DETECTION OF HELMINTH EGGS AND PROTOZOAN CYSTS IN WASTEWATERS

AYTEN DAVUTLUOĞLU

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DETECTION OF HELMINTH EGGS AND PROTOZOAN CYSTS IN WASTEWATERS

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Prof. Dr. Canan Özgen
Director

I certify that this thesis satisfies all the requirements as a thesis for the degree of Master of Science

Prof Dr Filiz Bengü Dilek
Head of Department

This is to certify that we have read this thesis and that in our opinion it is fully adequate, in scope and quality, as a thesis for the degree of Master of Science

Prof Dr Nevin Keskin
Co-Supervisor

Prof Dr Celal Ferdi Gökçay
Supervisor

Examining Committee Members

Prof. Dr. Filiz B. Dilek (METU,ENVE) _____

Prof. Dr. Celal F. Gökçay (METU,ENVE) _____

Prof. Dr. Nevin Keskin (HACETTEPE UNV,BIO) _____

Assoc. Prof. Göksel N. Demirer (METU,ENVE) _____

Assoc. Prof. Nurdan Özer (HACETTEPE UNV,BIO) _____

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AYTEN DAVUTLUOĞLU

Signature

ABSTRACT

DETECTION OF HELMINTH EGGS AND PROTOZOAN CYSTS

IN WASTEWATERS

Davutluoğlu, Ayten

M.S., Department of Environmental Engineering

Supervisor: Prof. Dr. Celal Ferdi Gökçay

Co-Supervisor: Prof. Dr. Nevin Keskin

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The withdrawal of water sources concluded the reuse of treated wastewaters, especially for non-potable purposes. Agricultural use of the reclaimed wastewaters is one of the reuse options.

However health considerations of the reuse of reclaimed wastewaters for public related purposes are underestimated, since wastewaters contain a variety of microbial pathogens, which may be transmitted to workers and consumers through the crops irrigated. Of these, parasitic eggs have a special place, as they are capable of surviving in the soil for months or even years, depending on environmental conditions.

There is insufficient accumulated information on the health related criteria for the reuse of treated wastewaters in Turkey.

The aim of this study was therefore to determine the helminthic eggs in raw sewage and in effluents of ASKİ municipal wastewater treatment plant in Ankara. The study involved examining to decide whether these organisms exist in the wastewaters at all, and if so in what concentrations.

Modified Bailenger's method, which published in the "WHO Laboratory Manual of Parasitological and Bacteriological Techniques" and "U.S.EPA ICR Microbial Laboratory Manual" were used in developing the specific methods used in this study.

Keywords: Reclaimed Wastewater, Irrigation, Helminth Egg, Protozoan Cyst

ÖZ

ATIKSULARDA HELMİNT YUMURTALARI VE PROTOZOAN KİSTLERİ TAYİNİ

Davutluoğlu, Ayten
Çevre Mühendisliği Bölümü
Danışman: Prof. Dr. Celal Ferdi Gökçay
Yardımcı Danışman: Prof. Dr. Nevin Keskin

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Dünyada su kaynaklarındaki azalma arıtılmış atıksuların yeniden kullanılması sonucunu doğurmuştur. Arıtılmış atıksular ile tarım alanlarının sulanması, kullanım seçeneklerinden birisidir.

Ancak arıtılmış atıksuların, çeşitli hastalık yapıcı mikroorganizmalar içerdiğinden dolayı, yeniden kullanılması sebebiyle ortaya çıkabilecek sağlık sorunları göz ardı edilmektedir. Patojen mikroorganizmalar atıksu ile sulanan tarım ürünleri aracılığı ile, tarlada çalışan insanlara ve tüketicilere bulaşabilirler. Patojenlerden biri olan parazit yumurtaları toprakta çevre şartlarına da bağlı olarak uzun aylar boyunca canlı kalabilmektedirler.

Ülkemizde arıtılmış atıksuların yeniden kullanılmasında, sağlıkla ilgili kriterler konusunda yeterli bilgi bulunmamaktadır.

Bu çalışmanın amacı Ankara'da evsel atıksuda ve ASKİ evsel atıksu arıtma tesisi çıkış suyunda helmint yumurtaları tayinidir. Çalışma kapsamında atıksularda helmint

yumurtalarının var olup olmadığı araştırılmış ve hangi konsantrasyonlarda bulundukları belirlenmiştir.

Çalışmada WHO tarafından, "Laboratory Manual of Parasitological and Bacteriological Techniques" de yayınlanan modifiye Bailenger metod ve USEPA tarafından "ICR Microbial Laboratory Manual" da yayınlanan metot kullanılmıştır.

Anahtar kelimeler: Arıtılmış Atıksu, Sulama, Helmint Yumurtası, Protozoa Kisti

To Cem

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CHAPTER 1

INTRODUCTION

Diminishing fresh water resources and increasing water demand have been pressing in the recent decade. As exploitation of remaining water resources are approaching to saturation, search for alternative sources, especially for non-potable purposes, is increasing. In many cases wastewater reuse seems an excellent source to fill the growing demand and for sustainability. Crop irrigation is one area where reuse may be most promising. The wastewater must not only be considered as simply a water source for crop irrigation, but should be treated as a source for nutrient supplement, mainly for nitrogen and phosphorus.

In many states in the US and throughout the world the use of reclaimed wastewater is spreading. Israel, Egypt, Mexico, Brazil are some of the countries which tend to use reclaimed wastewaters in agriculture.

The “raw sewage” and “municipal wastewater treatment plant effluent” is frequently used for irrigation in Turkey, both direct and indirectly. The largest volumes of wastewaters for use in agriculture are produced in Ankara, İzmir, Gaziantep, Kayseri and Eskişehir municipality wastewater treatment plants. In indirect use, such that occur in Ankara and Kayseri, effluents are first discharged to nearby streams and later stream waters are abstracted for use in agriculture. For example, 765,000m^3 of municipal sewage is treated daily at ASKİ Ankara wastewater treatment plant and discharged to Ankara Creek, which then is used to irrigate the surrounding fields, indirectly. However in İzmir and Gaziantep treated municipality effluents are directly used in irrigation.

All these examples of wastewater reuse in Turkey are not based on any sound scientific and health considerations. Wastewaters in agriculture may be a serious threat to the population since they contain variety of microbial pathogens, which may be transmitted to consumers through the crops irrigated. A wide variety of bacteria, viruses and parasites may be present in the domestic wastewater. Of these, parasitic eggs have a special place as these are extremely resistant to disinfection with chlorination and their minimal infective doses are very low, in the order of 1-10 cysts. A group of parasites occurring in domestic sewage is listed in Table 1.

Table 1. Parasites in Raw Wastewater

Organism	Clinic Feature	Disease	Number/liter
Giardia lamblia	diarrhea, malabsorption	Giardiasis	530 - 100,000
Cryptosporidium parvum	diarrhea	Cryptosporidiasis	5 - 5,180
Ascaris lumbricoides	diarrhea	Ascariasis	5 - 100
Trichuris trichiura	diarrhea, anemia	Trichiuriasis	41

Source: Modified from Yates, 2004, [1]

In general, parasitic cysts (the resting stage of the organism which is found in sewage) are larger in size than bacteria, although they can range in size from 2 µm to over 60 µm. Cysts are capable of surviving in the soil for months or even years, depending on environmental conditions.

Evidence supporting the spread of diseases through irrigation with reclaimed water is scarce. Although there have been many outbreaks associated with the consumption of contaminated fruits and vegetables, the source of the wastewater was not specified in any of those cases. Moreover, outbreaks that may have gone unrecognized or reported must undoubtedly exist. The mild disease symptoms associated with many

viral infections (i.e., short-lived gastroenteritis), which do not require medical intervention could have easily been overlooked in many of the outbreaks.

In conclusion, reclaimed water is likely to contain pathogenic microorganisms. Depending on the treatment processes used, the pathogens may be present in high enough concentrations to pose a potential threat to human health. Adverse impacts can be minimized by careful management of the irrigation practice to minimize public and worker exposure to the reclaimed waters and aerosols. [1]

CHAPTER 2

PURPOSE AND SCOPE OF THE STUDY

Although the health related criteria are extremely important in the reuse of treated wastewaters, yet there is insufficient accumulated information on this matter in Turkey. Intestinal nematode infections may be observed both in people who are working in the fields and in those who consume these products, and also in animals, which indirectly infect humans. There are currently no specific national standards for nematodes in reuse waters. The design of tertiary treatment facilities is therefore based on E.coli parameters in Turkey and elsewhere, as information on survival of parasitic eggs through the treatment processes is scarce in the literature.

Absence of a fixed, practicable method is a major problem in the constitution of the statistical data. The aim of this study was therefore restricted to determination of helminth eggs in raw sewage and in effluents of ASKİ municipal wastewater treatment plant in Ankara. The study involved examining to decide whether these organisms exist in the wastewaters at all, and if so in what concentrations.

CHAPTER 3

LITERATURE SURVEY

3.1. DISCHARGE CRITERIA

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 urbanization, population density, sanitary habits, season of the year, and rate of disease in the community.

There are considerable risks of human exposure to pathogens in irrigation with reclaimed wastewaters, but the health concern is proportional to the degree of human contact with the reclaimed water and the degree and efficiency of the wastewater treatment processes. In order to minimize health risks, tight controls should be enforced on the treatment and reuse of the reclaimed wastewater. Regulations are therefore set up to safeguard different cross sections of the population, *i.e*., starting with the agricultural workers down to the consumers.

3.1.1 Turkish Regulations

Turkish regulations encourage the use of treated wastewaters that ensure the quality criteria (Table 2) given in the "Technical Aspects Bulletin" for agricultural purposes, in the regions where the irrigation water is scarce and economically valuable. Wastewaters that are used for agricultural purposes are treated and examined according to the "Technical Aspects Bulletin" The appropriateness of a wastewater mass for the use of irrigation is defined by the common decision of "General

Directorate of State Water Works", "General Directorate of Provincial Bank" and the related departments of the "Ministry of Agriculture and Rural Affairs" [2].

Table 2 Turkish Water Reuse Standards in Crop Irrigation

Quality Criteria	Irrigation Water Class				
	Class I (very good)	**Class II (good)**	**Class III (usable)**	**Class IV (usable with caution)**	**Class V detrimental unusable)**
EC25 $\times 10^6$	0–250	250–750	750–2000	2000-3000	≥ 3000
Variable Sodium Percentage, % Na	≤ 20	20–40	40–60	60–80	≥ 80
SAR	≤ 10	10-18	18-26	≥ 26	
Sodium Carbonate Residue (RSC), meq/l mg/l	≥1.25 ≤ 66	1.25–2.5 66-133	≥ 2.5 ≥ 133		
Cl^-, meq/l mg/l	0–4 0–142	4–7 142–249	7–12 249–426	12–20 426–710	≥ 20 ≥ 710
SO_4^{-2}, meq/l mg/l	0–4 0–192	4–7 192–336	7–12 336–575	12–20 575–960	≥ 20 ≥ 960
Total Salts, mg/l	0–175	175-525	525-1400	1400-2100	≥ 2100
Boron, mg/l	0–0.5	0.5–1.12	1.12–2	>2	
NO_3^- or NH_4^+, mg/l	0–5	5–10	10–30	30–50	>50
Fecal Coliforms/100ml	0–2	2–20	20–100	100–1000	>1000
BOD_5, mg/l	0–25	25–50	50–100	100–200	>200
SS, mg/l	20	30	45	60	>100
pH	6.6–8.5	6.5–8.5	6.5–8.5	6.5–9	<6 or >9
Temperature	30	30	35	40	>40

Source: Water Pollution Control Regulation, Technical Aspects Bulletin, 1991, [2]

3.1.2 The USA Criteria

There are currently no federal standards for microorganisms or consistent treatment requirements in the US regarding the presence of pathogenic microorganisms in reclaimed water, thus each state may have its own rules and regulations governing reuse. Many states require the production of reclaimed water that is “pathogen (virus) free”, yet no data are available on enteric protozoan parasites and many new and emerging viruses are not identified or addressed [3].

3.1.3 Mexican Standards

Microbiological standards for wastewater reuse in Mexico for agricultural use have developed considerably over the last decade. The final revision of the microbiological standards came out in 1996, NOM-001-ECOL-1996, shown in Table 3. The standard establishes maximum permissible limits of contaminants in wastewater to be discharged into “national waters and soils". The standard, regardless of the discharge source, was designed to be achievable with the presently available technology and technology expected to be available in the near future. In order to be more realistic and to reduce the amount of monitoring required, the limits imposed with the standard were designed to protect "at-risk" groups. Evidently Mexican approach lacks a scientific base since no health risk assessment study is mentioned. The standard is aimed to reduce microbial and chemical contamination of rivers, lakes, aquifers and other water sources [4].

Table 3 Mexican Standard NOM-001-ECOL-1996 governing wastewater reuse in agriculture.

Irrigation	FC/100 ml (MPN)	Helminth egg /l
Restricted	$1000_m - 2000_d$	≤ 5
Unrestricted	$1000_m - 2000_d$	≤ 1

(m=monthly mean, d=daily mean)

Source: Peasey et al., 2000, [4]

Based on the Mexican and other recent studies, the following changes have been proposed in the Mexican standards for wastewater reuse in agriculture, Table 4.

Table 4 Proposed changes to Mexican Standard NOM-001-ECOL-1996

Irrigation	Mexican Standards		Proposed standards for Mexico		WHO Guidelines	
	FC/100 ml	egg/l	FC/100 ml	egg/l	FC/100 ml	egg/l
Restricted	$\leq 10^3$	≤ 5	10^3-10^4	0.1-1.0		≤ 1
Unrestricted	$\leq 10^3$	≤ 1	$\leq 10^3$	0.1-1.0		≤ 1

Source: Peasey et al., 2000, [4]

3.1.4 Israeli Criteria

Israel requires the water quality standards given in Table 5, for the application of treated wastewater to agricultural lands. Israeli standards are also concerned with the BOD content of the wastewater. The levels are important because if the BOD level is too high the likelihood of the solid matter produced plugging drip irrigators will be high [5].

Table 5 Israeli criteria for application of effluent for agricultural irrigation (1).

Constituents (mg/1)	Group A Cotton, sugar beet plants for seeds production	Group B Olives, peanuts, fruits where the skin is not eaten, grass for animals	Group C (2) Gardens, vegetables for cooking fruits for the conservation industry	Group D (3) Other plant (unlimited irrigation)
Effluent quality (4)				
BOD_5 - Total	60	45	35	15
BOD_5 - Filtered	-	-	20	10
Suspended Solids	50	40	30	15
Dissolved Oxygen	0.5<	0.5<	0.5<	0.5<
Coliform Counts/ 100 ml	-	-	250	12(80%) & 2.2(50%)
Residual Chloride	-	-	0.15	0.5
Mandatory treatment				
Sand Filtration (5)	-	-	-	Required
Chlorination - Minimal Contact Time, Minutes	-	-	60	120
Distance (m)				
Residential Areas	300 (sprinkling)	250	-	-
Paved Roads	30	25	-	-

Comments:

(1) The criteria are not limited to the treatment method; however, for an oxidation pond they should be adapted to a retention time higher than 15 days.

