (Appendix III).

CHAPTER FIVE

DISCUSSION

The total number of eggs or (oo)cysts/litre of wastewater (influent) was higher than that of the effluent from the sewage treatment plant. This may be due to the adherence of the eggs/cysts of helminths and protozoa to the suspended particles and/or settle out in the sedimentation tank.

The parasitological profile of the wastewater revealed thattwo protozoa (*Cryptosporidium parvum* and *Giardia lamblia*) and eight (8) helminths were found which were *Ascarislumbricoides, Taenia* spp,*Hookworm, Hymenolepis* sppand *Enterobius vermicularis.* The WHO limit for helminth ova in wastewater for use in crop irrigation is less than one (≤ 1) Helminth Ova (HO) per litre (WHO, 2010). Mean egg counts of helminths such as *Ascaris lumbricoides, Trichuristrichiura*, Hookworm and *Toxocara* spp in the untreated wastewater (influent) were higher than the effluent from the sewage treatment plant. There is a great public health risk of reuse of these waters for crop irrigation without prior adequate treatment. However, the effluent from the biosand filter contained less than one mean egg per litre. Earlier studies by Feachem *et al*. (1983) and Shantala *et al*.(2007) have shown that helminth ova can survive in water, soil, and crops for several months or years.

Eggs of *Ascaris lumbricoides* and cysts of protozoa particularly *Entamoeba histolytica* remain viable in stabilization pond effluents used to irrigate vegetables that are eaten raw (Shanthala *et al*., 2007). Such inadequately treated sewage used for irrigation has been identified as increasing the risk towards the incidence of helminthic infections in both the farmers and consumers in the area (Damen *et al*., 2007; Uneke, 2007). It was observed that farmers from villages close to the waste stabilisation ponds and sewage treatment plant were using the discharged wastewater to irrigate their vegetable gardens downstream. These

farmers are at high risk, because they have intensive contact with wastewater, as they do most of the fieldwork manually andbarefootas such are at risk of contracting hookworm infection. Crop consumers are at high risk, because in the wastewater irrigated fields, vegetables are grown that are sometimes eaten raw. Tomatoes and sugar cane are the major crops on the wastewater irrigated fields. Tomatoes are widely used in salads while sugar cane is chewed.

The presence of ova of *Taenia*spp in the wastewater indicates the risk of human cysticercosis in members of the community eating unwashed, raw vegetables irrigated with untreated wastewater. There is also risk of bovine cysticercosis if reared cattle drink from any body of water contaminated with wastewater(Damen *et al*., 2007; Uneke, 2007).

Cryptosporidium parvum oocysts and *Giardia lamblia* cysts were detected frequently and at maximum concentration of 130 oocysts/litre and 58 cysts/litre respectively. *Cryptosporidium parvum* oocysts persistently outnumbered *Giardia lamblia* cysts in both raw and treated sewage samples. In contrast to this study, other studies in Sweden, Norway and Canada reported constant detection of *Giardia lamblia* cysts in sewage (Ottoson *et al.,* 2006; Robertson *et al.,* 2006a) and a rather erratic occurrence of *Cryptosporidium parvum* oocysts (Payment *et al.,* 2001) as opposed to the significantly greater number of *Cryptosporidium parvum* oocysts compared to *Giardia lamblia*cysts observed in this study.

The higher concentration of *Cryptosporidium parvum* oocysts in raw wastewater suggests that cryptosporidiosis may be more widespread, and/or occurred with greater infection intensity than giardiasis. The number of parasites in the influent of a sewage treatment plant depends on the size of the community served as well as the rate of infection within the population (Smith and Rose, 1990). One advantage of examining parasites in sewage is that it gives a more representative indication of endemic infection than epidemiological data, as it takes into account all of the asymptomatic carriers and misdiagnosed cases that ordinarily escape detection (Jakubowski *et al.,* 1991).

The presence of high concentration of *Cryptosporidium parvum* oocysts (4 – 130 oocysts/litre) and *Giardia lamblia* cysts (1 – 58 cysts/litre) in the effluent of the sewage treatment plant is of public health concern because the effluent is directly discharged into the river or dam causing environmental contamination with these parasites. It poses health risk when the river water is used by communities living downstream of the river for household consumption and irrigation of crops (agricultural reuse).

