AN INVESTIGATION OF AGRICULTURAL USE POTENTIAL OF WASTEWATER SLUDGES IN TURKEY WITH RESPECT TO HEAVY METALS AND PATHOGENS

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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.

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ABSTRACT

AN INVESTIGATION OF AGRICULTURAL USE POTENTIAL OF WASTEWATER SLUDGES IN TURKEY WITH RESPECT TO HEAVY METALS AND PATHOGENS

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Landfilling is the most common method that is used in the final disposal of sludge all around the world as well as in Turkey. However increasing sludge quantities and limited landfilling areas make this method invalid. Use of sludge in agriculture presents a possible alternative for disposal. However, it also poses some risks to be evaluated.

In this respect, it is important to identify heavy metal and pathogen content of sewage sludges because of their adverse health effects. This study aims to determine the heavy metal contents and pathogen levels of sludges from four different wastewater treatment plants of Turkey. The selection of plants was done according to the different treatment technologies applied to wastewater and sludge in those plants. Heavy metal analysis of sludges was conducted by using microwave assisted digestion procedure and pathogen levels were done by methods from Standard Methods (SM), ISO and USA EPA.

After sampling and analysis, the results show that all the related metal concentrations are below the values that are set in the Soil Pollution Control Regulation of Turkey. However in sludges from Ankara and Kayseri wastewater treatment plants, Zinc and Nickel concentrations should be tracked carefully. The results related with pathogen levels in sludges show that dewatered sewage samples taken from Ankara, Kayseri and Kemer wastewater treatment plants do not meet neither Class A nor Class B fecal coliform limits set by USA EPA however lime stabilized dewatered sludge from İzmir wastewater treatment plant meet the requirement. In addition, *Salmonella* levels in Kayseri dewatered sludges exceed the limit value.

Keywords: sewage sludges, agricultural use, microwave digestion, heavy metals, pathogens

ÖZ

TÜRKİYEDEKİ ATIKSU ÇAMURLARININ TARIMDA KULLANIM POTANSİYELİNİN AĞIR METALLER VE PATOJENLER AÇISINDAN İNCELENMESİ

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Çamurun nihai bertarafında dünyada ve ülkemizde en sık kullanılan yöntem çamurun katı atık depolama sahalarına atılması şeklindedir. Ancak artan çamur miktarları ve kısıtlı depolama sahaları bu yöntemi giderek geçersiz kılmaktadır. Çamurların tarımda kullanımı alternatif bertaraf yöntemlerindendir. Ancak bu uygulama değerlendirilmesi gereken bazı riskleri ortaya çıkarır.

Bu kapsamda insan ve çevre sağlığına olan olumsuz etkileri açısından çamurların ağır metal ve patojen içeriğinin belirlenmesi gerekmektedir. Bu çalışma, Türkiye'deki evsel atıksu arıtma tesislerinden ortaya çıkan çamurların tarımda gübre olarak kullanılabilirliğinin belirlenmesini hedeflemektedir. Tesislerin seçimi, atıksuya ve çamura uygulanan farklı arıtma teknolojilerinin varlığına göre yapılmıştır. Çamurlardaki ağır metal analizleri mikrodalga çürütücü sistem ile, patojen seviyeleri ise Standart Metot, ISO ve USA EPA tarafından verilen metotlarla gerçekleştirilmiştir.

Numune alımı ve analizlerden sonra elde edilen sonuçlara göre metal konsantrasyonları Türkiye'deki Toprak Kirliliği Kontrolu Yönetmeliğinde oluşturulan sınır değerlerden düşüktür. Ancak Ankara ve Kayseri atıksu arıtma tesislerinden gelen çamur numunelerinde çinko ve nikel konsantrasyonları dikkatlice takip edilmelidir. Ankara, Kayseri ve Kemer atıksu arıtma tesislerinden gelen çamurlardaki patojen seviyeleri USA EPA tarafından verilen Sınıf A ve Sınıf B fekal koliform sınır değerini sağlamamaktadır ancak İzmir atıksu arıtma tesisinden gelen çamur numunleri bu sınırı sağlamaktadır. Ayrıca, Kayseri çamurlarındaki *Salmonella* seviyesi sınır değeri geçmektedir.

Anahtar Kelimeler: atıksu çamurları, tarımda kullanım, mikrodalga çürütme, ağır metal, patojen

To my family,

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ABBREVIATIONS

Cd : Cadmium
CFR : Code of Federal Regulations
CFU : Colony Forming Units
Cr : Chromium
Cu : Copper
EEC : European Economic Community
EPA : U.S. Environmental Protection Agency
H_3BO_3 : Boric acid
HCl : Hydrochloric acid
HF : Hydrofluoric acid
Hg : Mercury
HNO_3 : Nitric acid
MF : Membrane Filter
MPN : Most Probable Number
RSHC : Refik Saydam Hıfzısıhha Center
Ni : Nickel
Pb : Lead
PFU : Plaque Forming Unit
SPCR : Soil Pollution Control Regulation
SS : Salmonella and Shigella
TNTC : Too Numerous To Count
TS : Total Solids
WWTP : Wastewater Treatment Plant
XLD : Xylose Lysine Deoxycholate
Zn : Zinc

CHAPTER 1

INTRODUCTION

Sewage sludge is the solid, semi-solid, or liquid residues generated during the treatment of domestic sewage in a treatment works (National Research Council, 2002). Throughout the wastewater and sewage sludge industry, the term “sewage sludge” has largely been replaced by the term “biosolids”. “Biosolids” specifically refers to sewage sludge that has undergone treatment for beneficial use. The distinction between untreated sewage sludge and biosolids is biosolids have undergone processing during treatment (U.S. EPA, 1999).

Wastewater sludge disposal is a major urban environmental problem (Parkpain et al., 2000). In the past, incineration and landfilling were common practices for disposal. However, limited landfilling areas and the increasing cost of landfill disposal as well as the phasing out of other environmentally unacceptable disposal options, such as ocean disposal, are the reasons encouraging increased use of sludges in agriculture. Sludges contain organic materials that are often rich in nutrients such as nitrogen and phosphorus, and contain valuable micro nutrients (U.S. EPA, 2000). The long-term goal should be to utilize the nutrients and organic matter in sludges through land application. Although the recycling of the organic matter and nutrients contained in wastewater sludge through land application is a worthwhile objective, presence of heavy metals like lead (Pb), cadmium (Cd), chromium (Cr), copper (Cu), zinc (Zn) and mercury (Hg) and pathogens in sludges pose risks to human health, agricultural productivity and ecological health (Harrison et al., 1999).

Pollution problems may arise if toxic heavy metals are mobilized into the soil solution and are either taken up by plants or transported in drainage waters to associated water supplies (Zufiaurre et al., 1998). The potential input of heavy metals range from the run-off and atmospheric deposition to direct, unauthorized, down the drain disposal of hazardous and/or industrial wastes (Bright and Healey, 2003). Major industrial sources include surface treatment processes with elements such as Cu, Zn, Ni and Cr, as well as industrial products that, at the end of their life, are discharged in wastes (Karvelas et al., 2003).

The rapid and accurate determination of heavy metals in environmental matrices such as sludges, soils and sediments has led to the development and continuous improvement of various analytical methods concerning both sample digestion and the choice of most appropriate instrumental techniques to obtain exhaustive chemical information in the shortest time possible (Bettinelli et al., 2000). The most common methods used nowadays for the determination of heavy metals in environmental samples involve highly sensitive spectroscopic techniques.

Regarding the presence of pathogens, sludge has been of the greatest concern because of the pathogen uptake by plants and entry into the chain, movement through the soil and the contamination of groundwater and runoff and erosion containing pathogens and exposure to people contacting the contaminated water.

Because of these concerns, the use of sludge in agriculture is strictly controlled by regulations. The land application of wastewater sludge is regulated by U.S. EPA 40 CFR 503 in USA, 86/278 EEC in EU and Soil Pollution Control Regulation (SPCR) in Turkey. The aim of all these regulations is the safe recycling of sludge and an enhancement of its characteristics. In USEPA 40 CFR 503, there are three major categories of requirements establishing sludge quality and site management criteria for land application. Three requirement categories are pollutant concentrations versus ceiling concentrations for metals, Class A versus Class B requirements for pathogens and process-control criteria to reduce vector attraction versus physical barriers for vectors. In EU and Turkey, heavy metals are regulated but neither 86/278 EEC in EU and SPCR in Turkey have any restrictions on pathogens.

1.1. Aim of the Study

The aim of this study is to determine the possibility of the utilization of wastewater sludges in agriculture in Turkey regarding the heavy metal concentrations (Cu, Cd, Pb, Cr, Zn, Ni and Hg), indictor microorganisms and pathogen levels (fecal coliform, fecal streptococci, *Salmonella*, *Cryptosporidium*, *Giardia* and helminth eggs) in sludge.

1.2. Scope of the Study

This study examines the sludges from four domestic wastewater treatment plants in Turkey in terms of its suitability for agricultural use with respect to its heavy metal and pathogenic microorganism contents. For this purpose, municipal wastewater treatment plants in four cities (Ankara, İzmir, Kayseri and Tekirova) in Turkey with different wastewater and sludge treatment technologies, serving populations, and industrial inputs were selected.

First, a microwave extraction technique was optimized for the extraction of heavy metals from wastewater sludges. Following the extraction technique development, the sludge samples from the selected plants were investigated for their heavy metal contents. Microbiological parameters were analyzed in accordance to EPA, ISO and Standard Methods. Finally, heavy metal results and microbiological parameters are evaluated in relation to the limits set by Soil Pollution Control Regulation of Turkey, USA and EU regulations. In addition, results for heavy metal concentrations and microbiological parameters are evaluated in accordance with the treatment technologies applied as well.

CHAPTER 2

LITERATURE SURVEY

2.1. Wastewater Sludge

Within the objective of producing an effluent that does no harm to the watercourse and the surrounding ecosystem and reducing or eliminating waterborne diseases, modern wastewater treatment plants are reasonably effective at achieving these goals (Spinosa and Vesilind, 2001).

Modern wastewater treatment plants are subject to a number of physical, chemical and biological treatment processes. Wastewater treatment processes range from preliminary treatment, primary treatment, secondary treatment, advanced or tertiary treatment to sludge treatment (Spinosa and Vesilind, 2001; Epstein, 2003; Enezi et al., 2004). The solids produced after gravitational settling of the settleable solids in primary treatment are named as primary sludges and are characterized by a highly odiferous nature and high content of human pathogens. After primary treatment, the most popular secondary treatment method is the activated sludge process which has an objective of mixing the wastewater with the active biomass which assimilates the organic fraction and reduces the demand for oxygen. The process produces an excess growth of biomass called waste activated sludge or secondary sludge. Waste activated sludge does not contain the concentration of pathogens found in primary sludge but its inability to dewater and its high concentration of volatile solids make it difficult to treat (Spinosa and Vesilind, 2001).