(2) Irrigation should be terminated two weeks before the pick-up season, and no fruits that fall on the ground should be taken.

(3) Observation and examination frequency in accordance with the irrigation season.

(4) The criteria should satisfied at least 80% of the cases.

(5) Sand or equivalent filtration.

(6) All examinations should be done in official governmental laboratories.

Source: Sammis et al., 2000, [5]

3.1.5 WHO Criteria

World Health Organization is concerned with health aspects of the management of water resources and publishes documents on the safety and of re-use water. In order to prevent the transmission of intestinal diseases, it has been recommended by WHO that; only treated wastewaters should be used for crop irrigation; and the treated wastewaters should comply with the microbiological quality guidelines given in Table 6 [6].

Table 6 WHO, Recommended microbiological quality guidelines for treated wastewater used for crop irrigation

Category	Reuse conditions	Exposed group	Intestinal nematodes[b] (arithmetic mean no. of eggs per litre[c])	Faecal coliforms (geometric mean no. per 100 ml[c])	Wastewater treatment expected to achieve the required microbiological quality
A	Irrigation of crops likely to be eaten uncooked, sports fields, public parks	Workers, consumers, public	≤1	≤1000[d]	A series of stabilization ponds designed to achieve the microbiological quality indicated, or equivalent treatment
B	Irrigation of cereal crops, industrial crops, fodder crops, pasture and trees[e]	Workers	≤1	No standard recommended	Retention in stabilization ponds for 8-10 days or equivalent **helminth** and faecal coliform removal
C	Localized irrigation[f] of crops in category B if exposure of workers and the public does not occur	None	Not applicable	Not applicable	Pretreatment as required by the irrigation technology, but not less than primary sedimentation

Source: World Health Organization, 1989, [6]

WHO states that wastewaters may be applied to land using flood irrigation technique but this method of irrigation should not be used for growing vegetables. Flood irrigation probably exposes field workers to the greatest health risk. WHO also states that, sprinkler irrigation should not be used on vegetables and fruits unless effluents meet the guideline for "Group A" in Table 6. Sub-surface or localized irrigation, with the surface covered with plastic mulch, gives the greatest degree of health protection when using treated wastewater [6].

3.1.6 Comparison of the International Standards

A comparison of international standards might help to develop guidelines for a reference area within each particular project. Table 7 summarizes some of the worldwide existing standards for reuse in agriculture. In many countries, like USA and Spain, only regional standards exist. 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 [7].

Table 7 Comparison of selected water quality guidelines/standards

Parameter	California Ca/T22 (1978)	US EPA (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 LAWA*
Minimum treatment required	Advanced	Advanced	Stabilization ponds	Secondary	Stabilization ponds	Tertiary		Stabilization 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 (/100 ml)		14 (which is not detectible)	1000			50	1000	1000		
Helminths (eggs/l)	-	-	1		<1	0		1		
Main treatment process	Oxydation Clarification Filtration Disinfection	Filtration disinfection	Stabilization ponds or equivalent	Long storage, disinfection	Stabilization ponds or equivalent	Filtration disinfection		Stabilization ponds or equivalent		

Source; Kretschmer et al. 2000, [7]

3.2. HELMINTH EGGS

The intestinal nematode infections are mostly due to *Ascaris lumbricoides* (the human roundworm) and *Trichuris trichiura* (the human whipworm) in the world [6]

Nematode eggs generally conform to the same pattern, with thin eggshells containing either an un-embryonated mass of cells, or a larvae.

***Ascaris lumbricoides*:** A micrograph of *A. lumbricoides* is presented in Plate 1 to illustrate the general pattern.

A. lumbricoides is a parasitic nematode (roundworm). The size of the *A. lumbricoides* eggs varies between 45 to 70 µm in length and 35 to 50 µm in width. Their shapes are usually ovoid and typically in 40 by 60 µm in size. A thick shell gives the egg a brown or yellow-brown color. The shell coat is sometimes lost and decorticated eggs have a colorless shell with gray or black internal material. Female adults lay fertilized or unfertilized eggs. Infertile eggs are not infective. They have elongated, occasionally triangular, kidney shaped or other strange forms. The eggshell is often very thin and opaque in the interior, almost totally black in color. The sizes are typically 90 µm to 40 µm, but may range between 85 to 95 µm by 35 to 45 µm, larger than fertilized eggs [8].

Plate 1 ***Ascaris lumbricoides*** **egg,** [9]

Trichuris trichiura: These have characteristic elongated barrel-shape with two opercular plugs at each end of the eggshell (Plate 2). While the eggs are yellow to brown in color, "plugs" are colorless. Eggs are generally 54 x 22 µm in size but may range between 49-65 x 20-29 µm. Rarely, larger eggs, as large as 78 X 30 µm are met [10].

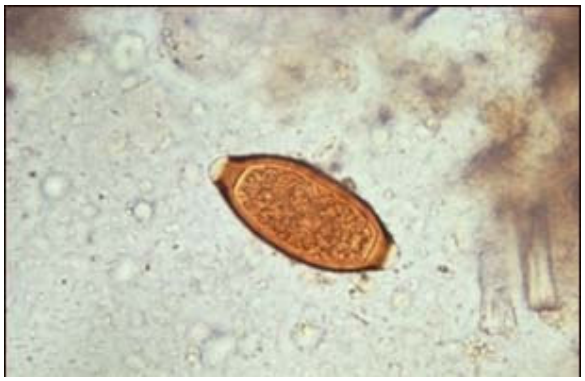

Plate 2 ***Trichuris trichiura*** **egg,** [11]

3.2.1. Significance of Ascaris and Trichuris Species in Human Health and in Wastewater Reuse

Humans worldwide are infected with *A. lumbricoides* and *T. trichiura*. The eggs of these roundworms (nematode) are "sticky" and may be carried to the mouth by hands, other body parts, fomites (inanimate objects), or foods. The egg of *A. lumbricoides* has a relatively thick shell wall, and is highly resistant to the external environment. Infections with *Ascaris* eggs are extremely common, with estimates of annual incidences of infections being greater than 1500 million cases or around one quarter of the world's population [12]. The Ascariasis and Trichuriasis are the scientific names of these infections. Ascariasis is also known commonly as the "large roundworm" infection and trichuriasis as "whip worm" infection. These infections are cosmopolitan, but ascariasis is more common in North America and trichuriasis in Europe. Relative infection rates on other continents are not available [13].

A. lumbricoides larvae may grow, as large as 31 X 4 cm. A vague digestive tract discomfort sometimes accompanies the intestinal infection, however in small children with more than a few worms there may be intestinal blockage because of the worms' large size. The larvae of ascarid species hatch in the human intestine and they migrate by venaporta to the tissues and locate in various organ systems of the human body, such as heart, lungs, etc., perhaps eliciting a fever and diverse complications.

The *Trichuris* sp. larvae do not migrate after hatching but molt and mature in the intestine. Adults are not as large as *A. lumbricoides*. Symptoms range from non-apparent through vague digestive tract distress to emaciation with dry skin and diarrhea (usually mucoid). Toxic or allergic symptoms may also occur.

The eggs of both worms are found in insufficiently treated sewage-fertilizer and in soils where they embryonate (i.e., larvae develop in fertilized eggs). The eggs may contaminate crops grown in soil or fertilized with sewage that has received improper treatment; humans are infected when such products are consumed raw. Infected food-

handlers may contaminate a wide variety of foods. Direct ingestion of the wastewater or aerosols created during spray irrigation may result in infection of workers. Another potential route of exposure is from the ingestion of ground water that has been contaminated by pathogens in irrigation water [1]. Target populations are particularly consumers of uncooked vegetables and fruits grown in or near soil fertilized with sewage. Eggs of *Ascaris* sp. have been detected on fresh vegetables (cabbage) [13].

3.2.2. Resistance of Helminth Eggs to Disinfectants

For wastewater reuse, the need for disinfection will depend on its intended uses. When reuse involves high-level risks of exposure for humans or livestock, that water will require high level of disinfection to achieve the sanitary levels.

WHO compiled a risk ranking of pathogens, reflecting the potential concentrations in wastewater, their resistance to treatment and infectious doses;

High risk: Helminths (for example, intestinal nematodes such as *Ascaris, Trichuris* and cestodes such as *Taenia, Hymenolepis*)

Lower risk: Bacteria (for example, those causing cholera, typhoid and shigellosis); Protozoa (for example, *Giardia, Cryptosporidium*)

Least risk: Viruses (for example, enteric viruses) [14].

Protozoa and helminths are generally found in lower numbers in wastewater compared to other pathogen groups. However, traditional disinfection methods such as chlorination are not particularly effective in reducing helminths and some protozoan numbers to acceptable risk levels. This reflects the resistance of these pathogens to treatment and their extremely low minimum infective doses.

Bacteria are the most common microbial pathogens found in wastewater. They are often used as an indicator of pathogen contamination and as a representative indicator to assess the efficiency of treatment and disinfection methods. However, as bacteria are generally the most sensitive group to disinfection and have high infective doses and they present a relatively low health risk. Although enteric viruses generally have a higher resistance to disinfection methods than bacteria, WHO estimates that the lowest pathogenic risk attached with these. Transmission of enteric viruses in the home gives early exposure and immunity to the viruses last for long periods compared with short to medium immunity for bacteria and little immunity for parasites [14].

Dessication:

Helminth eggs can survive in soil for long periods. The *Ascaris* eggs, for example, are the most resistant ones, which can survive for several years under suitable conditions. The eggs of *Trichuris* are more resistant than the *Taenia* and *Hymenolepis nana* eggs, and can survive for several months in the environment [15].

Temperature:

The most important factor affecting the survival of all helminth eggs is temperature, significant with rapid death resulting from temperatures below freezing and above 45 °C [16]. More thermally resistant organisms such as *Cryptosporidium* oocysts and *Ascaris* eggs have much longer survival times. However *Cryptosporidium* oocysts were inactivated more rapidly at all temperatures between 30º and 60º than were the eggs of *Ascaris*.

Chemical agents:

Pathogen reduction processes, such as aerobic digestion, composting, anaerobic digestion, lime stabilization and air-drying could inactivate *Cryptosporidium* oocysts but not *Ascaris* eggs. The main difference between the *Ascaris* egg and the *Cryptosporidium* oocyst is the outer shell. The *Ascaris* egg consists of three layers

that have been observed to be highly resistant to many chemical disinfectants. The *Crytposporidium* oocyst wall is thinner and appears less resistant than the *Ascaris* egg. Both of these organisms are susceptible to non-charged biosolids (ammonia released during alkaline stabilization), temperature, and cavitation processing (ultrasound or pulse power). In alkaline stabilization, it is the ammonia concentration that is the factor determining the effectiveness of alkaline agents to inactivate the helminth eggs and protozoan oocysts. In these conditions, *Ascaris* eggs require one magnitude greater concentrations of ammonia for inactivation than is required for the activation of oocysts, i.e., 1 to 3% ammonia for *Ascaris* eggs and less than 0.1% ammonia for oocysts [15].

3.2.3. Effectiveness of Treatment Technologies in Removing Helminth Eggs from Wastewaters

Treatment of wastewater can effect from 50% to almost 100% pathogen removal, depending on the treatment processes used. A summary of average pathogen concentrations reported to be present after several stages of sewage treatment is presented in Table 8. It can be seen that even tertiary treatment (consisting of primary sedimentation, trickling filter/activated sludge, disinfection, coagulation, direct filtration, and chlorination) does not remove all pathogens. It is important to consider the infective dose of the organism in relation to the final concentration when assessing the potential public health risk associated with use of reclaimed water. It is relatively unlikely that the two Salmonella cells would cause disease, considering that the infective dose is more than 1000 organisms. On the other hand, the final concentrations of viruses, such as Hepatit A, and Giardia are sufficiently high to cause several people to become ill if they ingest the water [1].

Table 8. Pathogen Removal by Wastewater Treatment

Effluent	Viruses #/liter	Salmonella #/liter	Giardia #/liter
Raw	500,000	42,500	104,500
Primary	129,250	935	59,405
Secondary	117,700	288	30,462
Tertiary	42	2	784
Infective Dose	1	>1,000	25 - 100

Source: Yates, 2004, [1]

Effects of the disinfection methods vary widely on the different species, present in the wastewaters. Table 9 and Table 10 gives the comparison of disinfection methods and other treatment processes for the removal of pathogens in the wastewaters.

Table 9 Comparison of Disinfection Methods

Disinfectant	Chlorine	Ozone	UV	Micro-filtration	Detention Lagoons
Effectiveness against					
Bacteria	High	High	High	High	Medium-High
Viruses	Low to Medium	High	High	Low to Medium	High if detendion >14 days
Parasites	Low	High	Not fully assessed	High	High if detendion >30 days

Source: Modified from US EPA, 1992, [14]

Table 10 Expected removals of enteric pathogenic microorganisms in various wastewater systems

Treatment process	Removal (log10 units)			
	Bacteria	**Helminth**	**Viruses**	**Cysts**
Primary Sedimentation				
Plain	0-1	0-2	0-1	0-1
Chemically Assisted	1-2	1-3	0-1	0-1
Activated sludge	0-2	0-2	0-1	0-1
Biofiltration	0-2	0-2	0-1	0-1
Aerated lagoon	1-2	1-3	1-2	0-1
Oxidation ditch	1-2	0-2	1-2	0-1
Disinfection	2-6	0-1	0-4	0-3
Waste stabilization ponds	1-6	1-3	1-4	1-4
Effluent storage reservoirs	1-6	1-3	1-4	1-4

Source: Westcot., 1997, [16]

The most effective and also the least costly method seems to be detention processes, especially on the removal of helminth eggs in wastewater systems (Figure 1). However the detention period should be optimized for the efficacy of the process.