The type of treatment processes play an important role in determining the reduction of parasites such as *Cryptosporidium parvum* and *Giardia lamblia* as indicated in this study. The (oo)cysts of both protozoan parasites were efficiently removed by the biosand filter. It is of utmost importance that pollution of water and vegetables is prevented by substituting the rapid sand filter used in the sewage plant with the biosand filter.

The rate of ova removal by biosand filter and waste stabilisation ponds for *Schistosoma, Taenia, Toxocara* species and *Giardia lamblia* was 100.00% while for *Cryptosporidium parvum* it was 98.89% and 92.5% for biosand filter and waste stabilisation ponds respectively. Experimental data suggests that waste stabilisation ponds may remove practically all the protozoan and helminth eggs producing a final effluent that meets the WHO guidelines and recent revisions for use of treated wastewater in agricultural unrestricted irrigation (Blumenthal *et al.,* 2000). The results of this study are in consonance with such studies and confirm the suitability of the biosand filter and waste stabilisation ponds for removing pathogens from wastewater. These performances were higher than those observed in conventional sewage treatment processes such as activated sludge or primary treatments where reductions of 70 – 99% of protozoan and helminth eggs were reported (Paton *et al.,* 2000; Ottoson *et al.,* 2006; Jimenez *et al.,* 2007; Reinoso and Becares, 2008). This shows that the biosand filter is more efficient in the removal of helminth ova and oocysts of *Cryptosporidium parvum* and *Giardia lamblia* as indicated by the statistically

significant difference when compared with the waste stabilisation pond and the sewage treatment plant. The analysis revealed that 52.61% of the parasite eggs, cysts and oocysts were removed by the sewage treatment plant, 97.25% was removed by waste stabilisation ponds while egg removal efficiency of the biosand filter was between 97.45 – 99.7%.

5.1 Conclusions

It wasobserved that wastewater in the study area was laden with oocysts of *Cryptosporidium parvum*, *Giardia lamblia* cysts and helminth ova.The study has shown that biosand filtration (slow sand filtration)of wastewateris a better alternative to rapid sand filter which is used in the conventional sewage treatment plant as it ensures removal or reduction of helminth ova and protozoan (oo)cysts. The use of biosand filtered effluent for irrigation farming in Zaria metropolis will prevent the parasitological pollution of vegetablesthat may be eaten raw.Statistical analysis showed that waste stabilisation ponds performed better than the conventional wastewater treatment plant; while the efficiency of biosand filter was not statistically different from that of waste stabilisation ponds in the removal of parasites eggs and (oo)cysts.

5.2 Recommendations

In view of the danger and health risks posed to farmers and vegetables consumers by untreated or inadequately treated wastewater, the following measures are recommended:

i. Appropriate form of treatment of wastewater (using biosand filter or waste stabilisation ponds) for helminth eggs and protozoan (oo)cysts removal should be used before application of effluent to the fields.

ii. Wastewater treatment authorities should rethink the relevance of *Cryptosporidium parvum* and *Giardia lamblia* contamination levels in wastewater and watersheds and to develop countermeasures in wastewater treatment plants.

iii. Government should embark on low cost interventions which should include information on hygiene behaviour for farmers, wearing of shoes and gloves while working in wastewater irrigated fields, regular treatment of farmers and their families with antihelminthic and antiprotozoan drugs and crop restrictions in wastewater irrigated fields.

iv. Furthermore, epidemiological studies on the occurrence and removal of pathogenic parasites from excreta and sewage are recommended in order that the public health risks can be defined and the most cost effective wastewater treatment options adopted or developed.