2.2. Wastewater Sludge Generation Rates

The more stringent requirement for the treatment of wastewater discharged from the domestic and industrial sectors has resulted in an increase in the construction of wastewater treatment plants and therefore sewage sludge production in recent years all over the globe (Wong et al., 2001; Spinosa, 2004). The following table represents the amount of sludge produced from different European Union countries and United States of America.

Table 1. Annual Sewage Sludge Production in EU countries and USA

Countries	Total Sludge Production (dry matter tonnes/yr)
Austria[c, e]	2.5×10^5
Czech Republic[c, e]	2.1×10^5
Denmark[d, g]	1.4×10^5
Finland[b, e]	1.6×10^5
France[a, e]	1.02×10^6
Germany[a, e]	2.5×10^6
Netherlands[b, e]	3.6×10^5
Poland[c, e]	3.97×10^5
Spain[b, e]	8.7×10^5
Sweden[h]	2.4×10^5
United Kingdom[a, e]	1.1×10^6
United States of America[a, f]	6.9×10^6

[a] *1998 data*
[b] *2000 data*
[c] *2001 data*
[d] *2002 data*
[e] *data obtained from europa.eu.int/comm/eurostat/*
[f] *data obtained from Bastian, 1997*
[g] *data obtained from Jensen and Jepsen, 2005*
[h] *data obtained from Hultman et al., 2000*

From Table 1, in the United Kingdom by the year 1998, 1.1×10^6 tonnes of dry solid sewage sludge equivalent was produced per year and is projected to be in the quantity of 1.5×10^6 tonnes of dry solid sewage sludge by 2005 (Bowler, 1999). In the United States of America, in 1998 approximately 6.9×10^6 dry tonnes of sewage sludge was produced and U.S. Environmental Protection Agency (EPA) estimated that 8.2×10^6 tonnes of sewage sludge would be generated by 2010 (EPA530-R-99-009). The production of sewage sludge is sharply increased in Hong Kong from 6.4×10^4 tonnes of dry solids in 1997 to about 1.7×10^5 tonnes of dry solids in 2005 (Wong et al., 2001). The amount of sewage sludge produced in China was about 0.4×10^6 dry tonnes of sewage sludge per year and estimated to rise 40-50% in 2010 (Wang, 1997). The amount of sludge production in metropolitan cities of Turkey is 8.0×10^4 tonnes of dry solids per year for Ankara wastewater treatment plant.

2.3. Sludge Stabilization and Disposal Methods

Considering the huge amount of sewage sludge produced annually, there is a general agreement that long-term goal should be to assess the beneficial use of sewage sludges. Basic options for biosolids utilization and sludge disposal are landfilling, incineration and application to the land. Prior to disposal options, biosolids stabilization is of major importance because it must be nonhazardous to humans, biologically inactive, free of offensive odorss and aesthetically acceptable.

2.3.1. Sludge Stabilization

The stabilization of above mentioned sewage sludges should be done to reduce the undesirable characters such as volatile solids content biologically and chemically, pathogen levels, and odor (Lucero-Ramirez, 2000). The technologies for sludge stabilization include 1) alkaline stabilization, 2) thermal drying, 3) anaerobic digestion, 4) aerobic digestion and 5) composting (Metcalf and Eddy, 1991). After sewage sludge treatment, the term biosolids specifically refers to sewage sludge that meets standards for beneficial use (U.S. EPA, 1999).

2.3.1.1. Alkaline Stabilization

Purpose of the alkaline stabilization is to reduce the number, prevent regrowth of pathogenic organisms, and reduces the number of odor-producing organisms and to suppress the availability of heavy metals in sludges by the addition of lime. Lime or other alkaline additives such as cement kiln dust, lime kiln dust, Portland cement and fly ash is applied to raise the pH level to make conditions unfavorable for the growth of organisms such as pathogens by providing adequate contact time (Haug et al., 1995; Wong and Su, 1996; Epstein, 2003). Pathogen reduction requirements can be achieved when the pH of the mixture of wastewater solids and alkaline material is maintained at or above 12 after 2 hours of contact (EPA, 2000). In addition, the application of coal fly ash reduces heavy metal availability (Wong and Su, 1996).

Lime stabilization can be a part of a sludge conditioning process prior to dewatering named as prelime stabilization or following a dewatering step referring as postlime stabilization. Both processes are reliable and low in capital cost. Although pathogens, odours are greatly reduced and immobilizing or fixing of specific metal ions are achieved, compared with digestion processes, sludge mass is not reduced, potential for the regrowth of pathogens happen if the pH drops below 9.5 while the material is stored prior to use and the process has a potential for odor generation both at the processing and end use site (Haug et al., 1995; U.S. EPA, 2000) when lime stabilization is used.

2.3.1.2. Anaerobic Digestion

Anaerobic digestion is a sequential process by which organic materials in sludges are fermented by a mixture of fermentative, acetogenic and methanogenic bacteria in the absence of free oxygen to gaseous end products such as methane and carbon dioxide and to a innocous sludge (Dohanyos and Zabranska, 2001; Lucero-Ramirez, 2000). Both mesophilic temperatures (30-38°C) and thermophilic temperatures (50-60°C) are used during the process. The final product is a stable sludge that can be used as a fertilizer in which the pathogen level, the volatile solid content, odour and the volume of sludge are significantly reduced (Dohanyos and Zabranska, 2001; Epstein, 2003). Anaerobic digestion has the potential to cause more odor than other treatment methods if not performed properly (http://www.ext.vt.edu/pubs/compost/452-304/452-304.html). According to USEPA 40 CFR 503 regulation, anaerobic digestion produces either a Class B type biosolids having fecal coliform level of 2×10^6 CFU/g TS at mesophilic temperatures which is more common or Class A biosolids having fecal coliform density less than 1,000 MPN/ g TS or *Salmonella* density less than 3 MPN/4 g of TS at thermophilic temperatures.

In literature, it was stated that proper mesophilic anaerobic digestion results in the reduction of fecal coliform bacteria level below 2×10^6 fecal coliform. *Salmonella* sp. can survive mesophilic anaerobic digestion. The number of organisms can be higher in the digested biosolids than in raw sludge. The survey conducted in New York, USA show that many pathogens and indicator microorganisms survived anaerobic digestion within the fecal coliform levels of 11,000-620,000/100 mL, fecal streptococci levels of 1,100-650,000/100 mL, *Salmonella* densities between 0,8-30/100 mL, *Giardia Lamblia* level of 0-120/100 mL (Epstein, 2003).

2.3.1.3. Aerobic Digestion

Aerobic digestion of sludges involves the direct oxidation of biodegredable matter by aerating the sludge results in the production of end by-products such as carbon dioxide, water and nitrogen (Stentiford, 2001; Epstein, 2003). High thermophilic temperatures are recently being used for the pathogen reduction requirements (Lucero-Ramirez, 2000).

Autothermophilic aerobic digestion process can provide the temperature/time exposure to reduce pathogen indicators. As stated by Epstein, 2003, aerobically digested sludges can meet pathogen requirement of Class B type biosolids having fecal coliform level of 2×10^6 CFU/g TS and be applied. In addition, aerobic digestion is able to reduce the bacteria level by 70-99.99%, viruses by 70-99% and protozoa and helminths by 70%. The control of odour by implementing gas scrubbers, the reduction of volatile solids content, and improvement in the dewaterability of sludges up to 25-30% cake solids are the principal advantages of this stabilization method (Stentiford, 2001).

2.3.1.4. Composting

The philosophy behind the stabilization method of composting is mixing the dewatered sludge with amendments like wood chips to adjust the moisture content and optimizing the conditions for the degrading microorganisms to enhance biological decomposition of organic content of sludges (Krogmann, 2001; Warman and Termeer, 2005). Main current composting technologies are open windrows, open aerated static piles and reactor systems. End products of aerobic degradation are mostly water, carbon dioxide, biomass and stabilized compost. During the degradation, process results in the increase in temperature up to 70^oC which is suitable for the pathogen reduction. The humus like composted material can be applied to land as a soil conditioner or an amendment for growing media (Krogmann, 2001).

2.3.1.5. Heat Treatment

The priniciple of heat treatment is heating the sludge for short periods of time under pressure to coagulate solids, to break down the gel structure and to reduce the water affinity of sludge solids. Sludge is heated in a pressure vessel to temperatures up to 260 ^{o}C at pressures up to 2760 kN/m^2 for approximately 30 min. when the sludge is subjected to high temperatures and pressures, the thermal activity releases bound water and results in the coagulation of solids. In addition, hydrolysis of proteinaceous materials occurs, resulting in cell destruction and release of soluble organic compounds and ammonia nitrogen. This process combines the positive effects of microbial reduction, hygienization and the improvement of dewaterability properities. Main advantages are the destruction of most pathogenic microorganisms, improvement of the solids content of the dewatered sludge ranging from 30-50%, and improvement in the heating value of volatile solids. The major disadvantages are associated with high capital cost and the production of sidestreams with high concentrations of organics, ammonia nitrogen, odours (Metcalf and Eddy, 1991). Pathogenic bacteria, helminth eggs and viruses are reduced to below detectable levels and therefore sewage sludge meets requirements for indicator and pathogen microorganisms when heat treatment is applied (Lucero-Ramirez, 2000).

2.3.2. Sewage Sludge Disposal Methods

2.3.2.1. Landfilling

Landfills are the physical facilities used for the disposal of residual solid wastes in the surface soils of the earth in a series of compacted layers on the land. The wastes are covered, usually daily, with a layer of soil (www.recyclethis.org /QP_Res_dictionary.html).

Landfill sites can be divided into a number of types depending on their nature and mode of operation. In monofills, sludge only material such as the solid in dewatered or liquid state is combined with a processing, dewatering or fixing material like cement kiln dust, lime and fly ash and deposited within the land in a regular manner. In this application dewatered sludge is placed on the land surface for final disposal with daily or final cover (Haug et al., 1995; Banks and Heaven, 2001). Co-disposal sites are those where sewage sludges are accepted for disposal along with wastes from other sources. Surface impoundments and lagoons are disposal sites where sludge with high water content is placed in an open excavated area (Banks and Heaven, 2001). Dedicated disposal sites receive repeated applications of sewage sludges for the purpose of final disposal rather than sludge utilization and often located on the territory of a wastewater treatment plant. Municipal sewage sludges can be used for two beneficial purposes at landfills. Municipal sewage sludge can be substituted for or mixed with topsoil and used to grow a vegetative cover on closed landfills or can also be substitued for soil used for daily cover at a municipal solid waste landfill (Haug et al., 1995). At sludge utilization sites, the fertilizer and soil conditioning properities of the sludge are used to grow crops however at dedicated disposal sites no crops are grown (Haug et al., 1995; Banks and Heaven, 2001).