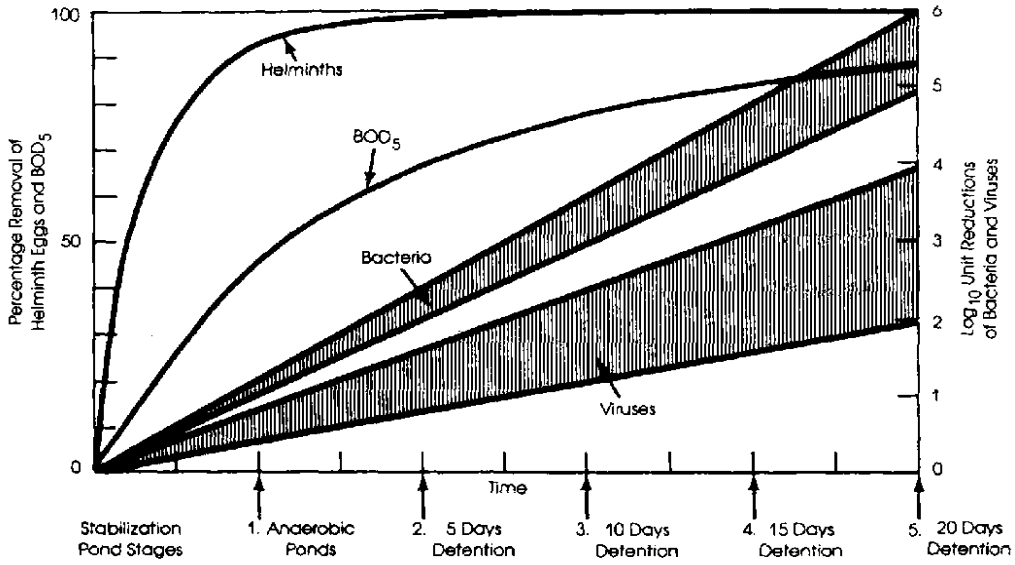

Figure 1 Generalized removal curves for BOD, helminth eggs, excreted bacteria, and viruses in waste stabilization ponds at temperatures above 20°C, [16]

3.3. PROTOZOA CYSTS

Giardia lamblia: The *Giardia lamblia* is the most commonly isolated intestinal parasite in the world. *Giardia* cysts, (Plate 3) are also present in high numbers in domestic sewage and are of particular concern due to their inherent resistance to disinfectants commonly used in treatment processes. Outbreaks of *Giardia* are associated with unfiltered, inadequately chlorinated water.

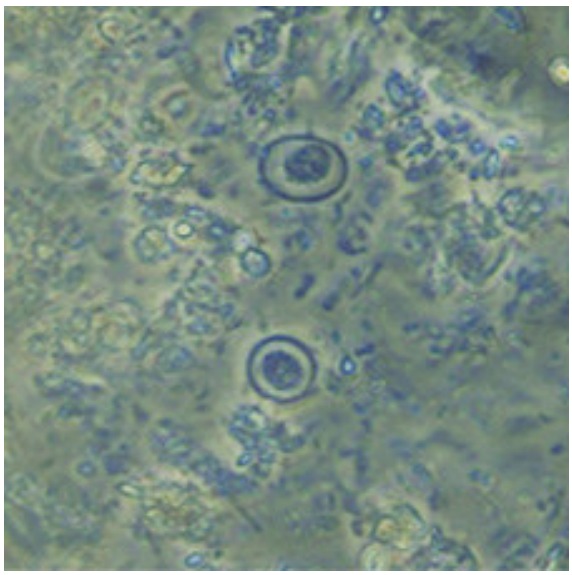

Plate 3 *Giardia lamblia* cysts, [17]

Cryptosporidium parvum: *Cryptosporidium parvum* is an intestinal parasite found worldwide. The oocysts, (Plate 4 and 5), are widely distributed in both sewage and drinking water. As with *Giardia* spp., the *Cryptosporidium* spp. oocyst is resistant to the levels of chlorine commonly used in water treatment [3].

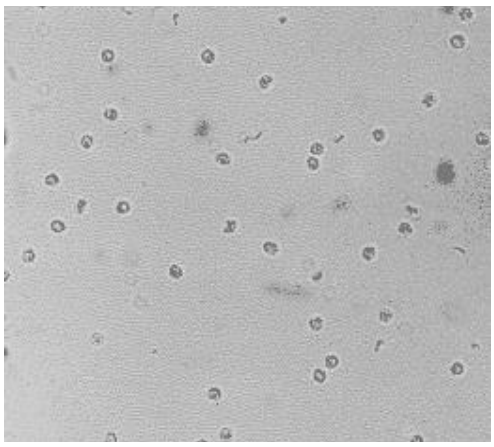

Plate 4 *Cryptosporidium parvum* oocysts and sporozoites in wet mount preparation, [18]

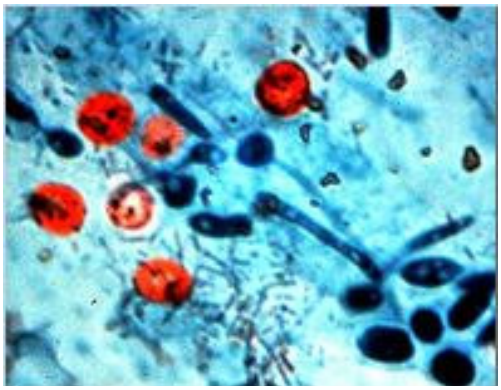

Plate 5 Stained *Cryptosporidium parvum*, [19]

3.3.1. Significance of Giardia and Cryptosporidium in Human Health and in Wastewater Reuse

Giardia lamblia infects the intestinal tract and can result in a variety of symptoms, such as chronic diarrhea, bloating, abdominal cramps, frequent greasy malodorous stools, fatigue, and weight loss. *Giardia* is transmitted by fecal/oral route. Giardia cysts are elliptically shaped and range in size from 7 to 14 microns [20]. They can be killed by long contact with chlorine during disinfection. The cyst stage is the form that can be found in the environment [21].

Cryptosporidium parvum is now one of the most commonly identified intestinal pathogens throughout the world. It affects humans and animals. It is shed in the feces in the form of an "oocyst". Oocyst is the environmentally resistant transmission stage of this parasite. It may remain in the environment for very long periods without loss of infectivity: A very robust oocyst wall protects the sporozoites inside against physical and chemical damage. Cryptosporidium oocyst is round and approximately 3-4 microns in diameter, but can fold to an even smaller size [22]. Occurrence of *C. parvum* infection is dependent on factors that include season, age and other demographic characteristics of a population. Among children aged 1 – 5 years with diarrhoea, *C. parvum* may be the most frequently found pathogen [23].

Infections may be asymptomatic or may cause watery diarrhea and abdominal cramps. The organism is transmitted by fecal-oral route. Outbreaks have most commonly been associated with person-to person (day care center) and waterborne (drinking water) modes of spread. Food-borne and animal (especially calves) to person spread has also been documented.

Infection with the *Cryptosporidium* organism may also contribute to the premature deaths of immunosuppressed individuals in outbreaks. Ordinary water disinfection methods cannot kill *Cryptosporidium* oocysts, and even the best filtration units may allow a few organisms to pass through in treated water [24].

3.3.2. Resistance of Protozoa Cysts to Disinfectants

As part of their life cycle, protozoa often have an environmentally resistant stage. Cysts survive over a wide range of pH values and are resistant to osmotic pressures. *Cryptosporidium*, for example, forms an oocyst that aids in its environmental survival; *Cryptosporidium* oocysts may survive for over twelve months in isotonic solutions in the laboratory [25]. Although susceptible to freezing and drying, oocysts can remain infectious for more than a year in aquatic environments. An overview of survival times for some enteric pathogens in water and sewage is presented in Table 11.

Table 11 Survival times of pathogens in water and sewage at 20–30ºC

Pathogen	**Survival time in fresh water and sewage (days)**
Viruses *	
Enteroviruses	<120 but usually <50
Bacteria	
Faecal coliforms *	<60 but usually <30
Salmonella spp *	<60 but usually <30
Shigella spp. *	<30 but usually <10
Vibrio cholerae **	<30 but usually <5
Protozoa	
Entamoeba histolytica cysts	<30 but usually <15
Cryptosporidium oocysts	>12 months
Helminths	
Ascaris lumbricoides eggs	Many months
* In seawater, viral survival is less and bacterial survival is very much less, than in freshwater. ** V. cholerae survival in aqueous environments is uncertain.	

Source: Westcot., 1997, [16]

An overview of survival times of some enteric pathogens in soil is presented in Table 12.

Table 12 Survival time of pathogens in soil at 20–30ºC

Pathogen	Survival time in soil (days)
Viruses	
Enteroviruses	<100 but usually <20
Bacteria	
Faecal coliforms	<70 but usually <20
Salmonella spp.	<70 but usually <20
Vibrio cholerae	<20 but usually <10
Protozoa	
Entamoeba histolytica cysts	<20 but usually <10
Cryptosporidium oocysts	>12 months
Helminths	
Ascaris lumbricoides eggs	Many months

Source: Westcot., 1997, [16]

Disinfection, is applied as the final stage in water treatment to inactivate the waterborne microbial pathogens, such as bacteria, virus, and protozoa. Microorganisms, however, differ greatly in their sensitivity to disinfectants. The following discussion on water disinfection, categorized by individual oxidants, summarizes the microbial inactivation research, which has been conducted or sponsored by EPA during the time period from 1980 to 1999 [26].

Chlorine: Chlorination is the most frequently used form of halogen disinfection for both drinking water and wastewater applications in Turkey. Being an oxidizer, chlorine typically destroys the outer membrane of the bacterial cell and renders it noninfectious. The free chlorine has been the major choice of disinfectant for municipal water treatment. This oxidizer is highly effective against most bacteria but only moderately effective against cysts and oocysts.

Protozoan Inactivation: Using an in vitro excystation technique to determine cyst viability, *Giardia* cysts exhibited resistance to free chlorine. Inactivation levels were determined after exposures to chlorine for 10, 30, or 60 minutes at different pH levels and temperatures. Inactivation of greater than two orders of magnitude was reported after 10 minutes of exposure to 1.5 mg/L of chlorine at pH 6.7, at 25 ^{o}C. Similar inactivation occurred after exposure to 1.5 mg/L of chlorine at pH 6 at 15 ^{o}C. An exposure time of 60 minutes at 5 ^{o}C, 2 mg/L of chlorine at pH 6 and 7 was required to achieve a greater than two order of magnitudes of inactivation. This report confirmed that inactivation rates are slower with decreasing temperature [27].

Chloramine: The use of monochloramine in drinking water treatment has gained increasing popularity as concerns have grown regarding adverse health effects attributed to chlorine disinfection by-products. Monochloramine is considered a weak biocide in comparison to free available chlorine, requiring exposure times of 25 to 100 times greater than chlorine to achieve comparable inactivation. The efficacy of disinfection for monochloramine is pH dependent and increases with decreasing pH values [28].

Protozoan Inactivation: ***M***onochloramine is the least effective disinfectant compared to free available chlorine, chlorine dioxide, and ozone for inactivating *Cryptosporidium parvum* oocysts. At 5 ^{o}C, an exposure to 80 mg/L of monochloramine for 90 minutes was required to achieve a one order of magnitude inactivation [28].

Chlorine Dioxide: Chlorine dioxide exists as an undissociated gas dissolved in water in the pH range from 6 to 9, and the disinfection efficiency increases within this range with increasing pH. Existing as an undissociated gas makes this oxidant more vulnerable to volatilization than free chlorine or monochloramine. Chlorine dioxide is a relatively stable disinfectant and is less likely to react with oxidant demand substances than free chlorine. Chlorine dioxide is a potent disinfectant and is

generally considered to have a biocidal efficiency greater than free chlorine or monochloramine [29].

Protozoan Inactivation Owens et al. (1999) compared three bioassay procedures for determining the viability of *Cryptosporidium parvum* oocysts exposed to chlorine dioxide. It was found that the biocidal activity of chlorine dioxide was pH dependent, with better inactivation occurring at higher pH levels [30].

Ozone: Ozone represents the most potent biocide examined in disinfection studies; it is also very unstable and highly reactive. Like chlorine dioxide, ozone exists in water as a dissolved gas. It is subject to losses due both to volatilization and reactions with demand substances present in the water. It would appear from most studies that ozone is relatively unaffected by pH within the range normally encountered in water treatment. Maintaining stable ozone levels is very difficult. Concentration of disinfectant, especially in pilot-plant studies, is often determined by averaging dissolved ozone residual measurements collected at various points in the ozone contactor. The average ozone concentration, C_{avg}, is then multiplied by the mean exposure time, T_2, to determine the product of the concentration (C) and exposure time (T) (CT) value (mg min/L) [31].

Protozoan Inactivation Encysted forms of protozoa represent of the most difficult forms of microorganisms to inactivate by disinfection. Consequently, the use of ozonation has gained popularity as a means of inactivating these organisms. Ozone was found to be effective in the inactivation of *Giardia* cysts [32]. The average CT value for a two order of magnitude inactivation at 5 ^{o}C, pH 7, was 0.55 mg min/L for *G. lamblia* cysts. For the same conditions at 25 ^{o}C, the mean CT value for *G. lamblia* was 0.17 mg min/L. In a pilot-scale ozonation study, Miltner et al. (1997) reported that at pH 7.65, 23 to 24 ^{o}C in ORW, a CT value of 0.75 mg min/L was required to

achieve two orders of magnitude inactivation of *G. muris* cysts as expressesd in **Figure 2** [32].

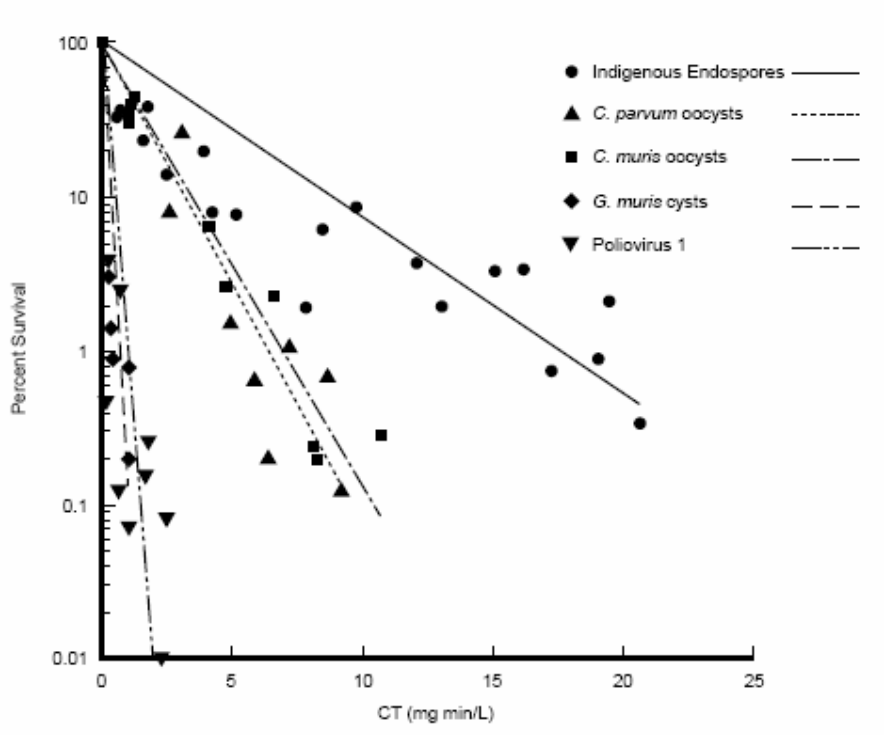

Figure 2 Comparison of inactivation of microbial populations exposed to ozone in filtered ORW. Temperatures = 23.6 to 25.2 °C, [32]

Ozone is one of the few chemical oxidants that has been shown to be capable of inactivating *Cryptosporidium* spp. under normal water treatment conditions Korich et al. (1990) demonstrated 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 min/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 [28]. Similar results for a 2.5 order of magnitude inactivation (CT value of 6.56 mg min/L) at 20 ^{o}C were reported for *C. parvum* [33]. In a pilot-scale

study using ORW, a CT value of 4.0 mg min/L inactivated approximately 1.4 orders of magnitude of *C. parvum* oocysts at pH 7.6, 23 to 24^{o}C [31].