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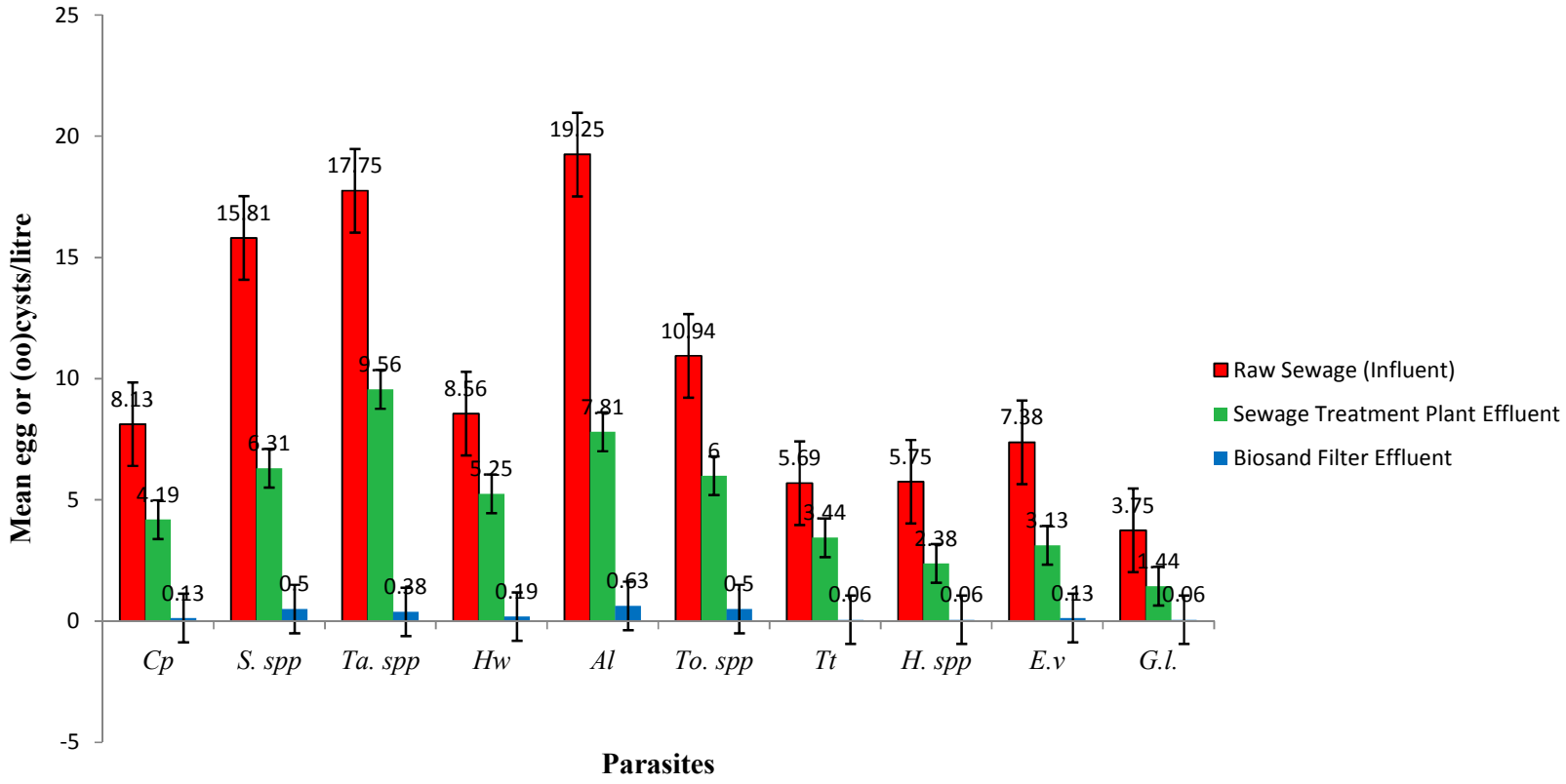
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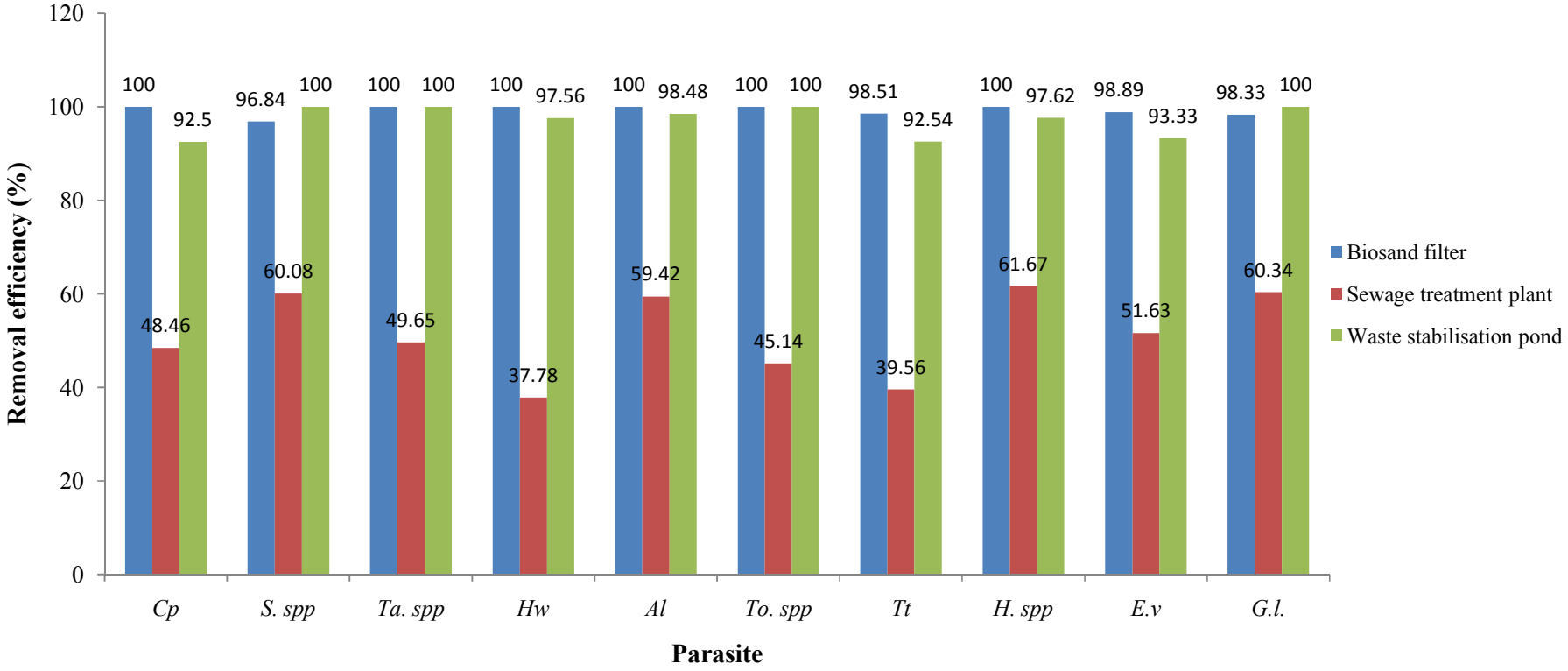
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APPENDIX I: Mean eggs or (oo)cysts/litre of parasites in raw sewage (influent) and effluents of Sewage Treatment Plant and Biosand Filter over the study period.