Many countries decided landfilling as a disposal option. Statistics from the official website of the Statistical Office of the European Communities (EUROSTAT) show that on annual basis in Denmark 13.20% of sludge produced was landfilled in 1998, in Sweden 17.65% of sewage sludges, in Austria 11.61% of sludges produced, in Netherlands 11.23% of sewage sludges, in Czech Republic 18.78%, in United Kingdom 8.03% of sludges produced were landfilled in year 2002, in Spain 17.94% of total sludge produced in 2000 was landfilled.

Present trends in management of sewage sludges show that landfill is the lowest priority and should be used when no alternative exists because of increasing sludge quantities and limited landfilling areas (Wong et al., 2001). The target should be the use of sewage sludges for beneficial purposes.

2.3.2.2. Incineration

Municipal sewage sludge can be incinerated in an environmental conscious manner in an alternative application to landfilling due to progressive exhausting of landfill sites. Incineration is the chemical reaction of oxygen with a combustible material yielding combustion products such as carbon dioxide, water and sulphur dioxide (Mininni, 2001). During incineration, the flue gases created contain the majority of the available fuel energy as heat. The organics in the waste will burn in gas phases when they have reached their necessary ignition temperatures and come into contact with oxygen. The actual combustion process takes place in gas phase in fractions of seconds and simultaneously releases energy (Autret et al., 2006).

Sewage sludges present a potentially exploitable non-fossil fuel and a source of green energy. The calorific value of biosolids is similar to that of brown coal. The average proportion of combustible organic matter in biosolids is 75%. From the energy potential calculation in biosolids produced in a year in United Kingdom, it was declared that biosolids produced in a year represents about 0.3% of national annual consumption accounting for 1 day's energy consumption per year. During incineration, the nitrogen fertiliser value is destroyed and phosphate is converted to recalcitrant forms from which cannot be extracted economically (Bruce and Evans, 2002).

Although it is a convenient way to reduce the volume and mass of sewage sludges and to recover energy, incineration of sewage sludges results in the formation of gaseous, liquid and solid emissions (Mininni, 2001; Khiari et al., 2006). During combustion, the formation of sulphur dioxide in the emissions results in the occurence of sulphuric and sulphurous acids in the atmosphere. Phosphorus present in the sludge is converted to calcium phosphate which constitutes 15% of furnace ash. Nitrogen can be converted to molecular nitrogen or to NO_x. Heavy metals present in the sewage sludge tend to transfer in the gaseous phase and to condense on fine particles during gas cooling. Bottom ashes and air pollution control residues that are mentioned above should be controlled by landfilling of ash and implementing flue gas cleaning system (Mininni, 2001).

Statistical surveys conducted in 2002 by EUROSTAT show that in Austria, Netherlands, United Kingdom and Norway, 50.17%, 57.73%, 19.80% and 15.62% of biosolids were incinerated as a disposal option, respectively (europa.eu.int/comm/eurostat/).

2.3.2.3. Land Application

The application of sludge on both agricultural and non agricultural land is a common practice around the world as a disposal option. Agricultural lands include sites where food crops (for human or animal consumption) and non food crops are grown. Nonagricultural lands include forests, rangelands, and public contact sites such as public parks, golf courses, and cemeteries (National Research Council, 2002). In the past, incineration and landfilling were common practices for disposal. However, limited landfilling areas and the increasing cost of landfill disposal as well as the phasing out of other environmentally unacceptable disposal options, such as ocean disposal, are the reasons encouraging increased use of sludges in agriculture (Harrison et al., 1999; Vasseur et al., 2000; National Research Council, 2002; Oliver et al., 2005).

While land application has the advantage of providing many of the nutrients required for plant growth, its use is influenced by a number of factors, including the source of the sludge, the organic matter content of the sludge, the form in which the sludge is applied and the prevailing conditions of the receiving soils (Urasa and Macha, 1999). Sewage sludges characteristically contain high levels of the major plant nutrients such as nitrogen, phosphorus and potassium, contain valuable micro nutrients such as Fe, Mn, Cu, Zn, Mo and Cl and are often enriched in organic matter (EPA, 2000; Warman et al., 2005). The disposal of sewage sludge on soils as a fertilizer or as a regenerative agent for soil is the most attractive application to reutilize the nutrients for crop production owing to sludges' high content of organic matter (Wong and Su, 1996; Zufiaurre et al., 1998; Wong et al., 2001). Besides being a low cost alternative method for agricultural fields, sludge application results have been shown to benefit crop production increasing the yield and the protein level of crops. In addition to these beneficial results obtained for the crops, soil amended with sewage sludges has been found to keep organic matter and plant nutrients higher for the next crop. Soil water/air holding capacities, soil bulk density, total microorganism population and structure, percent total aggregates, and percent water-stable aggregates are all improved by sludge application (Wang, 1997; Navas et al., 1998; Petersen et al., 2003).

Benefits from sludge application for agricultural purposes have to be weighed against the potential hazards associated with certain sludge-borne constituents such as heavy metals, organic contaminants and pathogens (Sajwan et al., 2003). Although the recycling of the organic matter and nutrients contained in wastewater sludge through land application is a worthwhile objective, presence of chemicals and pathogens in sludges pose risks to human health, agricultural productivity and ecological health (Harrison et al., 1999). Uncontrolled land application of sludge can have ecological harmful effects such as contamination of surface waters by phosphorus and ground water by leaching of nitrates and metals. Retention of sludge-borne heavy metals in soils and their accumulation in plant tissues have caused concerns about their extensive use on cropland. Furthermore sewage sludge tends to increase acidity of the soils as a result of proton release from organic matter decomposition and mineralization of NH_4-N. Increased soil acidity could cause greater solubility of metals and consequently their enhanced plant availability and leaching potential, particularly in soils with poor buffering capacity (Sajwan et al., 2003). Therefore attention should be paid to essential and non-essential trace elements to humans and animals and biological properties of biosolids, such as indicator and pathogenic microorganisms.

2.4. Heavy Metals in Sludges

2.4.1. General

There is an increasing tendency to benefit from waste charateristics of sewage sludges for land application in agriculture despite the fact these wastes may have other properties undesirable for agriculture or may contain significant concentrations of numerous contaminants (McBride, 2003). A serious restriction of the application of municipal wastes for agricultural purposes is due to their high content of toxic metals (Ciba et al., 1999). The potential accumulation of heavy metals in human tissues and biomagnification through the food-chain create both human health problems and environmental impacts (Alvarez et al., 2002).

Pollution problems may arise if toxic heavy metals are mobilized into the soil solution and are either taken up by plants or transported in drainage waters to associated water supplies (Zufiaurre et al., 1998). High levels of heavy metals can result in leading to changes in plant diversity such as weed invasion, productivity and reduced plant growth (Vasseur et al., 2000; Udom et al., 2004). The addition of heavy metals especially to low pH soils and their uptake by plants and ingestion by animals may have health risks (Joshua et al., 1998; Udom et al., 2004). In addition potential heavy metal contamination of soil associated with sludge applications affect bacterial diversity of an agricultural soil by imposing a chronic stress in microbial communities (Moffett et al., 2003).

2.4.2. Sources of Heavy Metals

The potential input of heavy metals ranges from atmospheric deposition to direct, unauthorized, down the drain disposal of hazardous and/or industrial wastes as well as domestic discharges, stream runoff and groundwater infiltration from soil to the sewerage system (Bright and Healey, 2003; Karvelas et al., 2003; Gil et al., 2004; Babel and Del Mundo Dacera, 2005). Industrial sources account for between 30 and 85% of heavy metals in municipal sewage, with domestic sources being food, tap water, detergents, soap, cosmetics, dust, medicine and toilet paper (Whitehouse et al., 2000). The main man made sources of metals in the environment are combustion of fossil fuels, mining and smelting operations, processing and manufacturing industries and waste disposal including dumping, release of domestic sewage and scrap metal handling. Farming and forestry also contribute to the contamination of the milieu by metals due to the use of fertilizers and pesticides (Sandroni et al., 2003).

The distribution ratio of total metal content between the sludge and the water phase depends upon the chemical properties of the metal and of the physicochemical properties of the sludge, and the conditions employed in the sludge treatment process, such as pH, temperature, redox potential, presence and concentration of complexing or precipitating agents (Fytianos et al., 1998). Heavy metals are assumed to be immobile in soil; some factors enhancing their mobility are the properties of the metals, soil texture, pH and competing cations in the soil solution. Heavy metal movement in sewage sludge-treated soils most likely occur where heavy disposal of sewage sludge is made on sandy, acidic, low organic matter soils, receiving high rainfall or irrigation water (Udom et al., 2004).

Source control of industrial and domestic discharges to the sewer systems or extractive removal of metals from the sludge reduces heavy metals concentration in sewage sludges. Control of the processes and materials used in production at the industries; removal and controlled disposal of hazardous constituents before they reach the waste stream; separation of highly contaminated industrial wastewater from the domestic wastewater are the part of the source control and pretreatment of the wastes before discharge to the municipal collection system can be the effective way to decrease heavy metals in sludges (Babel and Del Mundo Dacera, 2005).

2.4.3. Heavy Metal Content of Sludges

The trace elements in sludge that are of greatest concern are arsenic (As), Cd, Cu, Hg, molybdenum (Mo), Ni, selenium (Se), Zn and Pb. Concern for heavy metals originate from their toxicity, non-biodegredability and persistence (Babel and Del Mundo Dacera, 2005). Zn, Cu and Pb are important because they can be phytotoxic. Concern for Cd arises from its possible entry into the food chain, and kidney disorders are the first biochemical signs of Cd toxicity. If these metals move too rapidly in a particular soil, they can pollute ground water supplies, especially in areas with high water table (Udom et al., 2004). Jakobsen et al. (2004) emphasized in their research that obvious cadmium sources in in sewage sludges are from Ni-Cd batteries.

Pb is of somewhat lesser concern because of insolubility and lower bioavailability, unless it’s directly ingested. Air, water, dust, soil and diet are the primary sources (Epstein, 2003). Other metals normally present in wastewater sludge are manganese, iron, aluminum and chromium, as well as a few others less frequently encountered (Jason and McCreary, 2001).

Cu is widely used as an algicide and fungicide. Its use as a spray in vegetable crops is a common practice. Cu enters soil due to its use as a diet additive and thus excretion in the manure. Also industrial wastes add Cu to soil (Epstein, 2003).

The primary natural source of Zn is weathering of the ferromagnesian minerals and sphalerite and also introduced to agricultural soils from phosphate fertilizers as well as atmospherc deposition (Epstein, 2003).

Hg has been widely used as a fungicide in agriculture and horticulture. Hg compounds inhibit bacterial growth and have been used as antiseptics and disinfectants (Epstein, 2003).