Ultraviolet (UV) Irradiation: In a study about the effect of UV irradiation on *Giardia lamblia* cysts, the cysts were found to be resistant to high doses of germicidal UV irradiation [34]. There has been renewed interest in the use of UV light for inactivating waterborne protozoan parasites. Recent studies suggest that UV irradiation is an effective treatment option for inactivating oocysts of *Cryptosporidium* [35]

Concludingly; the ideal disinfectant hasn't yet been developed. The variable nature of microbial populations and their ecological habitat alter the efficacy and predictability of the disinfection process. The resistance of the microorganism depends on the power of the disinfectant, presence and magnitude of organic matter, turbidity, excessive numbers of organisms, exposure times, concentration of disinfectant, pH, temperature, and water hardness [36].

3.3.3. Effectiveness of Treatment Technologies in Removing Protozoa Cysts from Wastewaters

Water treatment facilities rely on filtration technology to remove the small protozoa, such as *Giardia Lamblia* cyst (6-8 μm), which are largely unaffected by chlorine. Filtering of water should also be effective in removing *Cryptosporidium* oocysts (3-4 μm). Reverse osmosis membranes and other point-of-use filters with an "absolute" (not "nominal") pore size of one micron or smaller will remove oocysts. The pore size of microfiltration is still too small for oocysts to pass through.

CHAPTER 4

MATERIALS AND METHODS

Several methods are available for the concentration of helminth eggs in wastewater prior to identification, however each method differs with respect to their advantages and disadvantages, such as, percentage recovery, cost, time consumed, reproducibility etc. These methods usually are based on one of two principles, *i.e.* floatation or sedimentation. The parasitic eggs are separated from underlying debris by flotation in a solution of comparatively high relative density. Whereas, in sedimentation, underlying material is first treated with a solvent such as ether or ethyl acetate, then centrifuged. The eggs are then separated in an interphase solution (normally) while the parasitic eggs sediment into a non-miscible layer, below. Both processes rely on centrifugal force.

4.1 CONCENTRATING HELMINTH EGGS AND PROTOZOA CYSTS IN ENVIRONMENTAL SAMPLES

Bouhoum & Schwartzbrod (1989) adapted a range of fecal analysis methods to wastewater samples and compared them [37]. They concluded that Bailenger's method, which was modified for wastewater, was the best method overall. It requires relatively inexpensive reagents and successfully concentrates the full range of species routinely found in wastewater [6]. World Health Organisation (WHO) published the modified Bailenger's method as "WHO Laboratory Manual of Parasitological and Bacteriological Techniques" to be used for the examination of treated wastewater samples to determine their compliance status with the guideline values given in Table 4.

The United States Environmental Protection Agency (U.S. EPA) developed the "Information Collection Requirements Rule (ICR)" in 1992. The most critical

element of the ICR involves collection of data on the concentrations of specific microbes, such as *Cryptosporidium*, *Giardia* and total cultivable viruses that are monitored in the surface waters, which are being used for potable water supply. The U.S. EPA published "ICR Microbial Laboratory Manual" which describes specifically the detection and enumeration of *Giardia* cysts and *Cryptosporidium* oocysts in ground, surface, and finished waters by a fluorescent antibody procedure [38].

Both the "WHO Laboratory Manual of Parasitological and Bacteriological Techniques" and "U.S.EPA ICR Microbial Laboratory Manual" were used in developing the specific methods used in this study.

4.1.1 Who Gravimetric Method (The Modified Bailenger Method)

The required reagents: zinc sulfate solution (33%, relative density 1.18); ethyl acetate (or ether); acetoacetic buffer (pH 4.5) (15 g sodium acetate trihydrate, 3.6 ml glacial acetic acid, made up to 1 liter with distilled water); 1 % Tween 80 detergent solution (or Triton X-100).

Materials: 1-5 L PET bottles for sample collection; centrifuge tubes; Pasteur pipettes and teats, measuring cylinder or graduated pipette,

Equipments: Centrifuge, microscope, vortex mixer, siphon;

Procedure: The method can be applied to both raw and treated wastewaters The following steps are described for raw wastewaters However it can also be used for treated wastewaters if the sample size is increased.

Sample collection and analysis: Sample volumes are 1 liter for raw wastewater and 10 liters for treated or partially treated wastewaters. The samples are analyzed within 24 hours after collection. If an immediate analysis is not possible, samples are kept in +4 °C in refrigerator. Freezing is avoided because helminth eggs are damaged in low temperatures.

Sample bottles are left for sedimentation for 1-2 hours, depending on the height of the bottle. Sedimentation time is calculated according to Stoke's Law. *Ascaris lumbricoides* egg settle down by a rate of 20 mm/min and *Trichuris trichiura* egg's settling rate is 16 mm/min at 20 °C. Supernatant is removed with a siphon until about 1/5 of the water is left over the sediment. At the end of the transfer processes, the container is rinsed with detergent solution and the rinsing solution is added to the collectate.

The sediment is centrifuged at 1000 ×g. Each centrifugation lasts for 10-15 minutes. Supernatants are removed carefully and pellet is collected in a single tube. The final product is centrifuged once more to remove the rinsing liquid.

The volume of pellet is measured. If the volume is more than 2 ml than the pellet is suspended in an equal volume of acetoacetic buffer. If the volume is less than 2 ml, volume is completed to 4 ml with acetoacetic buffer, because the total volume should be at least 4 ml to have an efficient phase separation in the next step. The solution is shaken by hand or mixed by vortex mixer.

Two volumes of ethyl acetate is added on the solution and mixed again. This solution is centrifuged at 1000 ×g for about 15 min. This process separates the sample into three distinct phases. The bottom phase contains heavy debris and helminth eggs, larvae and protozoa are trapped in the mid phase. Clear buffer is collected at the top, which is ethyl acetate layer. The fatty and other material moves into the ethyl acetate layer and forms a thick dark plug at the top of the sample. The plug is separated from the tube wall by running a needle around the side of the centrifuge tube. Then the supernatant is poured off smoothly without disturbing the bottom pellet. The volume of pellet is measured. The pellet is suspended in five volumes of $ZnSO_4$ solution. The volume of final product is recorded and is used to calculate the egg concentration. The solution is mixed thoroughly. An aliquot is taken quickly with a Pasteur pipette and transferred onto microslide for the last step.

Half a drop of Logul's Iodine solution may be added onto the microslide to enhance visualization. The drop is covered by a cover slip and mounted by nail polish and/or Canadian balsam to preserve the sample for further examination under microscope. The sample slide is then examined under microscope at 10×, 25× and 40× magnifications. A number of slides, usually three, are examined for added accuracy.

Calculation:

the number of eggs per liter is calculated from;

$$N = {}^{AX}\!/_{PV} \quad \text{.....Equation 1}$$

where:

N = number of eggs per litre of sample

A = number of eggs counted on the slide or the mean of counts from slides

X = volume of the final product (ml)

P = volume of the drop on the slide (ml)

V = original sample volume (liters)

Since a single drop is 0.05 ml. We assume that a half-drop of sample is placed on the slide so the volume "P" is 0.025 ml.

4.1.2 EPA Filtration Method

ICR Microbial Laboratory Manual

This test method provides a quantitative indication of the level of contamination in raw and treated drinking waters with the environmentally resistant stages of two genera of pathogenic intestinal protozoa: *Giardia* and *Cryptosporidium*.

With this method, the protozoa are concentrated from a large volume of water sample by retention on a yarn-wound filter. Retained particulates are eluted from the filter with an eluting solution and are concentrated by centrifugation. The *Giardia* cysts and *Cryptosporidium* oocysts are separated to some extent from other particulate debris by flotation on a Percoll-sucrose solution with a specific gravity of 1.1. [38]

After this step the method continues with Indirect Fluorescent Antibody (IFA) procedure. A monolayer of the water layer/Percoll-sucrose interface is placed on a membrane filter, indirectly stained with fluorescent antibody, and examined under a microscope and total IFA count is reported.

IFA stage is not applied in this study due to financial inadequacies.

Apparatus Used

The apparatus used in this study for sampling helminth eggs from wastewaters, which was adapted from EPA ICR Microbial Laboratory Manual, is shown in Figure 3 and 4.

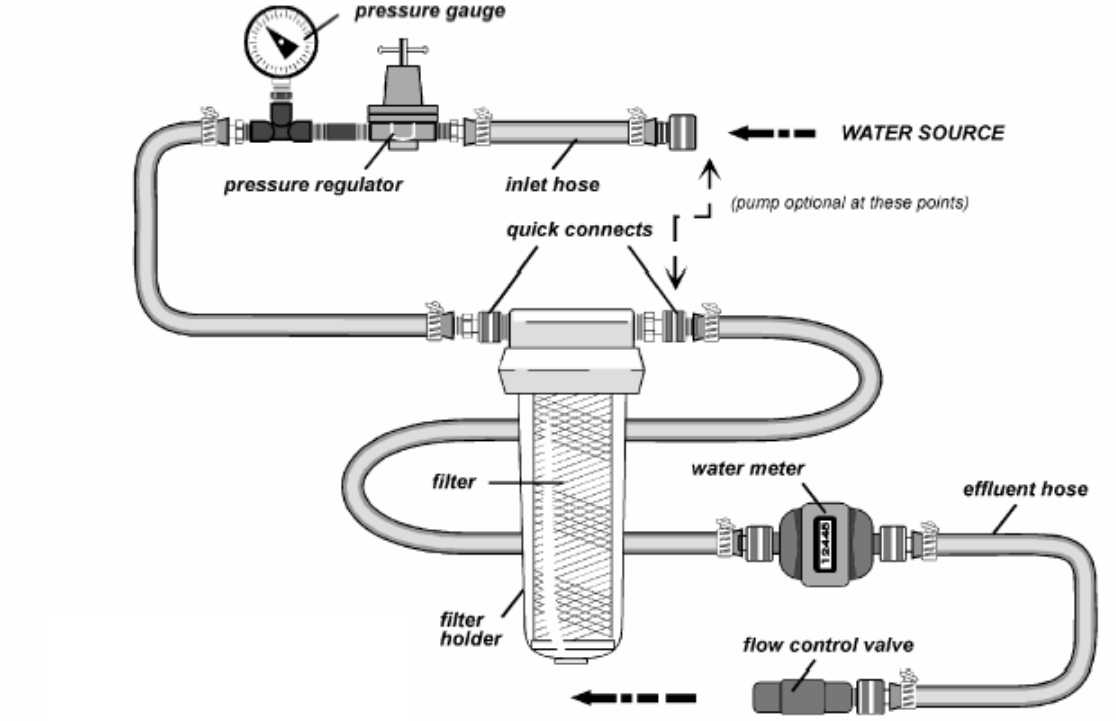

Figure 3 Apparatus designed for the concentration of parasite eggs and cysts suggested by EPA, [38]

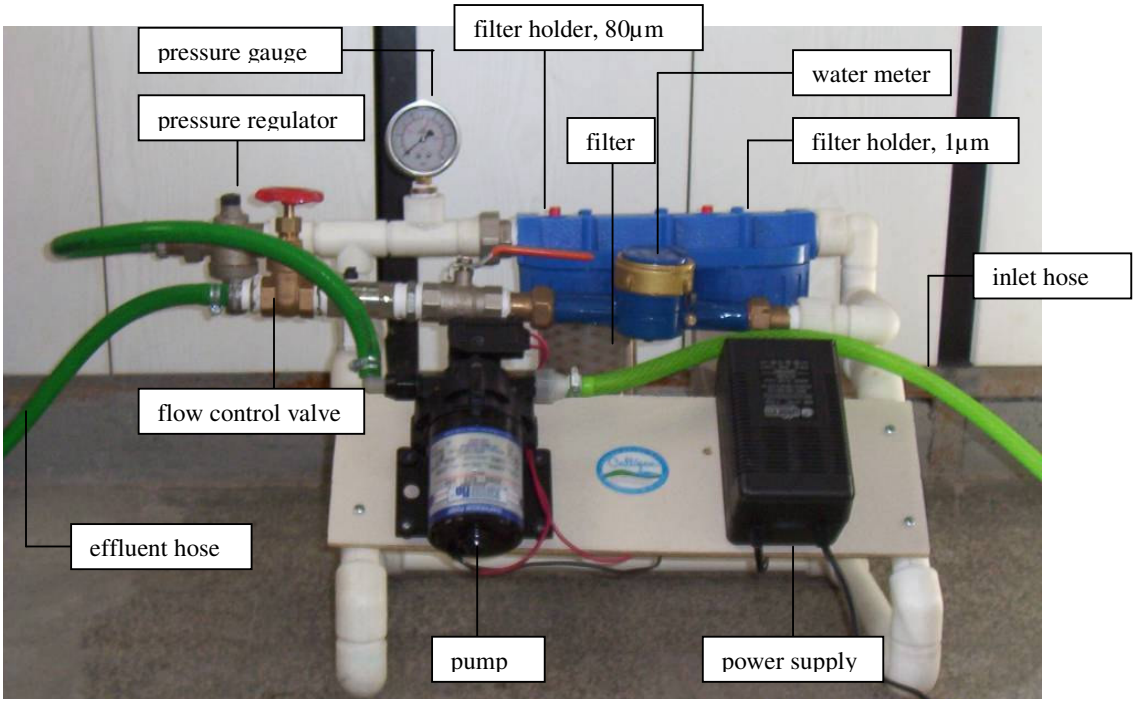

Figure 4 Apparatus assembled for the concentration of helminth eggs and cysts.

The filter was a 25.4 cm (10 inch) long having 1 µm nominal porosity, and yarn-wound polypropylene cartridge. Flow control valve was adjusted to 4 L/min. A pump was employed for sampling un-pressurized water sources. Other equipments used for sample processing were: a glass tray for filter washing, a knife/cutting tool, for cutting the polypropylene filter fibers, a centrifuge, with swing-out bucket rotors and a vortex.

An additional filter holder, in series with the first one, was added to the apparatus. A pre-filter with 80 µm porosity was placed into the first filter holder to capture coarse particles in the wastewater. Wastewater sample filtered through the pre-filter then passed through the 1 µm filter.

Other equipment which was used during preparation and processing of the micro slide mounts were a microscope, cover slips, fingernail polish, clear fixative are reagents.