Cp = *Cryptosporidium parvum*, S. spp = *Schistosoma* species, Ta spp = *Taenia* species, Hw = Hookworm, Al = *Ascaris lumbricoides*, To spp = *Toxocara* species, Tt = *Trichuris trichiura*, H. spp = *Hymenolepis* species, E.v = *Enterobius vermicularis*, G.l = *Giardia lamblia*).

Appendix II: Comparison of parasite removal efficiencies of biosand filter, sewage treatment plant and waste stabilisation pond.



(Cp = *Cryptosporidium parvum*, S. spp = *Schistosoma* species, Ta spp = *Taenia* species, Hw = Hookworm, Al = *Ascaris lumbricoides*, To spp = *Toxocara* species, Tt = *Trichuris trichiura*, H. spp = *Hymenolepis* species, E.v = *Enterobius vermicularis*, G.l = *Giardia lamblia*).

Appendix III: Mean and standard deviations comparing the parasite removal of the three treatment types (BSF, WSP and STP)

	BSF			WSP			STP		
	n	**M**	**SD**	**n**	**M**	**SD**	**n**	**M**	**SD**
Cryptosporidiumparvum	16	0.00	0.00	16	0.38	0.62	16	16.75	3.95
Schistosoma spp	16	0.00	0.00	16	0.00	0.00	16	25.25	11.87
Taenia spp	16	0.00	0.00	16	0.00	0.00	16	35.75	8.66
Hookworm	16	0.00	0.00	16	0.19	0.40	16	13.25	6.08
Ascarislumbricoides	16	0.00	0.00	16	0.19	0.40	16	33.75	15.04
Toxocara spp	16	0.00	0.00	16	0.00	0.00	16	24.00	7.70
Trichuristrichiura	16	0.06	0.25	16	0.31	0.60	16	13.75	0.96
Hymenolepis spp	16	0.00	0.00	16	0.06	0.25	16	9.25	5.85
Enterobiusvermicularis	16	0.06	0.25	16	0.38	0.50	16	12.50	5.92
Giardialamblia	16	0.00	0.00	16	0.00	0.00	16	5.75	2.22

n = number of sample; M = mean egg and/or (oo)cysts per litre of effluent; SD = standard deviation; BSF = biosand filter; WSP = waste stabilisation pond; STP = sewage treatment plant.

APPENDIX IV: One-way analysis of variance (ANOVA) summary table comparing BSF, WSP and STP on removal of parasite

		df	Sum of Squares	Mean Square	F	*p*
Cryptosporidiumparvum	Between Groups	2	248.167	124.083	122.049	< 0.001*
	Within Groups	45	45.750	1.017		
	Total	47	293.917			
Schistosoma spp	Between Groups	2	1.042	.521	6.818	0.003*
	Within Groups	45	3.437	.076		
	Total	47	4.479			
Taenia spp	Between Groups	2	1001.042	500.521	222.043	< 0.001*
	Within Groups	45	101.438	2.254		
	Total	47	1102.479			
Hookworm	Between Groups	2	615.375	307.688	579.935	< 0.001*
	Within Groups	45	23.875	.531		
	Total	47	639.250			
Ascarislumbricoides	Between Groups	2	1609.125	804.562	172.252	< 0.001*
	Within Groups	45	210.187	4.671		
	Total	47	1819.312			
Toxocara spp	Between Groups	2	513.375	256.688	428.805	< 0.001*
	Within Groups	45	26.937	.599		
	Total	47	540.312			
Trichuristrichiura	Between Groups	2	165.875	82.938	50.350	< 0.001*
	Within Groups	45	74.125	1.647		
	Total	47	240.000			
Hymenolepis spp	Between Groups	2	71.792	35.896	30.658	< 0.001*
	Within Groups	45	52.688	1.171		
	Total	47	124.479			
Enterobiusvermicularis	Between Groups	2	312.542	156.271	192.993	< 0.001*
	Within Groups	45	36.438	.810		
	Total	47	348.979			
Giardialamblia	Between Groups	2	.375	.188	1.901	0.161*
	Within Groups	45	4.437	.099		
	Total	47	4.812			

APPENDIX V: Tukey HSD post hoc test on the efficiency of BSF, WSP and STP in the removal of parasites from wastewater.