2.4.4. Heavy Metal Analyses in Sludge

Sample digestion for heavy metal analysis is mainly carried out by a wet procedure based on an acid digestion with a heated mixture of mineral acids. There are different heating systems that can be used for digestion such as, sand-bath, heating plate, pressure digestion bombs and aluminium blocks. Heavy metal extraction procedure suggested by Standard Methods (1995) includes the use of various acids and acids combinations. Acid combinations; HNO_3 alone, HNO_3 and HCl, HNO_3 and $HClO_4$, HNO_3, $HClO_4$ and HF; are added to sample to enhance the recovery of heavy metals from samples by boiling the samples over the hot plate. The introduction of microwaves, with both open and closed pressurized systems, has allowed a considerable reduction in the total time of analyses as well as in the risk of sample contamination (Sastre et al., 2002). The main differences between microwave digestion and digestion procedure conducted over hot plate are digestion times, recovery percentage, risks of contamination, quanitity of acids used for digestion and control of gas generation and working environment contamination.

In addition, a great variety of extraction schemes both simple and sequential called BCR and Tessier sequential extraction procedure assisted with microwave have been developed. The definition of speciation includes simple and sequential extraction to relate the species associated with particular phases of sludges help to gain more insight into metal behaviour and metal availability in the environment. In the all steps of the method, samples are centrifuged and the supernatant was decanted and stored, samples are then rinsed in distilled water and centrifuged for the next step. However difficulties with this scheme are the lack of phase selectivity, redistribution of analytes between phases and variability between operators (Fuentes et al., 2004).

2.4.5. Literature Studies on Heavy Metals vs Sludges

Several investigations on the quality of sludge in terms of its heavy metal content have been carried out all over the world. Bodzek et al. (1997) studied the use of sewage sludges in agriculture, and investigated heavy metal contents of sewage sludges of four sewage treatment plants in Poland which have anaerobic and aerobic digesters. It was reported that the concentration distribution of metals in sludges can be presented in the order of Zn>Cu>Pb>Ni>Cd.

Sewage sludge samples collected from five different wastewater treatment plants in Canada were studied by Bright and Healey (2003). Sewage sludges are stabilized by anaerobic sludge digestion and three of the wastewater treatment plants are equipped with dewatering facility. Sampling was done immediately after dewatering. The aim of the study is to document the heavy metal concentrations. With respect to heavy metals, Cu has the highest concentration of 1300 mg/kg followed by Zn, Pb, Cr, Ni and Cd.

Aulicino et al. (1998) evaluated heavy metal concentrations of digested sewage sludge samples from domestic wastewater treatment plants located in Italy. The chemical analyses of sewage sludges showed that concentrations of Zn and Cu are predominant for both anaerobic and aerobic digested sludge samples. Zn concentrations were 1520 mg/kg and 1472 mg/kg for anaerobic and aerobic digested sludges, respectively. Cu concentrations were 358 mg/kg and 560 mg/kg for anaerobic and aerobic digested sludges, respectively.

Düring and Gath (2002) reported in their study that mean heavy metal concentrations in sewage sludge of central Germany show similar distribution in the order of Zn>Cu>Pb>Cr>Ni>Cd>Hg from 1992 to 2000. Enezi et al. (2004) evaluated sewage sludges from a municipal wastewater treatment plant in Kuwait to meet the challenges of agricultural use of sewage sludges. Based on the results of this study, mean concentrations of heavy metals were Zn=2002 mg/kg, Cu=700 mg/kg, Pb=337 mg/kg, Ni=111 mg/kg, Cr=80 mg/kg, Hg=58 mg/kg and Cd=21 mg/kg.

Goi et al. (2005) monitored heavy metal contents in sludge coming from 10 different municipal wastewater treatment plants located in Italy. For almost all samples, they reported Cd and Hg concentration measurements below detection limit and they stated that metal concentrations (Zn, Cu, Cr, Pb, Cd and Ni) were found to be below the maximum concentrations permitted by European regulations.

The production, use and quality of sewage sludge in Denmark were studied by Jepsen and Jensen (2005). The concentrations of heavy metals in Danish sludge in the period of 1987-2002 are given. From 1994-2002, Zn concentration was the highest followed by Cu, Pb, Cr, Ni and Cd.

Wasted sludge samples collected from two wastewater treatment plants located in the Greater Thessaloniki Area in Greece were analyzed to evaluate the environmental hazard in the study of Mantis et al. (2005). Chemical anaylses including the determination of seven heavy metals in sludges were conducted in this study. They found that in both sludges examined, the most abundant metal was Zn whereas Cd was the lowest heavy metal.

Land application of sewage sludges in China was reviewed in the study conducted by Wang (1997). Mean heavy metal concentrations of twelve wastewater treatment plant sludges showed that Zn was usually the highest among the heavy metals; and Cd had the lowest concentration in sewage sludges.

Sewage sludges generated in five different cities in Galicia, Spain were examined by Nunez-Delgado (2002). The samples from selected cities consist of both raw sewage sludge samples with no pressure filtration or other treatment and the samples were partially dewatered. The sewage sludge samples were analyzed for Cd, Cr, Cu, Hg, Ni, Pb and Zn. For heavy metals Cd and Ni, the concentrations were below detection limit for almost all cities. Zn has the highest heavy metal concentration among the other heavy metals ranging from 320 mg/kg to 780 mg/kg. Pb content in sewage sludges varied from 200 mg/kg to 300 mg/kg. Cu concentration in sewage sludges were 200-840 mg/kg for five different cities. Cd and Ni concentrations were below detection level.

Oliver et al. (2005) conducted a survey to evaluate similarities and differences between sewage sludges produced and analysed in 1980s and in 2001. 2001 survey was conducted in 18 sewage treatment plants located across Australia. Comparing the 1980s survey heavy metal results with those conducted in 2001, they reported that median values of Cd, Pb and Zn were reduced by 87%, 77% and 58%, respectively. They stated that the cause of decrease in Pb concentrations was due to the change to lead-free petroleum which has been shown to decrease atmospheric Pb levels around cities and industrial areas. Reasons for decreases in other elements may be related to improved industrial processes and the increasingly stringent regulations governing contents of industrial wastes.

Debosz et al. (2002) conducted a survey to quantify the effects of anaerobically digested sewage sludge and composted household waste on selected soil properties, and to describe interactions with ambient climatic conditions. Selected sewage sludge had heavy metals concentrations of Cu, Cr, Cd and Hg as 360 mg/kg, 32.5 mg/kg, 2.4 mg/kg and 3.5 mg/kg, respectively.

Joshua et al. (1998) conducted a study about the potential of contamination of soil and surface waters from sewage sludge in Australia. Dewatered biosolids characteristics at the study area were investigated in this research. Heavy metals concentrations were given as Zn=3060 mg/kg, Cr=308 mg/kg, Ni=166 mg/kg, Pb=323 mg/kg, Cu=1257 mg/kg, and Cd=13 mg/kg.

Navas et al. (1998) described changes in physical and chemical properities of Gypsisols following the application of sewage sludges for land rehabilitation in Zaragoza, Spain. For this purpose, different doses of sewage sludge were applied to the experimental plots. The evaluation of heavy metals concentrations in sewage sludges taken from treatment plant was done. The concentrations of heavy metals were Zn=1036-1214 mg/kg, Ni=54.60-136 mg/kg, Cr=42.10-53 mg/kg, Pb=127-140 mg/kg and Cd=4.00-4.50 mg/kg. After the application of sewage sludges for land reclamation purposes, a pH decrease and a salinity increase were observed. They stated that increases in organic matter and nitrogen and in soil moisture will benefit the growth of vegetation in amended Gypsisols.

Dinel et al. (2000) studied the effect of direct land application of lime treated sewage sludges to soil systems. The study aimed to investigate and compare the chemical partioning of heavy metals in soils after the application of sewage sludges on soils. The sewage sludge used in this study was originated from wastewater treatment plants of the two cities around Canada. The order of heavy metals in sewage sludges was Zn>Cu>Pb>Ni>Cr. They finalized the report that the addition of cement kiln dust biosolids caused enhanced degradation and bioleaching of aliphatic soil organic matter components. They concluded that the reduction in trace metals' immobilization, mobility and leachability could be achived by biostabilizing the biosolids prior to their application on agricultural lands.

Karvelas et al. (2003) investigated the occurence and the fate of Cd, Pb, Mn, Cu, Zn, Fe and Ni during the wastewater treatment process in the wastewater treatment plant of the city of Thessaloniki in Greece. Sludge samples collected were primary sludge, activated sludge and digested/dewatered sludge. Zn appeared to be the most abundant metal whereas Cd exhibited the lowest abundance. They emphasized that Cu, Cd, Pb and Zn contents in digested sludge were 50-99% higher than their contents in undigested sludge. This is due to the weight loss of fresh sludge during anaerobic digestion.

Alvarez et al. (2002) determined the heavy metals concentrations at different sludge treatment steps including primary sludge, secondary sludge, dewatered and digested sludge and composts of five different wastewater treatment plants of the city of Seville, Spain. They indicated that bioavailability and toxicity are dependent on the chemical forms of the heavy metals. Therefore determination of extractable trace metal contents is crutial. They stated that these determinations are of great imporance to track the evolution of the chemical forms of heavy metals throughout the sludge treatment and to suggest their potential disposal options. The highest heavy metals concentrations were found in the digested and dewatered sludges and composts due to the weight loss of fresh sludge during sludge digestion. Zn levels were between 900-1600 mg/kg in digested and dewatered sludge. The group of metals with the second highest levels was comprised of Cu, Cr and Pb. For Cu, the values ranged from 204-326 mg/kg. In the case of Cr, metal concentration ranged from 54.4-439 mg/kg. Levels of Pb were between 179-223 mg/kg for digested and dewatered sludges. They concluded that most of the elements show a clear rise along the sludge treatment in the proportion of two less available fractions (oxidizable and residual metal) peaking in digested/dewatered sludges or compost samples. In contrast Zn shows the highest share of the available fractions (exchangable and residual).

A study carried out by Zufiaurre et al. (1998) on anaerobic digested sewage sludge samples collected at an urban wastewater treatment plant located in Zaragoza, Spain was based on the determination of several metals (Cd, Co, Cr, Cu, Ca, K, Fe, Mg, Mn, Ni, Na, Pb and Zn). With respect to heavy metals, Zn had the highest concentration followed by Cr, Cu, Ni and Pb. Cd has the lowest abundance. In general, metal speciation in sludge samples was associated with the oxidizable and residual fractions which have less mobility and availability. Exchangable and carbonate fractions of metals indicate higher bioavailability. None of the heavy metals limit the use of sewage sludges in agriculture.

Wang et al. (2005) reported heavy metals concentrations of wet anaerobic digested sludges collected from five municipal wastewater treatment plants. Samples were analyzed for the total concentrations of Cd, Cr, Pb, Cu, Ni and Zn. In general, these sludge samples had higher contents of Cu and Zn but relatively lower contents of Cr, Ni, Pb and Cd. Chipasa (2003) studied in a 2 year investigation on the accumulation and removal of selected heavy metals (Cd, Cu, Pb and Zn) by a biological wastewater treatment system. The aim of the investigation was to compare heavy metal contents in the effluent wastewater and to examine the heavy metals in the sludge before and after anaerobic digestion.