<u>The reagents are:</u>

1. Neutral Buffered Formalin Solution (10 %)

2. Phosphate Buffered Saline (PBS) –

3. Sodium Dodecyl Sulfate Stock Solution (1%) –

4. Tween 80 Stock Solution (1 %)

5. Eluting Solution (Buffered Detergent Solution) - Prepared by the following reagents; 100 mL 1% SDS, 100 mL 1% Tween 80, 100 mL 10X PBS, and 0.1 mL Sigma Antifoam A are mixed with 500 mL water. pH is adjusted to 7.4 using a pH meter. The final volume was completed to 1 L with additional water. This solution has a shelf life of one week. At least 3 L of eluting solution is required for each filter elution procedure.

6. Sucrose Solution (2.5 M).

7. Percoll-Sucrose Flotation Solution, Sp. Gr. 1.10 – A 45 ml Percoll was mixed with 45 ml water and 10 ml 2.5 M sucrose solution. The specific gravity should be between 1.09 and 1.10. Flotation Solution was stored at 4 °C and used within a week. The reagents were allowed to reach room temperature before use.

Sampling: Prior to sampling, the apparatus was rinsed with 20 gal (76 L) of sample water. In the meanwhile, the pressure regulator was adjusted so that pressure gauge reads no more than 30 pounds per square inch (PSI), (2 Bars). When flushing of the apparatus was complete, the filter housing was emptied. A fresh filter cartridge was then placed into the holder and 100 L (26.4 gal) of treated wastewater was passed through the filter with suction.

The residual water remaining in the filter holder and the filter are put the into a sample bag and transported to the laboratory. When the filter(s) arrived at the laboratory, they were immediately stored at 2-5 °C. The initiation of sample collection and elution from the collection filter was performed within 96 hrs.

Washing By Hand: Filter is removed from the sample bag and placed into a glass tray. The residual solution was poured into a pooling beaker. Using a razor knife, filter fibers were cut lengthwise down to the core. Blade was discarded each time after the fibers were cut. The filter fibers were divide into a minimum of six equal portions with one-sixth consisting of those cleanest fibers nearest the core; until the final one-sixth consisting of the outer-most, the dirtiest, filter fibers.

Beginning with the cleanest fibers (the one-sixth nearest the core), the fibers were washed in three consecutive 1.0 L volumes of eluting solution, by kneading them in the eluting solution. The elution process was subsequently continued with the remaining fiber fractions. The minimum total wash time for each fiber lot was 30 min. After all the fibers have been washed, the three 1.0 L volumes of eluate were

combined with the residual filter water in the pooling beaker, and fibers were discarded.

Eluate Concentration: The combined eluate and residual water were concentrated into a single pellet by centrifugation at 1,050 ×g for 10 min using a swing-out bucket rotor and plastic conical centrifuge bottles. The pellet was re-suspend in sufficient elution solution by mixing vigorously on a vortex mixer. After pooling all the pellets in one conical bottle, a final centrifugation was applied at 1,050 ×g for 10 min and the packed pellet volume was recorded. The Pellet was re-suspend in an equal volume of 10% neutral buffered formalin solution by vortexing. If the packed pellet volume was less than 0.5 ml, enough eluting solution was added to bring the pellet solution volume to 0.5 ml by adding enough 10% buffered formalin solution. The final re-suspended pellet volume should be 1.0 ml. At this point, a break could be inserted if one was not going to progress immediately the next steps. If a break is inserted at this point, the formalin treated sample was stored at 4 °C for not more than 72 hours.

Flotation Purification: In a clear plastic 50 ml conical centrifuge tube(s), a 0.5 ml volume of re-suspended pellet of packed pellet was vortexed with sufficient volume of eluting solution to make a final volume of 20 ml. By using a 50 ml syringe and 14-gauge cannula, a 20 ml of vortexed suspension of particulates were underlayed with 30 ml Percoll-sucrose flotation solution (sp. gr. 1.10). The preparation was then centrifuged at 1,050 ×g for 10 min. taking care not to disturb the pellet suspension/Percoll-sucrose interface.

Using a 25 ml polystyrene pipette, rinsed with eluting solution, the top 20 ml of particulate suspension layer, the interface, and 5 ml of the Percoll-sucrose below the interface, were drawn off. All these volumes were then placed into a centrifuge tube. Additional eluting solution was added to the plastic conical centrifuge tube to make up a final volume of 50 ml and centrifuged at 1,050 ×g for 10 min.

The volume of pellet was measured. If the volume was less than 2 ml, the volume was completed to 4 ml with acetoacetic buffer. Else if the volume was more than 2 ml, than the pellet was suspended in an equal volume of acetoacetic buffer. The solution was shaken by hand or mixed by vortex mixer. Two volumes of ethyl acetate was added on this solution and mixed again. This product was centrifuged at 1000 ×g for about 15 min. This process separates the sample into three distinct phases. The bottom phase contains heavy debris, helminth eggs, larvae and protozoa, in the mid phase clear buffer was present and top phase was the ethyl acetate layer. The fatty and other material moves into the ethyl acetate layer and forms a thick dark plug at the top of the sample. The plug was separated from the tube wall by running a needle around the side of the centrifuge tube. Then the supernatant was poured off smoothly without disturbing the bottom pellet. The volume of pellet was measured.

The pellet was suspended in five volumes of $ZnSO_4$ solution. The volume of final product was recorded and was used to calculate the egg concentration. The solution was mixed thoroughly. An aliquot was taken quickly with a Pasteur pipette and transferred onto a micro-slide for the last step. In this step, half a drop of Logul's Iodine solution may be added onto the sample to enhance the visualizing. The drop was covered by a cover slip for inspection under microscope. The cover slip was mounted by sealing the edges by a nail polish and/or Canadian balsam strip, in order to preserve the sample for further examinations. The slide mounts were examined by using an optical microscope, under 10×, 25× and 40× magnifications. For greater accuracy, always more than one slide, preferably three were examined in parallel.

4.2 SAMPLE COLLECTION

It is known that municipal wastewater treatment plant effluents are used in Ankara for indirect irrigation. In indirect use, effluents are first discharged to nearby streams and later stream waters are abstracted for use in agriculture. For example, daily

765,000m^3 municipal sewage is treated at ASKİ Ankara wastewater treatment plant and discharged to Ankara Creek, which then is used to irrigate the surrounding fields.

The Ankara Central Wastewater treatment plant (ACWTP) has been taken over by (ASKİ) in the year 2000. The plant is serving for four million equivalent populations at the moment and is designed to serve for six million equivalent populations by the year 2025 [39].

The plant has been constructed at the west side of the city, 45km far from the center. The plant is placed on the downstream of Ankara Creek nearby Tatlar village, as seen in Figure 5. Activated sludge, anaerobic sludge stabilization, and belt filtered mechanical sludge dewatering processes are being practiced in the plant, (Figure 6).

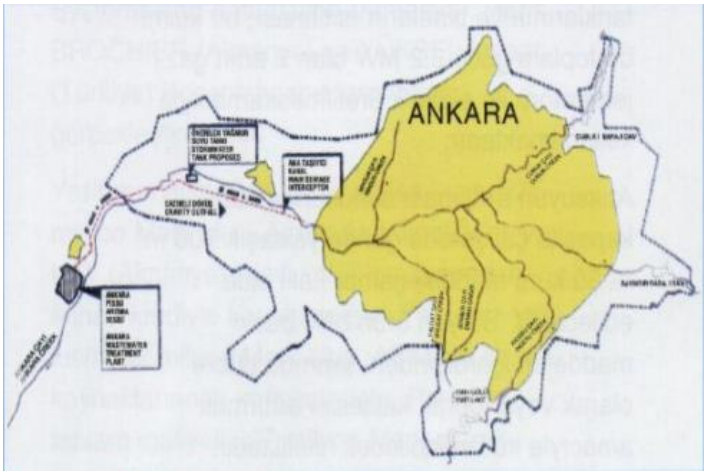

Figure 5 Location of ASKİ municipal wastewater treatment plant

Figure 6 General layout of the treatment plant

The pretreatment stage of the process consists of coarse and fine screens, and in grit chamber. The debris, such as paper, wooden particles, tin cans, plastic materials, sand and grease etc. is being removed in the stage. The remains are sent to the municipal solid waste disposal site. The influent samples were taken from the outlet of the grit chamber, (Figure 7), after the massive garbage had been removed.

Figure 7 Grit chamber

The discharged waters from the grit chambers are directed to the primary sedimentation tanks, shown in Figure 8. Suspended organic and inorganic solids are removed in primary settling process. The settled particles are sent to the raw sludge

thickeners. An efficient settling tank is able to remove 60-70 % of the suspended solids. The retention time in primary sedimentation tank is 1.5 hrs and the depth of the tank is about 4 m. According to the stokes law, *Ascaris lumbricoides* egg settle down at a rate of 20 mm/min at 20 °C. Settling rates of *Trichuris trichiura* and hookworms eggs are 16 mm/min and 6 mm/min respectively [6]. A simple calculation concludes that helminth egg removal cannot be effective in primary settling process. Consequently discharge of the primary sedimentation tank was collected as pre-treated water sample.

Figure 8 Primary sedimentation tanks

The pre-treated water is next subjected to excessive aeration in an aeration tank for biological treatment. Following the aeration the wastewater and activated sludge mixture arrives at secondary clarifiers. The major portion of the settled activated sludge here is recycled back to the aeration tank and excess portion is directed to raw sludge thickeners.

The primary sludge and excess activated sludge from secondary clarifiers are combined and thickened in sludge thickeners. Although probability of finding helminth eggs in the thickened sludge was high, aliquot was too thick to analyze with the methods described earlier.

The finished clean water from secondary clarifiers is discharged to the receiving body; Ankara Creek, as shown in Figure 9. Just before the discharge point, the effluent was sampled from the sluices, as shown in Figure 10.

Figure 9 Discharge to Ankara Creek

Figure 10 Secondary Clarifier

CHAPTER 5

RESULTS AND DISCUSSION

The Coliform bacteria are indicators of fecal contamination in waters. However absence of Coliform bacteria does not necessarily mean the absence of helminth eggs and protoozoan cysts, which are more resistant to environmental conditions and more potent in disease production than bacteria. Hence parasitic eggs and cysts were examined in Ankara municipal wastewater treatment plant effluent to define the contamination level of the finished effluents and to define the risk of use of treated wastewaters in agricultural irrigation. Wastewater samples were collected six times within 6 months and examined in terms of helminth eggs and protozoan cysts. Available detection techniques were checked by recovery experiments.

5.1 RECOVERY

Two separate recovery experiments were conducted. Where the WHO, and later modified EPA methods were checked for their efficacy in their recoveries.

For the checking of WHO method, *Ascaris lumbricoides* species were obtained from infected human feces diagnosed in Hacettepe University Hospital, Parasitology Laboratory. Three control samples were prepared namely, A (1 liter), B (5 liters) and C (5 liters) with tap water containing 100 eggs/l as seed. Samples were concentrated according to WHO procedure. The A and B samples were floated in 33% $ZnSO_4$ solution, as suggested in the WHO method, and C sample was floated in supersaturated NaCl solution, which was performed in Hacettepe University, Department of Biology, Parasitology Laboratory. During the microscopic examination, no eggs were detected in sample A. Calculations for B and C resulted in 27 eggs/l and 45 eggs/l respectively.

Table 13 Recovery experiment for WHO "Laboratory Manual of Parasitological Techniques"

Control Name	Initial Volume	Initial Parasite Concentration	Flotation Solution	Recovered Concentration	% Recovery
A	1 liter	100 eggs/l	$ZnSO_4$	0	0 %
B	5 liters	100 eggs/l	$ZnSO_4$	27 eggs/l	27 %
C	5 liters	100 eggs/l	NaCl	45 eggs/l	45 %

For checking the EPA method *Ascaris lumbricoides* species were obtained from infected human feces diagnosed in Hıfzısıhha Institute, Parasitology Laboratory. A 100 liters of control sample was prepared with tap water containing 4 eggs/l as seed. Sample was concentrated according to EPA method and the concentrated pellet was treated as in the WHO method. Finally the sediment was floated in supersaturated NaCl solution. Microscopic examination of the pellet resulted in 1.8 eggs/l, suggesting a 45 % recovery.

5.2 WASTEWATER ANALYSES

The influent and effluent of Ankara municipal wastewater treatment plant were examined to detect the level of incoming parasitic contamination and the outgoing concentration following the treatment process. An initial trial was done in November 2003. The observed images were compared with true *Ascaris* egg images obtained from the web (Plate 6 and 7). From the comparison of microscopic images it was found that a commonly encountered image in the concentrated effluents, resembling *Ascaris* eggs, were found to be false, due to their inappropriate sizes (Plate 8). This is deliberately included here to indicate a common flaw that may occur in identification.

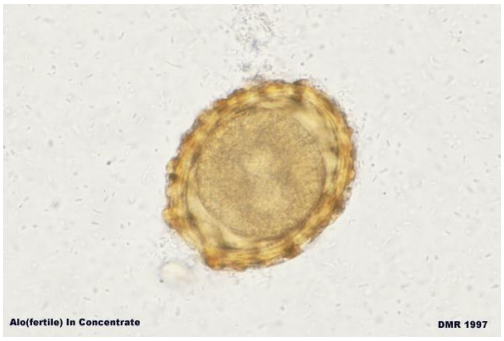

Plate 6 *Ascaris* egg WHO "Laboratory Manual of Parasitological Techniques", [6]

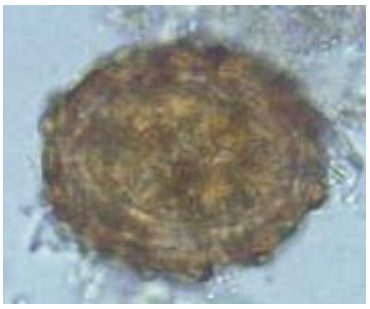

Plate 7 *Ascaris* egg, [40]

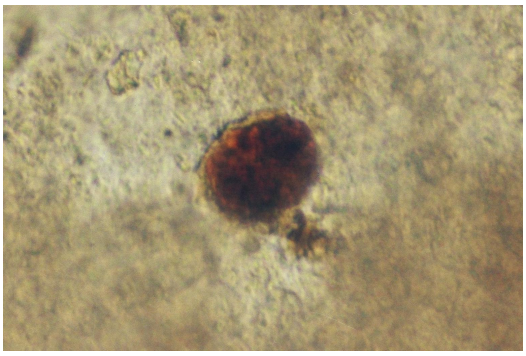

Plate 8 False unfertile *Ascaris* egg image about 30 µm, 600X

The rest of the samples were collected between April and September 2004 regularly about once a month. Some of the microslides were fixed, using fingernail polish

and/or Canada balsam to keep for further examinations. Concentrated samples were kept in 5 % formalin solution, as advised in the WHO manual.

Raw wastewater, raw sludge and pretreated wastewaters were expected to have high helminth egg and protozoan cyst counts. Besides, they had high solids content. Hence those samples were treated with respect to WHO method, which is suitable for raw sewage concentration. On the other hand, treated water was supposed to have lower helminth egg and protozoan cyst concentration, so these samples were concentrated according to EPA method, which is normally developed for the drinking waters.