Dependent Variable	(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Cryptosporidiumparvum	BSF	WSP	-0.375	0.356	0.549	-1.24	0.49
		STP	-5.000*	0.356	0.000	-5.86	-4.14
	WSP	BSF	0.375	0.356	0.549	-0.49	1.24
		STP	-4.625*	0.356	0.000	-5.49	-3.76
	STP	BSF	5.000*	0.356	0.000	4.14	5.86
		WSP	4.625*	0.356	0.000	3.76	5.49
Schistosoma spp	BSF	WSP	0.000	0.098	1.000	-0.24	0.24
		STP	-0.312*	0.098	0.007	-0.55	-0.08
	WSP	BSF	0.000	0.098	1.000	-0.24	0.24
		STP	-0.312*	0.098	0.007	-0.55	-0.08
	STP	BSF	0.312*	0.098	0.007	0.08	0.55
		WSP	0.312*	0.098	0.007	0.08	0.55
Taenia spp	BSF	WSP	0.000	0.531	1.000	-1.29	1.29
		STP	-9.688*	0.531	0.000	-10.97	-8.40
	WSP	BSF	0.000	0.531	1.000	-1.29	1.29
		STP	-9.688*	0.531	0.000	-10.97	-8.40
	STP	BSF	9.688*	0.531	0.000	8.40	10.97
		WSP	9.688*	0.531	0.000	8.40	10.97
Hookworm	BSF	WSP	-0.188	0.258	0.748	-0.81	0.44
		STP	-7.688*	0.258	0.000	-8.31	-7.06
	WSP	BSF	0.188	0.258	0.748	-0.44	0.81
		STP	-7.500*	0.258	0.000	-8.12	-6.88
	STP	BSF	7.688*	0.258	0.000	7.06	8.31
		WSP	7.500*	0.258	0.000	6.88	8.12
Ascarislumbricoides	BSF	WSP	-0.188	0.764	0.967	-2.04	1.66
		STP	-12.375*	0.764	0.000	-14.23	-10.52
	WSP	BSF	0.188	0.764	0.967	-1.66	2.04
		STP	-12.188*	0.764	0.000	-14.04	-10.34
	STP	BSF	12.375*	0.764	0.000	10.52	14.23
		WSP	12.188*	0.764	0.000	10.34	14.04

* = Significant mean difference at the 0.05 level. BSF = biosand filter; WSP = waste stabilisation pond; STP = sewage treatment plant.

Tukey HSD post hoc test on the efficiency of BSF, WSP and STP in the removal of parasites from waste water (Cont'd).

Dependent Variable	(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Toxocara spp	BSF	WSP	0.000	0.274	1.000	-0.66	0.66
		STP	-6.938*	0.274	0.000	-7.60	-6.27
	WSP	BSF	0.000	0.274	1.000	-0.66	0.66
		STP	-6.938*	0.274	0.000	-7.60	-6.27
	STP	BSF	6.938*	0.274	0.000	6.27	7.60
		WSP	6.938*	0.274	0.000	6.27	7.60
Trichuristrichiura	BSF	WSP	-0.250	0.454	0.847	-1.35	0.85
		STP	-4.062*	0.454	0.000	-5.16	-2.96
	WSP	BSF	0.250	0.454	0.847	-0.85	1.35
		STP	-3.812*	0.454	0.000	-4.91	-2.71
	STP	BSF	4.062*	0.454	0.000	2.96	5.16
		WSP	3.812*	0.454	0.000	2.71	4.91
Hymenolepis spp	BSF	WSP	-0.062	0.383	0.985	-0.99	0.86
		STP	-2.625*	0.383	0.000	-3.55	-1.70
	WSP	BSF	0.062	0.383	0.985	-0.86	0.99
		STP	-2.562*	0.383	0.000	-3.49	-1.64
	STP	BSF	2.625*	0.383	0.000	1.70	3.55
		WSP	2.562*	0.383	0.000	1.64	3.49
Enterobiusvermicularis	BSF	WSP	-0.312	0.318	0.592	-1.08	0.46
		STP	-5.562*	0.318	0.000	-6.33	-4.79
	WSP	BSF	0.312	0.318	0.592	-0.46	1.08
		STP	-5.250*	0.318	0.000	-6.02	-4.48
	STP	BSF	5.562*	0.318	0.000	4.79	6.33
		WSP	5.250*	0.318	0.000	4.48	6.02
Giardialamblia	BSF	WSP	0.000	0.111	1.000	-0.27	0.27
		STP	-0.188	0.111	0.221	-0.46	0.08
	WSP	BSF	0.000	0.111	1.000	-0.27	0.27
		STP	-0.188	0.111	0.221	-0.46	0.08
	STP	BSF	0.188	0.111	0.221	-0.08	0.46
		WSP	0.188	0.111	0.221	-0.08	0.46

* = Significant mean difference at the 0.05 level. BSF = biosand filter; WSP = waste stabilisation pond; STP = sewage treatment plant.