The study was concluded in the result that heavy metals in sludge are present as metal precipitates in the sludge flocs; complexes of soluble metal and biopolymers; accumulated soluble metal in the microbial cells and soluble metal ions. The study indicated that that the order of increase in heavy metal contents in digested sludge was Zn<Pb<Cu<Cd.

Fuentes et al. (2004) covered the different types of sludges (aerobic, anaerobic, unstabilised and sludge from a waste stabilisation pond) and determine the heavy metals in the sludges produced in wastewater treatment in Spain. A sequential extraction method consisting of four steps including the detection of exchangable, reducible, oxidisable and residual fractions of heavy metals was used. The aim was to establish the influence of stabilisation method on the mobility of the heavy metals associated to each phase. Total heavy metal concentrations of anaerobic sludge was obtained as Cr=3.809 mg/kg, Zn=871 mg/kg, Cu=337 mg/kg, Pb=167 mg/kg, Ni=29 mg/kg and Cd=18.3 mg/kg, and the results for aerobic digested sludge were Zn=487 mg/kg, Cu=204 mg/kg, Pb=58 mg/kg, Cr=38 mg/kg, Ni=17 mg/kg and Cd=1.10 mg/kg. They concluded that higher degree of mineralisation and stabilisation of sludges showed a lower metal availability index since all the heavy metals in sludges were associated to the oxidisable and residual fractions, which are the least mobile. Unstabilised sludge contained the highest accumulations of heavy metals in the most easily assimilable fractions (exchangeable and reducible).

Direct land application of sewage sludges in agriculture and land reclamation is an economic and environmentally sustainable option for the disposal and reutilization of the nutrient value in sewage sludges. Sewage sludges contain a significant amount of nitrogen, phosphorus, organic matter and other trace elements, represents a good source nutrients for plant growth and a good soil conditioner. But one of the major concerns in the agricultural use of sewage sludges is the enriched heavy metals; therefore it is always in the interest of many environmental agencies in many countries to search the heavy metal content of sewage sludges. Surveys conducted by above mentioned studies show different trends by means of meeting required limit values given by agencies. The concentration of heavy metals in wastewater sludges are affected by the industrial effluents. Applicability results for the sludge utilization show variations due to different production and consumption behaviours in different regions and industrial source inputs in wastewaters. Proper operation of sludge treatment and disposal facilities can directly affect the heavy metals content of sewage sludges. The chemical form of the metal in sludges define its mobilization capacity, transport and bioavailability in sludges and from the results of the above mentioned studies, these chemical forms show changes during the treatment systems.

2.5. Pathogens in Sludges

2.5.1. General

Primary objectives of handling and managing wastewater sludges are to encourage beneficial reuse of wastewater sludge and to ensure that adequate controls are developed to protect the environment and public health. As sludge contains high levels of organic matter and nutrients, their use is limited due to the presence of microorganisms such as bacteria, parasites and viruses because of their survival in the environment and potential risks of groundwater, drinking water and crop contamination during the disposal of sludge on soil (Hu et al., 1996; Aulicino et al., 1998; Santamaria and Toranzos, 2003; Capizzi-Banas et al., 2004).

The primary pathogens found in wastewater and biosolids can be grouped into four major categories: bacteria, enteric viruses, protozoa and helminths. Major pathogens found in wastewater sludges are listed in Table 2. Main sources of pathogens in wastewater are from human and animal wastes discharged into the sewer system and in addition surface runoff combined with the sewer will contain mammalian and avian pathogens (Epstein, 2003). Global and regional conditions such as climate, the state of public health, the presence of hospitals, tanneries, meat processing factories, and abbatoirs found in the area can also affect the type and numbers of pathogens (Dumontet et al., 2001; Epstein, 2003). Some of the factors which influence the survival of pathogens include pH, temperature, competition from other microorganisms, sunlight, contact with host organisms, proper nutrients, and moisture level (EPA, 1999). The principal factors causing pathogen decay or loss of viability during treatment of sewage sludges are temperature, retention period, reactor configuration, microbial competition, pH value and chemical interactions (Smith et al., 2005).

Indicator organisms; Members of two bacteria groups, coliforms and fecal streptococci are used as indicators of possible sewage sludge contamination because they are commonly found in human and animal feces. They indicate the possible presence of pathogenic (disease causing) bacteria, viruses, and protozoans that also live in human and animal digestive systems. Since it is a time consuming and an expensive test to identify the presence of a large variety of pathogens, water is usually tested for coliforms and fecal streptococci instead. Fecal coliforms are a subset of total coliform bacteria and are more fecal specific origin. Fecal streptococci generally occur in the digestive systems of humans and other warm-blooded animals. Although fecal streptococci are not ideal as indicators of fecal contamination, these organisms are relatively easy to enumerate and survive longer than fecal coliforms (Lucero-Ramirez, 2000).

Table 2. Major Pathogens in Municipal Wastewater and Sewage Sludge (Epstein, 2003)

Pathogen Class	Examples	Disease
Bacteria	*Campylobacter jejuni*	Gastroenteritis
	Enteropathogenic *Escherichia Coli*	Gastroenteritis
	Mycobacterium tuberculosis	Tuberculosis
	Salmonella spp.	Salmonellosis Gastroenteritis
	Salmonella typhi	Typhoid fever
	Shigellae	Shigellosis Bacterial dysentery
	Vibrio chlorea	Cholera
	Yersinia spp.	Yersinosis
Viruses	Coxsackievirus	''flu like'' symptoms
	Echovirus	''flu like'' symptoms
	Hepatitis viruses	Infectious hepatitis
	Polio virus	Poliomyelitis
	Reovirus	Acute gastroenteritis
	Rotaviruses	Acute gastroenteritis
Protozoa	*Balantidium* coli	Balantidiasis
	Cryptosporidium spp.	Gastroenteritis
	Entamoeba histolytica	Amoebic dysentry
	Giardia lamblia	Giardiasis
Helminths	*Ascaris sp.*	Ascariasis
	Necator americanus	Ancylostomiasis
	Taenia sp.	Taeniasis
	Trichuris trichura	Trichuriasis

The fecal coliform to fecal streptococcus ratio is a good test for determining the origin of the bacteria in the analysis of wastewater. A fecal coliform to fecal streptococcus ratio less than or equal to 7:10 indicates the origin of waste to be from animals other than human or the ratio greater than or equal to 4:1 indicates the origin to be human waste (Cox et al., 2000).

Rather than testing the samples directly for pathogens, which can be difficult, expensive and even hazardous, the possibility of the existence of fecal contamination can be assessed by detecting indicator microorganisms such as fecal coliform and fecal streptococcus.

Pathogenic bacteria; The pathogenic bacteria of major concern are *E.Coli* (pathogenic strains), *Shigella spp.*, *Salmonella spp.* and *Vibrio cholerae* (Epstein, 2003). *Salmonella* are the most widespread bacterial pathogen of significant global public health concern that is likely to cause an important sewage sludge contamination. *Salmonella* sp. is Gram-negative, flagellate and motile rods and is facultative anaerobes. Two serotypes of *Salmonella*, *S. typhi* and *S. paratyphi* (A, B, C) are most dangerous to people (Lucero-Ramirez, 2000).

Viral pathogens; More than 100 different types of viruses excreted by humans may be absorbed on sludge organic matter and thereby protected from inactivation. In addition to human viruses, animal viruses present from birds, dogs, and cats may reach sewage system, may then contaminate wastewater, to the detriment of human health. Among viruses of human concern found in sewage and sewage sludge, the occurrence and prevalence of hepatitis A virus, hepatitis E virus and polio virus have been extensively studied (Dumontet et al., 2001).

Protozoan parasites; Giardia lamblia and *Cryptosporidium parvurm oocysts* are protozoan parasites that can infect the digestive tract of humans and other warm blooded animals. Semi-aquatic mammals can serve as hosts, transmitting the disease to humans who consume contaminated water. Domestic mammals (particularly ruminants) can serve as infective hosts and contaminate a drinking water supply (EPA, 1999). The formation of a resistant cyst during the life cycle provides protozoan parasites to survive from chlorination and filtration of water to cause a diarrheal illness (Marshall et al., 1997; Steiner et al., 1997).

Helminth eggs; Helminths exist in at least two forms. The first is an actively growing form inside the host (i.e., the worm), which produces eggs or ova. The ova pass from the host in the feces and constitute, or develop into, a second form (the larvae), which is resistant to adverse conditions and infects a new host and establishes new growth (Lucero-Ramirez, 2000). Significance of helminth eggs in human health and in wastewater is the eggs of worms are found in insufficiently treated sewage fertilizer and eggs may contaminate crops grown in soil or fertilized with sewage and then humans are infected when such crops are consumed raw (Davutluoğlu, 2005). The pathogenic helminths whose eggs are of major concern in wastewater and sludge include *Ascaris lumbricoides*, *Ascaris suum*, *Trichuris trichiura*, *Toxocara canis*, *Toxocara cati*, *Taenia saginata*, *Taenia solium*, and *Hymenolepis nana*. *Ascaris lumbricoides* eggs are particularly important as indicator of the hygienic quality of biosolids as Ascariasis is one of the most widespread excreta-related infections in low-income areas and are the most resistant among the gastro intestinal diseases (Sanguinetti et al., 2005).

2.5.2. Pathogens vs. Type of Sludges

The pathogens in domestic sewage are primarily associated with insoluble solids. Primary wastewater treatment processes concentrate these solids into sewage sludge, so untreated or raw primary sewage sludges have higher quantities of pathogens than the incoming wastewater. Biological wastewater treatment processes such as lagoons, trickling filters, and activated sludge treatment may substantially reduce the number of pathogens in the wastewater. These processes may also reduce the number of pathogens in sewage sludge by creating adverse conditions for pathogen survival (EPA, 1999; Epstein, 2003). Because land application of sewage sludges requires disinfection and stabilization of sewage sludges, many investigators studied the virological quality of sludge produced by sewage treatment plants.

Aulicino et al. (1998) evaluated both anaerobically and aerobically digested sewage sludges from domestic wastewater treatment plants located in Italy for the presence of fecal coliform, salmonella, enteric viruses and helminth eggs. Fecal coliform densities varied from 2.0×10^2 to 2.0×10^6 MPN/g TS for aerobic digested sludges and from 2.0×10^2 to 7.0×10^6 MPN/g TS for anaerobic digested sludges. Only two of the samples out of ten samplings showed Salmonella existence. Helminth eggs were isolated only from two anaerobic digested sludges out of 10 samples.

Sahlström et al. (2004) surveyed the presence of bacterial pathogens in eight Swedish treatment plants with four different treatment methods. Salmonella was found in 38 samples out of 69 samples that account for 55% of samples. Mean levels of Coliforms in mesophilic anaerobic digested sludge is approximately 5×10^4 CFU/g TS.