In each sampling process, 1 liter of raw and partially treated wastewater were collected and concentrated according to the procedure in WHO manual. A 100 liters of finished water was sampled and filtered according to the EPA method.

The microslides were examined at Hacettepe University, Department of Biology, Parasitology Laboratory, under supervision of Prof Dr Nevin Keskin and research assistant Aslı Özkırım. The following results were achieved.

The first sampling, 09/04/2004:

Samples were taken from the discharge of the grit chamber (influent), the overflow weir of the primary sedimentation tank (pretreated wastewater), the outlet of sludge thickener (raw sludge), and the overflow weir of the final clarifier (effluent). The effluent was collected and concentrated according to the EPA method. The influent, pretreated water and raw sludge were processed according to the WHO method. All the samples were floated by 33% $ZnSO_4$ solution. The *Giardia* cysts were observed in the influent, by Prof Dr. Güven Uraz. However the detection of cysts using conventional methods was not possible for an uneducated analyst. So, scanning for *Giardia* cysts and *Cryptosporidium* oocysts was abandoned.

A deformed *Ascaris* egg (Plate 9, 10 and 11) and an *Enterobius* egg (Plate 12 and 13) and a damaged *Trichuris trichiura* egg (Plate 14 and 16) were detected in the effluent. Pollens (Plate 18) and spores were detected in the effluent as well. Notably, any cysts or eggs were not recovered from samples of raw sludge and partially treated wastewater.

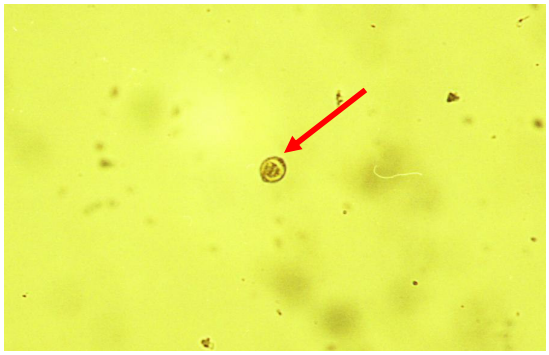

Plate 9 Decorticated *Ascaris* egg, 125X

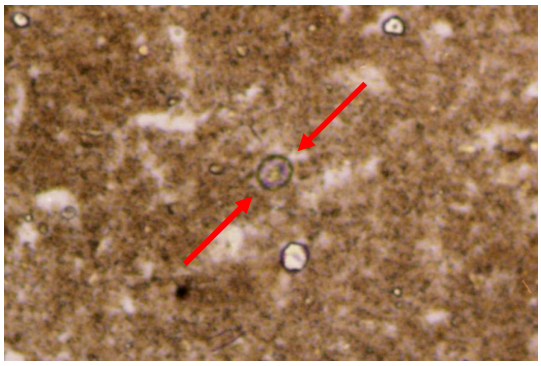

Plate 10 *Ascaris* egg, 125X

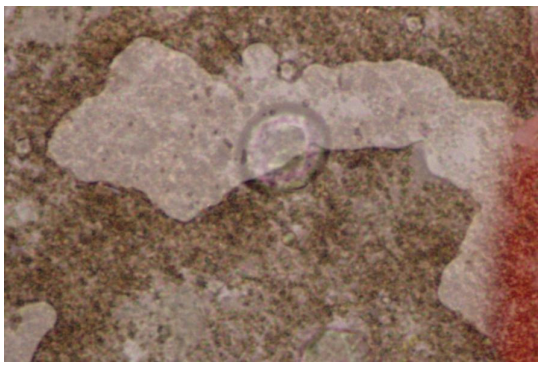

Plate 11 *Ascaris* egg, 200X

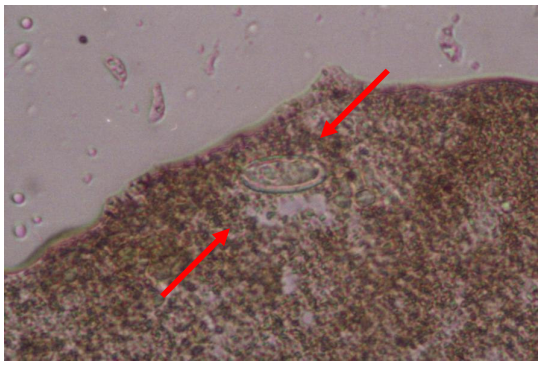

Plate 12 *Enterobius* egg, 125X

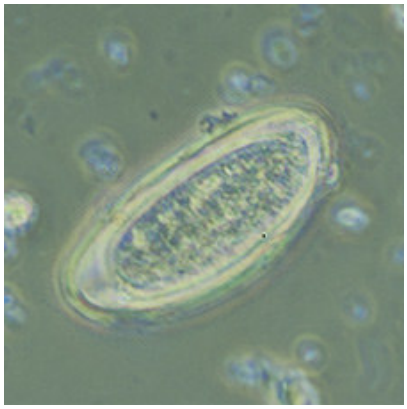

Plate 13 The *Enterobius* eggs are elongated and flattened on one side with a thick colorless shell, measuring from 50-60 µm by 20-30 µm, [41]

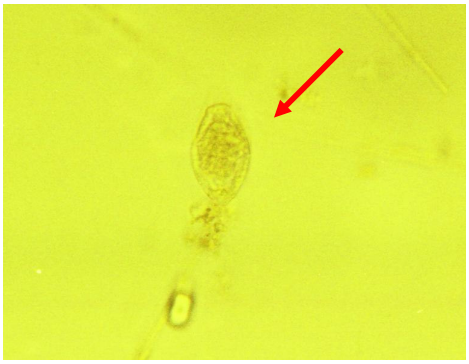

Plate 14 ***Trichuris trichiura,*** **500X**

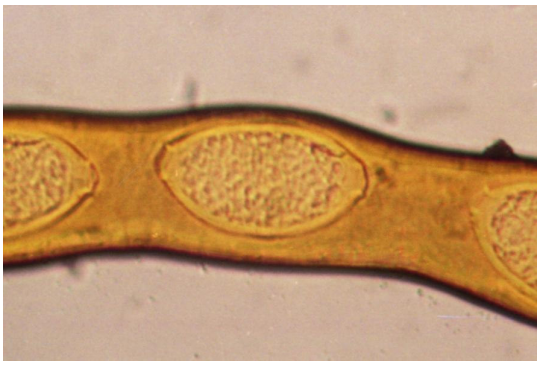

Plate 15 ***Trichuris trichiura*** **egg from demonstration microslides, 500X**

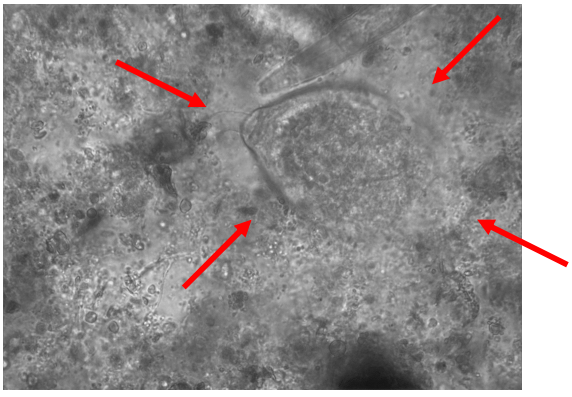

Plate 16 ***Trichuris trichiura,*** **500X**

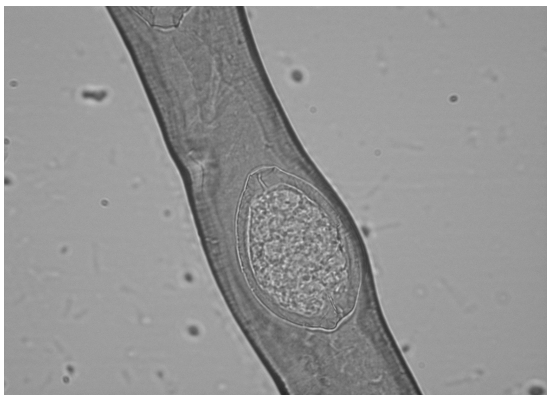

Plate 17 *Trichuris trichiura* egg from demonstration microslides, 500X

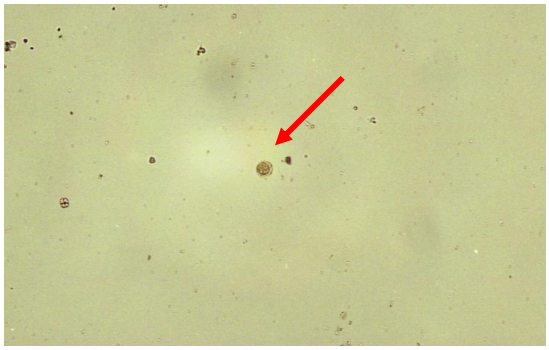

Plate 18 Pollen, 125X

The second sampling, 12/05/2004:

This sample was taken from Ankara Creek, since the creek water was also being used for irrigation in the surrounding fields and as sewers from squatter housings in the district are directly discharged to the Ankara creek. The samples were analyzed according to both the EPA and the WHO methods. The flotation was carried out by the use of supersaturated NaCl solution. Numerous saprophytic protozoan, spores and plant debris were detected in the samples. A positive result could not be obtained in terms of parasitic contamination, in these samples.

The third sampling, 09/06/2004:

In June, samples were collected from the grit chamber, primary sedimentation tank and secondary clarifier, in municipal wastewater treatment plant. Influent and partially treated wastewaters were concentrated by using both the WHO and EPA methods, whereas effluent was concentrated by using the EPA method only. This time, the "effluent pellet", was enriched by using the Percoll-sucrose flotation protocol of the EPA manual. The Percoll-sucrose flotation separates the protozoan cysts at the upper phase and the heavy debris plus helminth eggs at the bottom phase. Contrary to the protocol, the bottom phase of the product after the final centrifugation step, was not discarded, it was re-centrifuged in eluting solution for the helminth egg scanning. Especially a rich variety of organisms were observed in this bottom portion, e.g. lots of pine seeds (Plate 19 and 20) and plant pollens (Plate 21) were met.

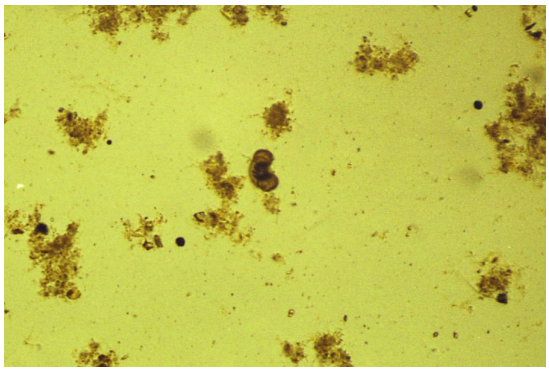

Plate 19 Pine grain, 200X

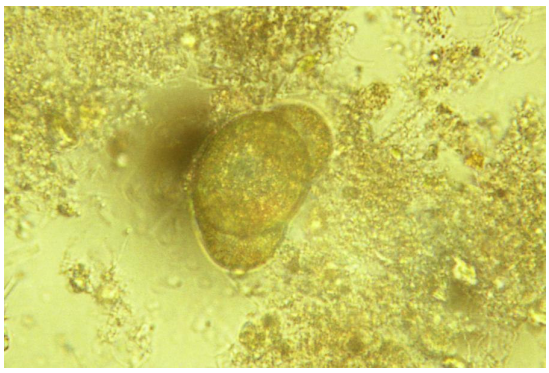

Plate 20 Pine grain, 500X

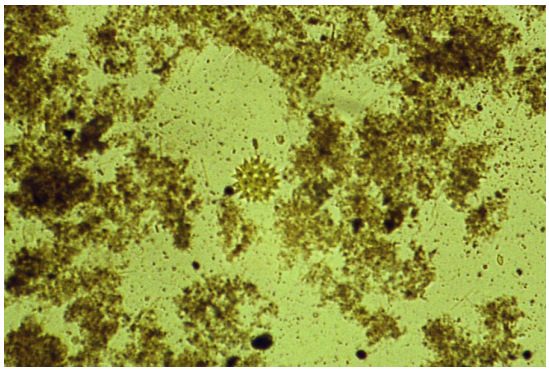

Plate 21 Plant pollen, 200X

Although *Taenia* eggs are often regular in shape, a deformation can be observed while eggs are extracted from the body of an adult *Taenia saginata* cestode (Personal communication with Esen Bostancı) [42]. The body in Plate 22 shows a deformed *Taenia* egg, detected in the effluent sample.

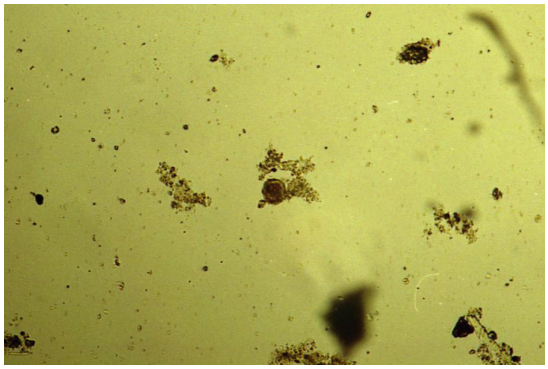

Plate 22 Deformed *Taenia* egg, 200X

Additionally, few nematodes, some carrying eggs inside their body, were detected in this bottom fraction (Plate 23). Approximate lengths of these nematodes were 0.4 mm. This size is particularly small compared with the human intestinal nematodes. It is concluded that this nematode should have been originated from the soil and it should have been introduced to the raw wastewater before the treatment plant inlet. Yet, regardless of their origin, presence of any nematode and eggs in the effluents, should point out the inadequacy of the treatment process and possibility of intestinal nematodes and their eggs being present in the effluent.

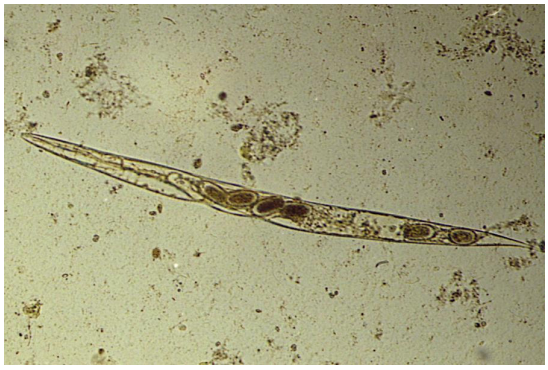

Plate 23 Soil nematode, 200X

Fungi spores are likely to be confused with decorticated *Ascaris* eggs. Nevertheless, circular inner material of the fungi spores, which are in contact with the outer shell at

a single point (Plate 24 and 25), differs from the *Ascaris* eggs, since a decorticated *Ascaris* egg does not include such a contact point (Plate 26 and 27). Normal fertile eggs may lack the mammillated layer and are referred as decorticated eggs.

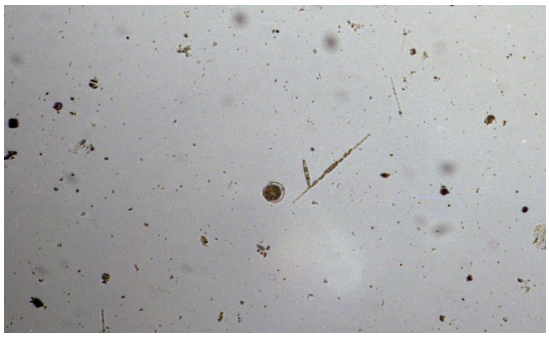

Plate 24 Fungus spore, 200X

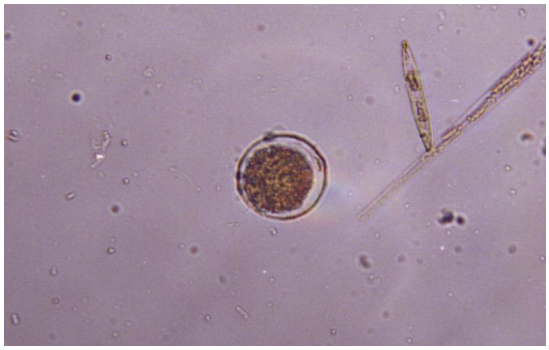

Plate 25 Fungus spore, 500X

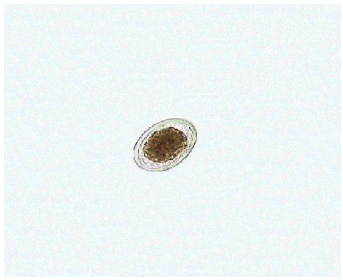

Plate 26 *A.lumbricoides* decorticated egg, [43]

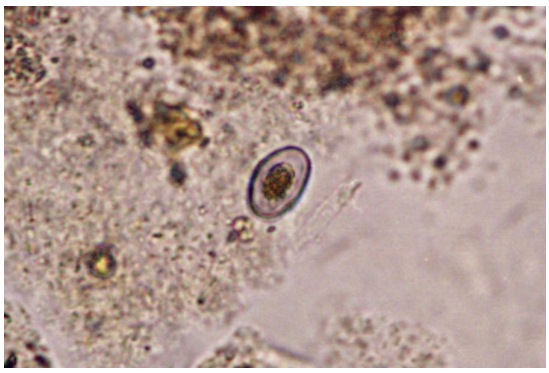

Plate 27 Decorticated *Ascaris* egg, 200X

A pseudoparasite was a protoskolex, originated from domestic animals, namely *Echinococcus granulosus*. This parasite's final host is dogs and it completes its lifecycle in ruminants' livers, such as goat, cow etc.

A group of *Echinococcus granulosus* ova found in the effluent samples (Plate 28 and 29) indicates animal-originated contamination in the wastewater, probably from the surrounding villages of Ankara creek.

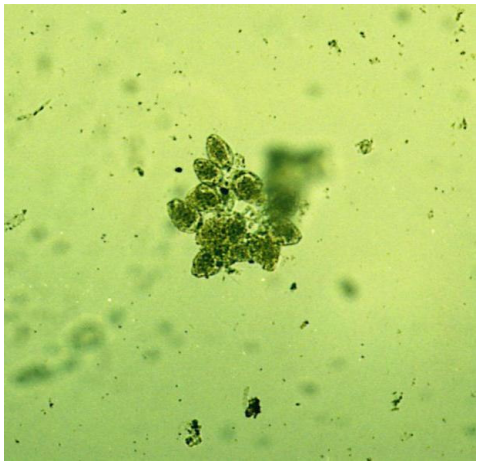

Plate 28 *Echinococcus granulosus* eggs, 125X

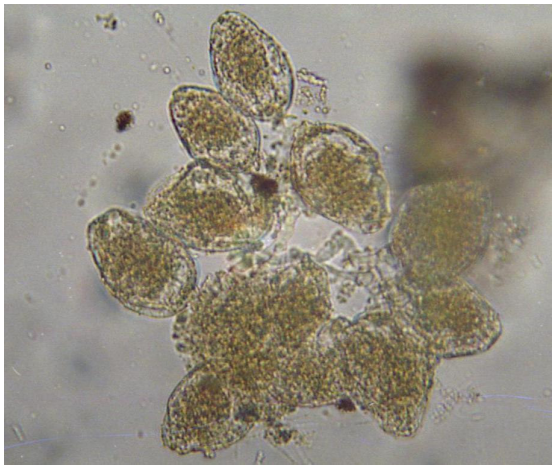

Plate 29 *Echinococcus granulosus* **ova 89 µm long, 500X**

Throughout the rest of the analyses of this sample, infertile *Ascaris* eggs were identified (Plate 30).

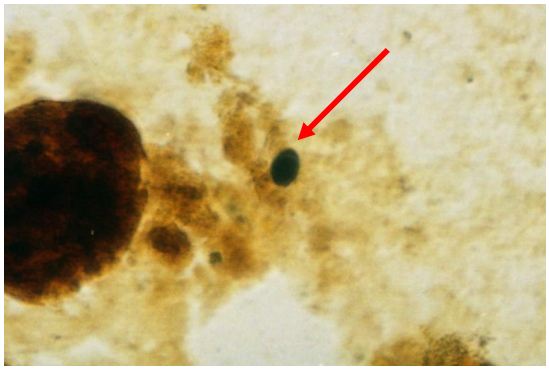

Plate 30 ***Ascaris*** **egg, infertile, 125X**

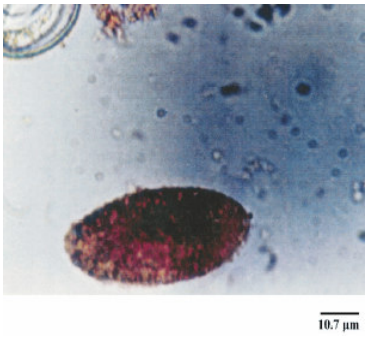

Plate 31 *Ascaris* egg, infertile, [44]

Logul's Iodine dye solution aided in identifying other organisms, such as *Hymenolepis nana* egg (Plate 32).

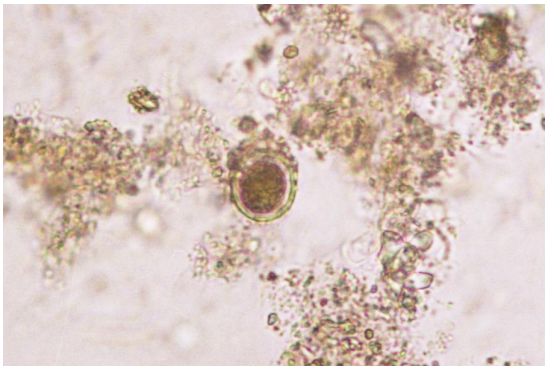

Plate 32 *Hymenolepis nana* egg, 200X

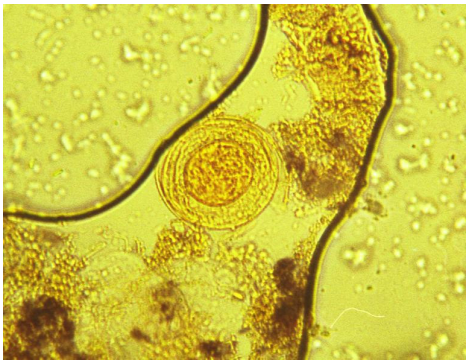

Plate 33 *Himenolapis nana* egg from patient feces, 600X

Fusiform shaped saprophyte protozoan and pollens (Plate 34 and 35), and coccidian cysts (Plate 36) were the other common organisms that were encountered in all the samples.

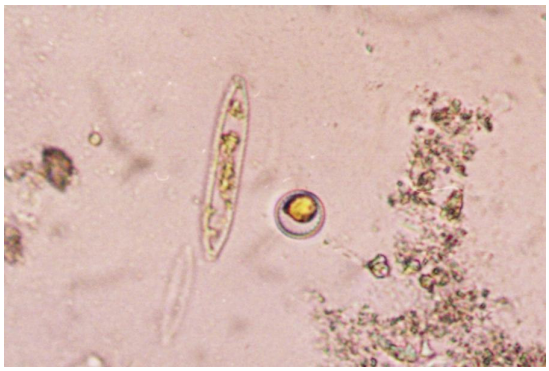

Plate 34 Pollen and fusiform shaped saprophyte protozoa, 200X

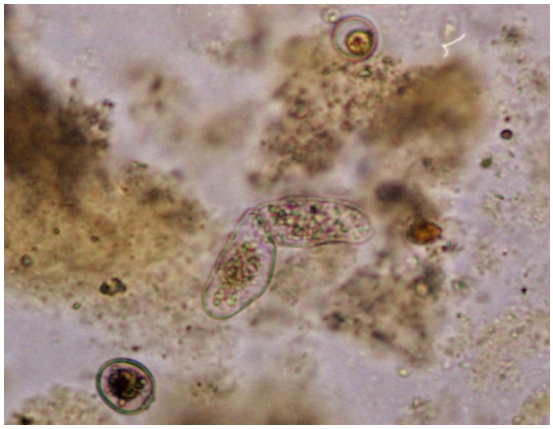

Plate 35 Saprophytic protozoa, two conjugating cysts and pollens, 200X

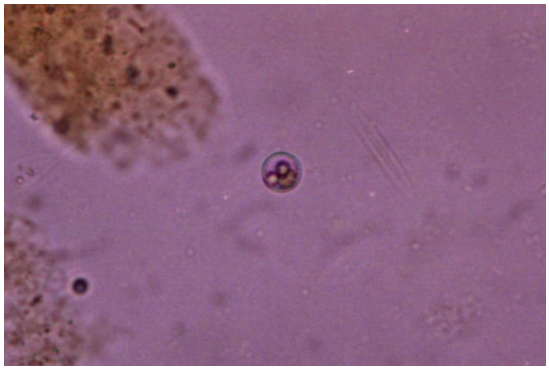

Plate 36 Oocyst of *Eimeria* sp, 200X

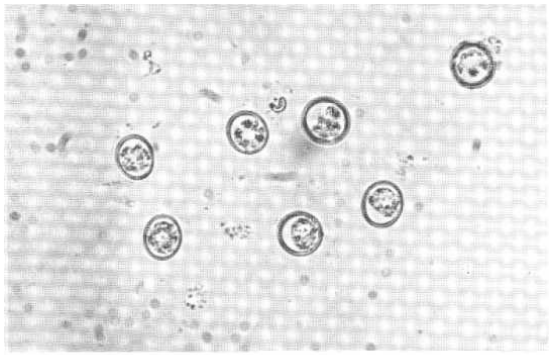

Plate 37 Oocyst of *Eimeria zurnii,* ruminant parasite eggs, [45]

The fourth sampling, 02/07/2004:

The treatment plant was visited for the fourth time on the beginning of July. Raw, partially treated and treated wastewaters were sampled. The WHO protocol was applied for the raw wastewater and the EPA protocol was applied for the partially treated and treated wastewaters. *Ascaris* eggs, as well as pine pollens, saprophyte protozoan, and skolex groups were observed in this sample.

The fifth sampling, 22/07/2004:

At the end of July, successful results were acquired denoting the success of the WHO method. Viable (Plate 38 and 39) and infertile (Plate 40) *Ascaris* eggs were encountered in the raw wastewater and viable *Ascaris* eggs in partially treated wastewater (Plate 41).

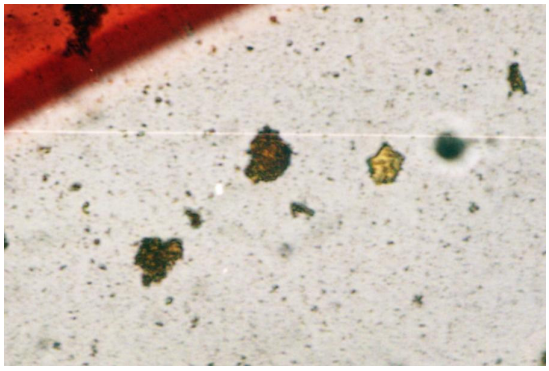

Plate 38 Embriyonated *Ascaris* egg, 125X

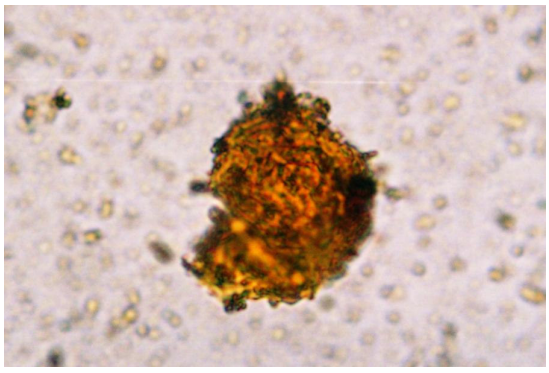

Plate 39 Embriyonated *Ascaris* egg, 500X

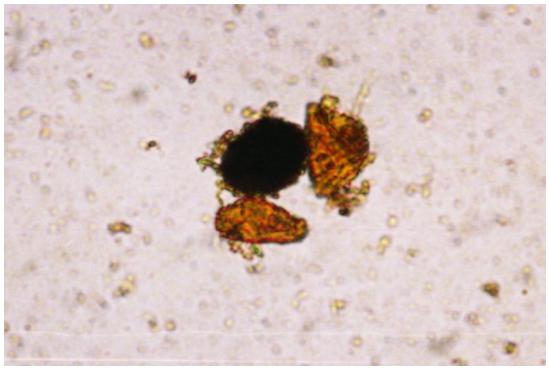

Plate 40 Infertile *Ascaris* egg, 200X

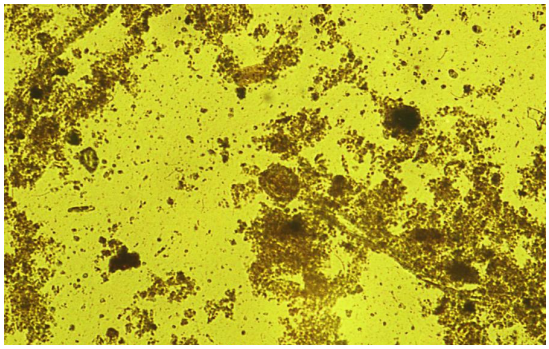

Plate 41 *Ascaris* egg, 125X

In addition to these findings, physically damaged *Ascaris* eggs were observed in the effluent sample (Plate 42). Mammillated layer of the *Ascaris* egg gets thinner in time. Such developments were observed on microslides, prepared with the patient feces (Plate 43, 44, 45 and 46).