George et al. (2002) reported in the study for the estimation of fecal coliform removal efficiency of various types of treatments that typical abundance of fecal coliforms in raw sewage is in the level 10^6-10^8 CFU/100 mL. Classical treatment reduces fecal coliform densities by 1-3 orders of magnitude.

Straub et al. (1993) informed data about the influence of aerobic and anaerobic digestion on pathogen reduction and concluded that *Salmonella* concentration in anaerobic digested sludge varied from 3 to 10^3/g TS, fecal coliforms ranged from 10^2-10^6/g TS and *Giardia* spp. varied from 10^2-10^3/g TS. *Salmonella* concentration in aerobic digested sludge was 3/g TS, fecal coliforms ranged from 10^5-10^6/g TS and *Giardia* spp. was not detected.

Hong et al. (2004) evaluated the efficiency of microwaves in destructing pathogens in sewage sludges, sludge samples used for the test were obtained from a wastewater treatment plant in USA and results indicated that fecal coliform level in anaerobic digested sludge was 1.78×10^5 CFU/g TS. The study indicated that microwave radiation application for 60 seconds readily reduces fecal coliforms to non detectable levels.

Watanebe et al. (1997) reported that fecal coliform level in digested sludge was 10^3 MPN/g TS and number of salmonella in mesophilic anaerobic digested sludge was ranged from 1.8 to 30 MPN/g TS. Bukhari et al., 1997 investigated occurunce of *Cryptosporidium* spp oocysts and *Giardia* spp in sewage effluent from seven treatment works. Of these samples, 27.2% of samples were positive for the presence of *Giardia* and *Cryptosporidium.* When considering individual sewage treatment works, the percentage of influents positives for *Cryptosporidium* ranged from 0-63.6% and 70-99.9% for *Giardia* cysts.

Berg and Bergman (1980) determined the concentration of fecal coliform, fecal streptococci and viruses in mesophically and thermophically digested anaerobic sludges in a wastewater treatment plant from the City of Los Angeles. Fecal coliform levels varied from 1.5×10^6 CFU/100 mL to 1.0×10^7 CFU/100 mL for mesophilic anaerobic digested sludge and $<3.0\times10^2$-9.5×10^4 CFU/100 mL for thermophilic anaerobic digested sludge, fecal streptococci was 1.5×10^6-4.0×10^6 CFU/100 mL for mesophilic anaerobic digested sludge and $<6.7\times10^2$-6.0×10^4 CFU/100 mL and recoveries of viruses range between 30-400 PFU/100 mL for mesophilic anaerobic digested sludge and <1.4-17 PFU/100 mL for thermophilic anaerobic digested sludge.

Studies indicated in the reference book of Epstein (2003) show that densities of fecal coliform, fecal streptococcus and *Salmonella* sp. in four wastewater treatment plants with aerobic digestion. The range of fecal coliform densities was from 5×10^4 MPN/g TS to 4×10^6 MPN/g TS with an average density of 1.7×10^6 MPN/g TS. Fecal streptococcus densities ranged from 3 MPN/g TS to 3×10^4 MPN/g TS with an average density of 8.5×10^5 MPN/g TS. Salmonella densities varied from plants to plants. Two of the four plants had densities of 80-82 MPN/4g TS, and the other two had densities of 2340-3840 MPN/4g TS.

Sidhu et al. (2001) reported that wastewater biosolids generally contain Salmonella at a level of 10^2 -10^3/g TS. However, the concentration of Salmonella in dewatered, anaerobically digested wastewater sludge can be more than 10^5/ g TS.

The agricultural utilization of sewage sludge is more common in many countries mentioned above. As seen from the results, most of the pathogens are reduced in number but not completely from the samples ready to be applied on land. They are accumulated by sedimentation processes in the sewage sludge. Moreover, the pathogen level in sludge is influenced by the health of the population, type of stabilization method applied to the sludge. The typical abundance of pathogens during treatment is affected by the retention time of sludges (Epstein, 2003). Although numbers of pathogens are reduced after the stabilization of sewage sludges, sewage sludge produced in many countries did not have a quality that fulfils criteria for unrestricted use in agriculture.

2.6. Other Parameters Related to Land Application of Sludge

Although heavy metal characteristics and biological properities of biosolids play an important role in the land application of biosolids, some physical and chemical properities affect the method of application. The important physical characteristics are solid content and organic matter content. The importance of physical characteristics of biosolids is the effects on plant growth and effects on the availability and the accumulation of plant nutrients and trace elements. Chemical properities such as pH, soluble salts, plant nutrients (N, P) and organic chemicals affect the plant growth as well (Epstein, 2003).

2.6.1. Plant Nutrients

Plant nutrients are among the most important chemical characteristics of biosolids Farmers value biosolids for the nitrogen (N) and phosphorus (P) content (Epstein, 2003).

The nitrogen components in biosolids are predominantly organic. These have been identified as proteinaceous, amino acids and hexosamines. When biosolids are applied to land, organic nitrogen goes into numerous transformations which affect plant growth, microbial activity and reactions through the soil. The transformation of nitrogen in soils are affected by the moisture content of soil, temperature of soil, rate of mineralization, oxidation, aeration, soil porosity, biosolids characteristics and rate of microbial activity. The rate of nitrogen mineralization is important in determining the rate of biosolids application, potential for crop uptake, and potential for leaching. USEPA regulation requires that the rate of biosolids application be in relation to the crop requirement for nitrogen. This restriction is required to prevent excess nitrogen and prevent leaching to groundwater (Epstein, 2003).

Phosphorus is an essential plant nutrient. Deficiency in P is resulted in the existence of soil fertility problem throughout the world. Excessive amounts of P tend to immobilize other chemical elements such as Zn and Cu that are essential to plant growth and can result in nonpoint source pollution of surface waters. Organic P must undergo mineralization in the soil before plant can take it up. Inorganic P is predominant in biosolids. When biosolids are applied at rates consistent with the nitrogen requirement of the crop, excessive P is applied. The accumulation of P results in eutrophication and potentially impact water bodies (Epstein, 2003).

2.6.2. Organic Matter and Organic Chemicals

Land application replenishes valuable organic matter, which occurs in less than optimum amounts in soils. The addition of organic matter can improve soil tilth, the physical condition of soil as related to its ease of tillage, fitness as a seedbed, and its impedance to seedling emergence and root penetration. The increase of water infiltration into the soil and soil moisture-holding capacity, soil compaction reduction, the increase of the ability of the soil to retain and provide nutrients, reduction in soil acidification can be listed as the advantages of the addition of organic matter (Epstein, 2003).

Biosolids contain toxic organic chemicals principally as discharges from industrial sources but also from atmospheric deposition. When organic compounds enter the wastewater treatment system, they can undergoe reductions or transformations prior to being deposits in biosolids that will be applied to land. The organic compounds of greatest concern are toxic chlorinated compounds, alkylphenol ethoxylates, volatile organic compounds, dioxin or dioxin like compounds, phthalates, polycyclic aromatic hydrocarbons (PAHs) and pesticides. The chlorinated compounds of major concern are polychlorinated biphenyls (PCBs) which are very persistent and bioaccumulate. When biosolids containing toxic organic compounds are applied to land, the compounds undergo numerous transformatons and reactions. These can effect their movement through the soil to water sources, uptake by plants, volatilization to the atmosphere, accumulation in soil biota (Epstein, 2003).

2.7. Regulations for Treated Sewage Sludge Land Application in U.S., European Union and Turkey

2.7.1. U.S. Regulation of Treated Sewage Sludge

In the United States, the use and disposal of treated sewage sludge (biosolids), including domestic septage, are regulated under 40 CFR Part 503. This regulation, promulgated on February 19, 1993, was issued under the authority of the Clean Water Act (CWA) as amended in 1977 and the 1976 Resource Conservation and Recovery Act (RCRA) (U.S. EPA, 1999).

Subparts of the regulation established standards which consist of general requirements, pollutant limits, management practices, and operational standards, frequency of monitoring, recordkeeping and reporting requirements for the final use (land application) or disposal (surface disposal and incineration) of sewage sludge generated during the treatment of domestic sewage in a treatment works (Iranpour et al., 2004, Biosolids Management Handbook, 1999).

The rule applies to publicly owned treatment works (POTW) with a design flow of 1 mgd or greater, POTWs serving for the population of 10,000 people or greater facilities (Iranpour et al., 2004).

In 40 CFR Part 503, land application is defined as the beneficial use practices include application to agricultural land (the production of food, feed and fiber crops), non agricultural land (forests, parks and golf courses), disturbed lands (mine spoils, construction sites and gravel pits) and home lawns and gardens (Biosolids Management Handbook.

The Part 503 Biosolids rule limits the use of sewage sludges for the land application in three categories; pollutant concentrations, pathogen densities and vector attraction potential (U.S. EPA, 1999).

Specific pollutant concentrations were set for nine heavy metals. Table A1 represented in Appendix A, lists the standards for metals. Bulk sewage sludge can be applied to land if the pollutant ceiling concentration and cumulative pollutant loading rate or pollutant concentration limits are met. Bulk sewage sludge applied to lawns and home gardens must meet the pollutant concentration limits. Sewage sludge sold or given away in bags or other containers must meet the pollutant concentration limits or the ceiling concentration and be applied at an annual sewage sludge product application rate that is based on the annual pollutant loading rates (U.S. EPA, 1999).

The pathogen reduction requirements are operational standards for two classes of pathogen reduction: Class A and Class B biosolids. Class A sewage sludge must meet the fecal coliform density of less than 1,000 MPN/ g TS, and that must be satisfied immediately after the treatment process is completed or the *Salmonella* density of less than 3 MPN/4 g of TS, and that must be satisfied immediately after the treatment process is completed. In addition, one of the following treatment processes shown in Table A2 in Appendix A, listed as alternatives must be met to reduce pathogen densities below specified detection limits for *Salmonella* sp. <3 MPN/4 g TS, enteric viruses <1 PFU/4 g TS, and helminths <1 viable organism/4 g TS. All sewage sludges that are to be sold or given away in a bag or other container for application to the land, or applied to lawns or home gardens must meet Class A pathogen requirements (U.S. EPA 1999).

The regulations for Class B require that at least seven samples should be collected at the time of use or disposal and analyzed for fecal coliforms during each monitoring period. The geometric mean of the densities of these samples will be calculated and should meet the restriction of fecal coliforms<2,000,000 MPN/g TS or fecal coliforms<2,000,000 CFU/g TS or the sewage sludge must be treated by Processes to Significantly Reduce Pathogens (PSRP) listed in Table A3 in Appendix A, or PSRP equivalent process. In addition, for any land applied sewage sludge that meets Class B pathogen reduction requirements, but not Class A requirements, the site restriction requirements given in Table A5 in Appendix A should be met (Biosolids Management Handbook). Class B biosolids require a significant reduction of pathogen densities but direct human exposure to Class B still pose a health risk (Iranpour et al., 2004).