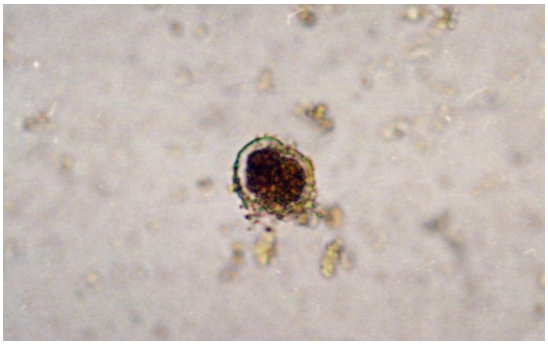

Plate 42 ***Ascaris*** **egg exploded. Mechanical centrifuge effect, 200X**

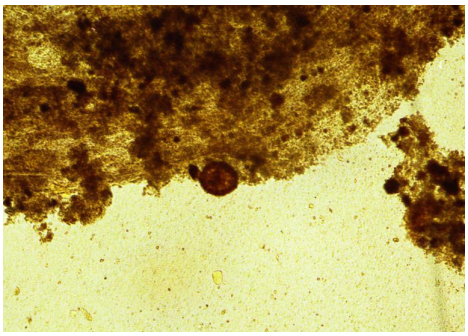

Plate 43 ***Ascaris*** **egg from patient feces, photographed on 03/05/2004, 135X**

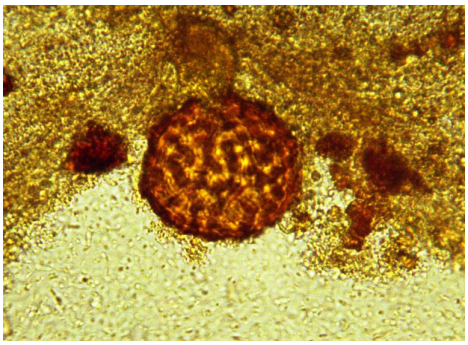

Plate 44 ***Ascaris*** **egg from patient feces, photographed on 03/05/2004, 600X**

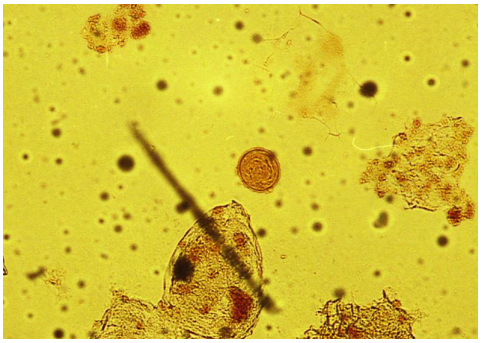

Plate 45 ***Ascaris*** **egg from patient feces, photographed on 08/07/2004, 135X**

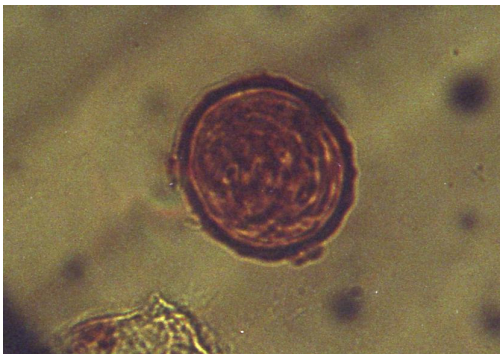

Plate 46 ***Ascaris*** **egg from patient feces, photographed on 08/07/2004, 600X**

The sixth sampling, 27/08/2004:

The last samples were collected at the end of August. Infertile *Ascaris* eggs (Plate 47 and 48) and decorticated fertile *Ascaris* eggs (Plate 49 and 50) were detected in the finished water. Besides several types of pollens (Plate 51) and algae (Plate 52 and 53) were observed.

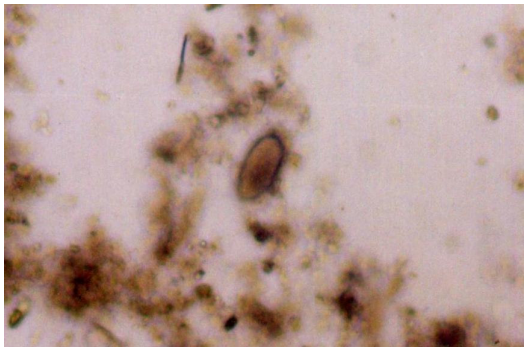

Plate 47 Infertile *Ascaris* egg, 125X

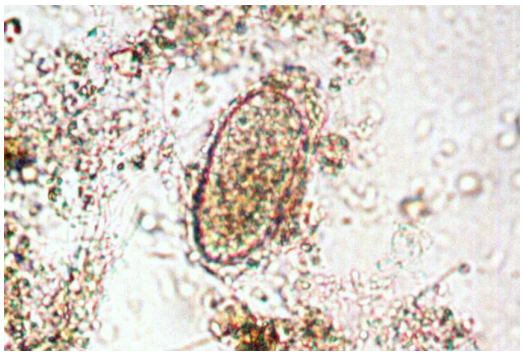

Plate 48 Infertile *Ascaris* egg, 500X

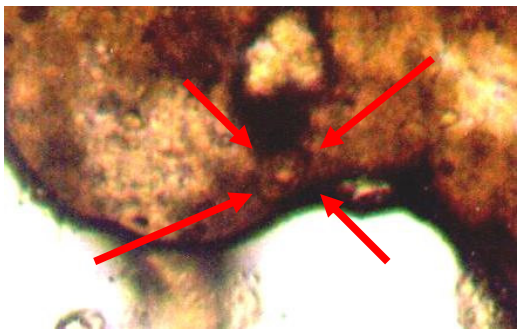

Plate 49 Decorticated *Ascaris* egg, embrionated, 125X

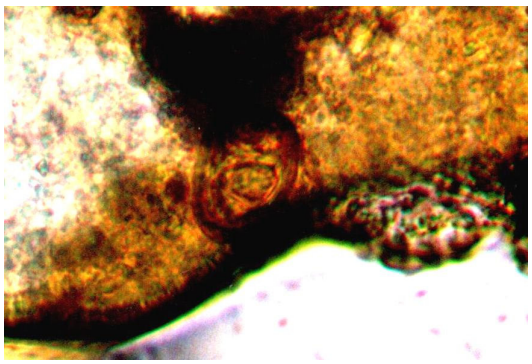

Plate 50 Decorticated *Ascaris* egg, embrionated, 500X

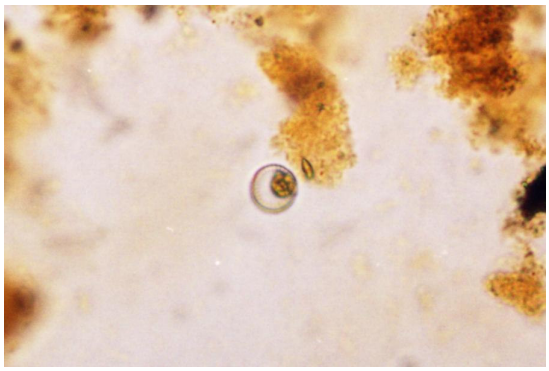

Plate 51 pollen, 500X

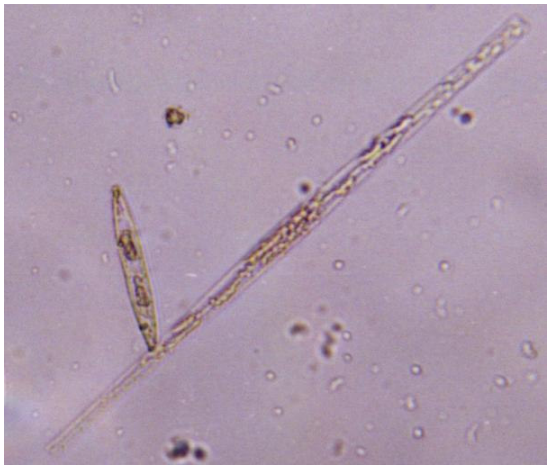

Plate 52 Algea, 830X

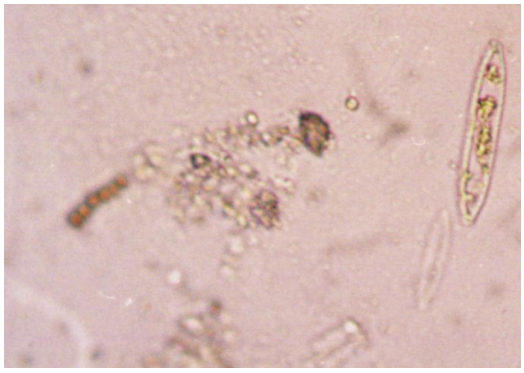

Plate 53 Algea, 500X

Based on the Equation 1 and the initial sample volume of 100 l, the number of egg concentrations were calculated for the collected effluent samples. The results were given in Table 14. Calculated concentrations of the helminth eggs in the wastewater effluent were all below the WHO standards (1 eggs/l). Table 14 indicates that ASKİ Central Wastewater Treatment Plant (ACWTP) effluents are fit for A, B, and C (unrestricted) categories of crop irrigation, which are defined by WHO, in Table 4

Table 14 *Ascaris* egg concentration, obtained from wastewater treatment plant effluent analyses.

Date of sampling	Sampling point	# of *Ascaris* eggs per microslide	Initial sample volume (liter)	Final concentrate volume (≈)	# of eggs per liter of water
10/11/2003	ACWTP eff	0	100	1 ml	0
09/04/2004	ACWTP eff	1	100	1.5 ml	0.6
12/05/2004	Ankara creek	0	100	6 ml	0
09/06/2004	ACWTP eff	1	100	2 ml	0.8
02/07/2004	ACWTP eff	1	100	1.5 ml	0.6
22/07/2004	ACWTP eff	2	100	1 ml	0.8
27/08/2004	ACWTP eff	2	100	1 ml	0.8

CHAPTER 6

CONCLUSION

Turkey uses wastewaters both directly and indirectly as an alternative source of water for crop irrigation. However the occurrence of parasitic eggs in the wastewaters is the main health problem considering the reuse practices in crop irrigation. Unfortunately very little or no data is available about the current situation in our country. Performances of particular units in a treatment plant for parasite removal are not known. Also a simple and easy detection method is not practiced for helminth and protozoa detection. Hence, in this study, detection methods were practiced; and the presence and concentration of intestinal parasites were examined in the Ankara Central Wastewater Treatment Plant Effluent.

Two methods were chosen for the concentration and detection of parasites from the wastewaters. The WHO method: "A Laboratory Manual of Parasitological and Bacteriological Techniques [6]" was chosen for analyzing raw and partially treated wastewaters. And EPA "ICR Microbial Laboratory Manual" [38], which is prepared for drinking water analyses in terms of protozoan cysts, was used only to concentrate wastewater treatment plant effluent. Because helminth egg concentrations in the wastewater treatment plant effluent were expected to be low, higher volumes of effluents could be sampled. However, procedure for detection of protozoan cysts in the EPA Manual could not be practiced due to financial problems. The concentrated effluent samples were examined only in terms of helminth eggs according to WHO manual. Hence a combination of WHO and EPA manuals were used for the effluent samples.

The WHO method is easy and cost effective. However, it has some limitations such as low recovery efficiency. In the calculation step of the WHO method, it is assumed that eggs are uniformly distributed in the final flotation step. For a more accurate

result, the initial sample volume can be increased, in this way; the number of positive samples from treated wastewater would be increased.

Due to low helminth egg concentrations in the effluent, the WHO method, suggesting 10 liters sample volume for treated wastewaters, was not found adequate for wastewater treatment plant effluent. Hence higher volume of sample was employed for effluent analyses. Although EPA manual is prepared for drinking water analyses, this method (proposes 100L sample volume) was chosen to concentrate treatment plant effluents having low helminth egg concentrations and low turbidity.

The WHO method recommends 1 liter for raw wastewater to be used as the working volume but it is found to give better results with the higher volumes such as 5 liters in the recovery studies. However, more than 10 liters of sample volume may result unexpectedly low recovery efficiency. Because increase in the sample volume would also increase the suspended solids content in the concentrated pellet and the interference of the solids would lead lower detection efficacy. Similarly, artificially prepared sample "A", having a one liter volume and 100 helminth eggs per liter initial concentration, resulted in zero recovery, owing to the fact that experimental errors could not be eliminated when such low volumes of samples were being processed through this method. The WHO method uses 33% $ZnSO_4$ as flotation solution. However supersaturated NaCl solution was found to give even higher recovery of eggs and also it is more cost effective. The WHO method resulted in 27% and 45% recoveries with initial 5 liters of sample volume and 100 eggs/l of helminth egg concentration by using 33% $ZnSO_4$ and supersaturated NaCl flotation solutions respectively. These recovery efficiencies were low considering the initially high helminth egg concentrations and low sample volumes.

A 100 liters of tap water was seeded with helminth eggs to achieve 10 eggs/l concentration. Checking on the EPA method resulted in 45% recovery in this sample, with comparatively low in helminth egg counts (4 eggs/l) and with lower turbidity

with respect to raw wastewater. The sample was representing municipal wastewater treatment plant effluent.

The WHO method did not present reliable results in the influent samples, partially treated water samples and raw sludge samples, even though egg concentrations were expected to be high in these. The raw sludge could not be processed using the WHO method due to high solids content, and it cannot be processed with the EPA method because raw sludge is not filterable using 1 µm nominal porosity filter. Consequently samples from raw sludge were not collected anymore.

Sampling from Ankara Creek did not provide reasonable information regarding the parasitic contamination of the waters, owing to the high concentration of interfering organic materials and suspended solids and high dilution in the creek. Sampling volume may be increased to 500 l, or higher for the creek waters or a completely different protocol should be developed.

The effluent sampling in June (the third sampling) was processed using the EPA Percoll-sucrose flotation procedure; as opposed to NaCl and $ZnSO_4$ flotation solutions employed in previous and further samples. Although a rich diversity of organisms were observed with Percoll-sucrose flotation, an extraordinarily high helminth egg concentrations could not be observed in these samples. Although protozoan cysts in the wastewater effluent were probably concentrated with Percoll-sucrose flotation procedure; they could not be detected via the conventional microscopic methods. Even the influent samples were being concentrated with the WHO method, processing high volumes of influent by using EPA method did not worked well since influent carried high amounts of suspended solids which subsequently clogged the filters. This hindered sampling and consequently no notable results were acquired.

The parasite eggs were deformed in general, probably due to the chemical and physical treatments, applied during the sample concentration procedures. This deformation may lead false negative results, so should be considered by the analyst.

Even though the general pattern of helminth egg concentrations in the effluent samples (Table 14) indicates a low level of parasitic contamination in the treatment plant effluents, caution should be taken since these figures may double or quadruple when recovery efficiencies are taken into consideration.

CHAPTER 7

RECOMMENDATIONS FOR FUTURE WORK

This study should also be extended to other treatment plants in Turkey, particularly to those regions where high rates of parasitic diseases are encountered.

Waste sludges should also be examined in terms of parasitic pathogens

The effects of sludge treatment processes on removal efficacies should be evaluated

A detailed study should be conducted in Ankara on the occurrence of protozoan parasites in wastewaters

The IFA assay from the EPA "ICR Microbial Laboratory Manual" should be adapted and applied in the detection of protozoan parasites

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