Irrespective of the class of pathogen reduction, all biosolids must meet one of the vector attraction reduction options. The objective of vector attraction reduction is to prevent disease vectors such as rodents, birds, and insects from transporting pathogens away from the land application site. Ten alternative methods for meeting the vector attraction reduction requirement imposed by Part 503 is represented in Table A4 in Appendix A. These options reduce the attractiveness of the biosolids to vectors with specified organic matter decomposition processes (e.g., digestion, alkaline addition) and prevent vectors from coming into contact with the biosolids (e.g., biosolids injection or incorporation below the soil surface within specified time periods) (http://www.ext.vt.edu/pubs/compost/452-302/452-302.html).

In addition to the two major levels of biosolids disinfection; Class A and Class B, the term Exceptional Quality (EQ) biosolids has been accepted as a convenient way to describe the sludges which meet the Class A pathogen reduction requirements in Table A2 in Appendix A, the pollutant concentration limits in Table A2 in Appendix A and one of the vector attraction reduction options in Table A4 in Appendix A, EQ biosolids can be freely applied to the land (Iranpour et al., 2004).

Class A, Class B and EQ biosolids should meet monitoring, recordkeeping and reporting requirements.

Radioactivity and dioxin issues and organic chemicals in biosolids are not covered in the existing regulation.

2.7.2. European Union (EU) Regulation of Treated Sewage Sludge

The land application of wastewater sludge is regulated by 86/278/EEC in EU. The aim of the directive is to regulate the use of sewage sludge in agriculture to prevent harmful effects on soil, vegetation, animals and man (86/278/EEC). The directive has 18 articles and related annexes (86/278/EEC) which limit the values for concentrations of heavy metals in soil and sludge and the amount of heavy metals which may be added annually to agricultural land based on a 10-year average. In addition to the limit values, there are some policies for the sludge and soil analysis covering the relevant parameters, the frequency of the analysis (86/278/EEC). In a working document published in 2000, additional regulations and revisions for organic pollutants, pathogens and treatment processes have been proposed. These are: 'AOX' so-called 'sum of halogenated organic compounds'; linear alkylbenzene sulphonates (LAS); di(2-ethylhexyl)phthalate (DEHP); 'NPE' (nonylphenol and nonylphenol ethoxylates with 1 or 2 ethoxy groups); polynuclear aromatic hydrocarbons (PAHs); polychlorinated biphenyls (PCBs); and polychlorinated dibenzo-p-dioxins and -furans (PCDD/Fs) (Working document, 2000).

All member states have a chance to adapt more stringent standard values according to the 86/278/EEC directive. Table A5 in Appendix A, indicates the differences in the limitations on heavy metal concentrations between the member states (National Research Council, 2002). As indicated in Table A5 in Appendix A, the countries in which the limitations on heavy metal concentrations are the most stringent are Denmark, Finland, the Netherlands, and Sweden. Greece, Luxembourg, Ireland, Italy and Spain have set limit values similar to those in the directive. The United Kingdom legislation differs by not providing any limit values for heavy metals in biosolids but rather specifies the maximum annual average loads of heavy metals to soil that are similar to the directive represented in Table A6 in Appendix A (National Research Council, 2002).

In addition, the regulations on biosolids use include limit values for pathogens represented in Table A7 in Appendix A in France, Italy, and Luxembourg and, for organic compounds in Austria, Belgium Flanders, Denmark, France, Germany, and Sweden, neither of which are included in the 86/278/EEC directive (National Research Council, 2002).

In all member states, regulations on the use of sludges specify limit values for heavy metals in soil that are similar in most cases to the requirements set in the directive as shown in the Table A8 in Appendix A. Some countries have defined limit values for several categories of soil pH or limit the maximum load of heavy metals to agricultural lands on a 10-year basis. For example, maximum quantities of sludges that can be applied on land have been set between 1 metric ton by the Netherlands for grasslands and 10 metric tons by Denmark per hectare and per year (National Research Council, 2002).

The use of biosolids in soils where the concentrations of heavy metals exceed the limit values suggested in Table A8 in Appendix A would be allowed only on a case-specific basis, and member states would have to ensure that those limit values are not exceeded as a result of the use of biosolids. If the concentrations of one or more heavy metals in biosolids are higher than the concentration limits suggested in Table A5 in Appendix A or if the concentrations of one or more organic compounds in biosolids are higher than the concentration limits proposed, the use of biosolids should not take place (National Research Council, 2002).

Austria, Belgium-Flanders, Denmark, France, Germany and Sweden have included limits for organic compounds; polychlorinated biphenyls (PCBs), Adsorbable Organohalogens (AOX), Linear Alkyl Sulfonate (LAS), Diethylhexylphthalate (DEHP), Nonyl Phenol Ethoxylate (NPE) and Toluene (National Research Council, 2002).

2.7.3. Turkish Regulation of Treated Sewage Sludge

The standards related to the land application of sludge in Turkey was set under the name of Soil Pollution Control Regulation (SPCR) and put into effect in December 2001. This regulation has 21 articles defining the technical and administrative principles of sewage sludge use in land application and 7 annexes declaring standards for the heavy metal concentrations in soil according to pH, maximum allowable heavy metal concentrations in sludge which is applied to soil, and maximum load of heavy metals applied to soil on a 10-year basis, limit values for pollutants other than heavy metals (SPCR, 2001). The limit values for heavy metals and other pollutants (including polycyclic hydrocarbons, organochlorinated compounds, PCBs) in soil and also heavy metals in sludge can be found as Table A9- A12 in Appendix A. The regulation also contains forms and methods for soil and sludge analysis and permission form for sewage sludge usage. In addition, SPCR strictly prohibits the use of untreated wastewater sludges.

2.7.4. Comparison of US, EU and Turkish Regulation

SPCR of Turkey differs from regulations in U.S. and EU in several aspects. The main difference in Turkish regulation from the US regulation is the absence of the limits for pathogen densities. However, the EU 1986 main directive does not specify limits on pathogens, member states adopted standards on pathogens. Pathogen limits in U.S. are defined for Fecal Coliform, *Salmonella*, Helminth ova and Enteric viruses whereas many member states in EU specify limits for Enteroviruses, Enterobacter and *Salmonella*. The proposed regulation developed in 2000 in EU are more specific towards pathogen reductions, treatment processes and site restrictions in land application (Iranpour et al., 2004). For example, limits for E. Coli and *Salmonella* will be defined and added according to the proposed limitation given in Working Document (2000).

Turkey has adopted similar limit values with the EU 1986 Directive for the heavy metal concentrations in soil according to pH, maximum allowable heavy metal concentrations in sludge which is applied to soil, and maximum load of heavy metals applied to soil on a 10-year basis except for Chromium which is not regulated in EU 1986 Directive. Some heavy metals are regulated stricter in EU (Cu, Cd, Hg and Zn) and others in USA (Pb). United States regulations include three more metals (As, Mo, and Se) regulated in addition to the metals regulated in EU.

CHAPTER 3

MATERIALS AND METHODS

3.1. Study Area and Sampling of Sludges

Samples of sewage sludge were collected from four wastewater treatment plants of various sizes. They were Ankara, İzmir, Kayseri and Tekirova wastewater treatment plants. The plants differ both in the wastewater treatment and sludge treatment technologies employed, serving populations and in industrial inputs. Another important issue in selecting study areas is the ease of access of the sewage samples to the laboratory. Main process characteristics of the wastewater treatment plants considered and sludge sample collections from these plants are described in the following sections. Table 3 is the summary of some properties of selected wastewater treatment plants.

3.1.1. Ankara Wastewater Treatment Plant

The wastewater treatment plant of Ankara serves about 3,000,000 residents by treating daily 250,000 m^3 of municipal wastewaters. Treatment plant receives about 15% industrial inputs from Ostim and İvedik Organized Industrial Zones. The treatment process includes pretreatment station, grit and scum removal, primary sedimentation, conventional activated sludge with sludge retention time of 4 days. Total sludge production is 220 ton/day. Sludge generated from primary and secondary clarifier are combined and thickened in a thickener tank and then sent to mesophilic anaerobic digester with sludge retention time of 22 days to stabilize the sludge. Finally the sludge is dewatered in belt filters. Dewatered sludges have been spreaded on land for drying since 2005. Flow chart of the wastewater treatment plant of Ankara is shown in Table C1 in Appendix C.

Table 3. Properties of selected wastewater treatment plants

Properties	**Ankara WWTP**	**İzmir WWTP**	**Kayseri WWTP**	**Tekirova WWTP**
Serving population	3,000,000	4,000,000	525,000	20,265
Inflow rate (m^3)	250,000	600,000	135,000	
Industrial inputs (%)	15	20	25	none
Wastewater Treatment Units	S+GC+PST+ AT+SST	S+GC+PST+ NRT+AT+SST	S+GC+OT+PST+ NRT+AT+SST	GC+OP+SST+C
Sludge treatment units	T+AD+BF	T+BF+LS	T+AD+BF	T+BF
Sludge production (ton/day)	220	700	52	9

(*S: Screen, SST: Secondary settling tank, OT: Oil trap, PST: Primary settling tank, AT: Aeration tank, NRT: Nutrient removal tank, T: Sludge thickening, AD: Anaerobic sludge digestion, BF: Belt filter, LS: Lime stabilization, OP: Oxidation pond*)

Dewatered sludge samples of approximately 3 kg were transferred to the laboratory with plastic boxes immediately after sampling and stored at the refrigerator at 4°C. Bacterial analysis began immediately after sampling. Sampling was conducted to represent the seasonal changes in selected parameters. 5 sampling was done in May, July, October, and November in 2005 and in January, 2006 for heavy metal analysis. For the detection of microbiological parameters; fecal coliforms and fecal streptococci, sampling was done in July, October, and November in 2005 and in January and April in 2006. For *Cryptosporidium* and

Giardia, detections were done for samples taken in July, October, November and December, 2005 and in April, 2006.

3.1.2. İzmir Wastewater Treatment Plant

The wastewater treatment plant of İzmir serves about 4,000,000 residents by treating daily 600,000 m^3 of municipal wastewaters. Treatment plant receives about 20% industrial inputs mainly generated from food preparation and processing, the leather and textile industries, metallurgy, marbel production, alcoholic beverage productions. The treatment process includes fine screen, grit removal, primary sedimentation, advanced biological treatment with biophosphorus tank followed by aeration tank with hydraulic retention time of 1.1 hours for anaerobic sludge, 3.3 hours for anoxic sludge and 3.4 hours for aerobic sludge. Total sludge production is 700 ton/day. Sludge collected from primary and secondary clarifiers are collected in a tank then transferred to the thickening unit after which, belt filter press is used for dewatering. The final treatment applied to the dewatered sludge is the lime stabilization. Dewatered sludge is mixed with 10% by dry weight basis of lime. Then lime stabilized dewatered sludges have been landfilled as a final disposal option. Processes used for wastewater and sludge treatment are schematically represented in Table C2 in Appendix C.

Lime stabilized dewatered sludge samples of approximately 3 kg were transferred to the laboratory with plastic boxes surrounded with ice bags within 18 hours after sampling and stored in the refrigerator at 4^{o}C. Bacterial analysis began immediately after the transportation to the laboratory. Lime stabilized dewatered sludge sampling was conducted to represent the seasonal changes in selected parameters. 5 sampling was done in March, May, and December in 2005 and in February and July, 2006 for heavy metal analysis. For the detection of microbiological parameters, 3 sampling was done in December, 2005 and in

February and July, 2006. For *Cryptosporidium* and *Giardia,* detections were done for samples taken in February and June 2006.

3.1.3. Kayseri Wastewater Treatment Plant

The wastewater treatment plant of Kayseri serves about 525,000 residents by treating daily 135,000 m^3 of municipal wastewaters. Treatment plant receives about 15% industrial inputs generated from wood processing and furniture production and textile industries. The treatment process includes pretreatment station, grit and scum removal, primary sedimentation, nitrogen and phosphorus removal units and an extended aeration tank with sludge retention time of 20 days. Total sludge production is 52 ton/day. In Kayseri, primary sludge is transferred to sludge thickening tank from which it is transferred to anaerobic sludge digestion with sludge retention time of 20 days for stabilization. Stabilized sludge is transferred to a second thickening tank, and then is sent to belt press for dewatering. Secondary sludge is directly transferred to belt filter press for dewatering. Flow chart of the wastewater treatment plant of Kayseri is shown in Table C3 in Appendix C.

Dewatered sludge samples of approximately 3 kg were transferred to the laboratory with plastic boxes surrounded with ice bags within 18 hours after sampling and stored in the refrigerator at 4^{o}C. Bacterial analysis began immediately after the transportation to the laboratory. Dewatered sludge sampling was conducted to represent the seasonal changes in selected parameters. 3 sampling was done in April and December in 2005 and in May, 2006 for heavy metal analysis. For the detection of microbiological parameters, 2 sampling was done in December, 2005 and in May, 2006. For *Cryptosporidium* and *Giardia,* detections were done for samples taken in December, 2005 and in May, 2006.

3.1.4. Tekirova Wastewater Treatment Plant

The wastewater treatment plant of Tekirova serves about 20,265 residents. Treatment plant does not receive any industrial inputs. The treatment process includes grit removal, oxidation pond with sludge retention time of less than 15 days to increase oxygen concentration and then the effluent is chlorinated. Total sludge production is approximately 8-9 ton/day. Sludge collected from secondary clarifier is then transferred to the thickening unit after which, belt filter press is used for dewatering. In the scheme, no stabilization process is applied to sludges currently. Processes used for wastewater and sludge treatment are schematically represented in Table C2 in Appendix C.

Dewatered sludge samples of approximately 3 kg were transferred to the laboratory with plastic boxes surrounded with ice bags within 18 hours after sampling and stored in the refrigerator at 4^{o}C. Bacterial analysis began immediately after the transportation to the laboratory. Dewatered sludge sampling was conducted to represent the seasonal changes in selected parameters. 2 sampling was done in March, May, and December in 2005 and in February and July, 2006 for heavy metal analysis. For the detection of microbiological parameters, 3 sampling was done in January, April and June, 2006. For *Cryptosporidium* and *Giardia,* detections were done for samples taken in January and June, 2006. Due to the seasonal changes in wastewater quantity, in winter, dewatering facility was not operated therefore activated sludge samples were taken in April, 2006.

As mentioned above, sampling and analyses frequency of sludge samples were differed in microbiological parameters and heavy metals. In the initial steps of experimental step of the study, heavy metal analyses are finished completely. After the completion of analysis of heavy metals, detection of microbiological parameters in sewage sludges of four wastewater treatment plants were finished.

3.2. Heavy Metal Analyses

An extraction procedure for heavy metals from sludges was based on a microwave assisted wet digestion procedure using several mineral acids to extract the heavy metals from sludges. The analytical determination of heavy metals Cd, Cu, Cr, Pb, Zn, Ni and Hg which were regulated under SPCR in Turkey was carried out by ATI Unicam 929 flame atomic absorption spectrometry with electrode discharge lamps. Hg analyses of optimization trials were done by Perken Elmer AAnalyst 800, equipped with FIAS 100 flow injection hydride system in TÜBİTAK and the rest of the analyses for Hg were conducted in Central Laboratory of Middle East Technical University.

The microwave digestion system that is used to extract heavy metals from sludges is *Berghof Speedwave MWS-2 Microwave Pressure Digestion Unit* suitable for conducting chemical digestion under extreme pressure and temperature conditions can be utilized for digestion by using nitric acid (65%), hydrochloric acid, hydrofluoric acid or combinations of these acids. *Berghof Speedwave MWS-2* system consists of pressure digestion vessels manufactured completely from Teflon. This means that, in addition to chemical resistance to all mineral acids, a high mechanical stability is supplied at high digestion temperatures. The pressure digestion vessels with 60 mL capacity are resistant to 40 bar of pressure and can be operated at temperatures up to 220 ^{o}C.

The system uses an infrared thermometer built into the microwave oven allowing a reliable recording and rapid control of the temperature of the contents of the vessel. The temperature in the vessels is recorded by measuring the infrared radiation of the sample. This supplies a sufficient, rapid and accurate temperature measurement of the sample to be heated and complete absence of contamination.

A method development of microwave digester should be done to get accurate results for heavy metal concentrations. An optimization strategy was followed according to the literature surveys and manufacturer's recommendations. The optimization of the microwave digestion system was succeeded by adjusting the operating parameters, heating time, temperature and power with reference sludge having certified concentrations of heavy metals (Cat #: CRM029-050) supplied by Resource Technology Corporation (USA) and sediment reference material (IAEA-SL-1) having certified concentrations of heavy metals supplied by International Atomic Energy Agency. Different programs labeled as A, B, C, D and E were tested with the certified reference sludge (Cat #: CRM029-050) and sediment reference material (IAEA-SL-1) and various acids and acid combinations. Certified reference sediment which we initially had was only used in the earlier runs with programs A and B until we get a certified reference sludge sample. Program C is actually same as program A, only difference is the use of reference sludge instead of reference sediment material. A satisfactory microwave-assisted digestion could be obtained by using one or several of the following reagents: sulphuric acid (H_2SO_4) with or without a catalyst, boric acid (H_3BO_3), hydrochloric acid (HCl), hydrofluoric acid (HF), nitric acid (HNO_3) and perchloric acid (H_2O_2) using a total digestion time (Melaku et al., 2005; Mester et al., 1999; Chakraborty et al., 1996). The addition of HF strongly breaks down silicates and mineral contents of the samples. However, HF can give rise to problems in glassware and torch damage of ICP. This problem can be resolved by using small volume of HF acid and addition of saturated boric acid solution to remove the excess of HF. The use of HNO_3 alone or combination with HCl, H_2O_2, and H_2SO_4 is responsible for complete digestion of inorganic materials in samples as well aid for organic materials in samples (Melaku et al., 2005).

Program A was set according to the manufacturer's suggestions on digestion applications for different samples and selected acid combination was 5 mL HNO_3 and 5 mL HF and reference material was sediment. Program A consisting of 3 stages is given in the Table 4. Program B again having 3 stages set and suggested by the manufacturer especially for the sediment material shown in Table 4 was experimented with acid combination 5 mL HNO_3 and 5 mL HF. In Program C, microwave digestion of reference sewage sludge and acid combinations of 5 mL HNO_3 and 5 mL HF was programmed under the guidance of the reference study done by the manufacturer. Program D having 3 stages used the acid selection (6 mL HNO_3) and the program combinations from the reference study done by Sandroni et al., 2002 represented in Table 4. Besides the acid selection from the study of Sandroni et al., 2002, Program D was tested with the acid combination of 5 mL HNO_3 and 5 mL HF as well. Program E represented as a summary in Table 4 had 2 steps and each step had 3 stages. Finally, in Program E, reference sewage sludge material was digested with the acid combinations of 2 ml HNO_3, 6 ml HCl, 0.5 ml HF for the first step, and 5 ml H_3BO_3 for the second step of the program and the program was done in line with the suggestion by the manufacturer.

Preliminary working step for the optimization attempts was the selection of the acid combination and it was done without the use of any reference material. Before choosing the acid combination nitric acid and hydrofluoric acid, 9 mL HNO_3 and 3 mL HCl was tested with the sewage sludge sample taken from Ankara municipal wastewater treatment plant. The microwave program set was chosen according to the manufacturer's suggestions same as Program A and Program C. Additionally, digestion procedure SM 3030 D with acid combinations; 10 mL HNO_3, 5 mL HF, 5 mL $HClO_4$ and 10 mL HCl given in Standard Methods (1995) was applied to the sewage sludge samples. After the application of labeled programs, the recovery of each metal is calculated based on the mean certified value for reference material [(measured concentration (mg/kg)]/[mean certified values (mg/kg)] ×100 and the method giving the highest recovery for as many metals as possible had been selected as the best method. The decision on the selection of appropriate recoveries for microwave digester depends on defining a reliable rate of recoveries for each metal. In our study, sample digestion recoveries after the application of microwave program assumed to be succesfull if the system enabled a rate of recovery of heavy metals in the region of 85%-120%.

Table 4. Microwave assisted digestion optimization trials

Program Stages	Time (min)	Temperature (^{o}C)	Power (W)	Experiment material
A				Certified reference sediment 5 mL HNO_3 + 5 mL HF
Stage 1	40	200	800	
Stage 2	25	100	400	
Stage 3	1	20	400	
B				Certified reference sediment 5 mL HNO_3 + 5 mL HF
Stage 1	5	160	900	
Stage 2	20	210	900	
Stage 3	20	20	400	
C				Certified reference sludge 5 mL HNO_3 + 5 mL HF
Stage 1	40	200	800	
Stage 2	25	100	400	
Stage 3	1	20	400	
D				Certified reference sludge 6 mL HNO_3 5 mL HNO_3 + 5 mL HF
Stage 1	6	160	900	
Stage 2	15	175	900	
Stage 3	15	100	400	
E				
Step 1				Certified reference sludge 2 mL HNO_3+ 6 mL HCl+ 0,5 mL HF+ 5 mL H_3BO_3
Stage 1	5	140	750	
Stage 2	5	160	850	
Stage 3	20	175	900	
Step 2				
Stage 1	15	160	800	
Stage 2	15	100	400	

After the selection of the appropriate microwave program for the digestion of the sludge samples, the extraction procedure was followed by sample preparation step. Firstly, sludge samples were dried in the furnace at 103 ^{o}C for 24 hours. Samples were then prepared by accurately weighing around 0.5 g of dried and homogenized sludge samples into clean Teflon vessels. Three to 5 replicates were taken for each sample. The addition of selected acids was followed as dictated by the method of microwave assisted digestion system. After the digestion procedure, sludge samples were observed visually to assess the complete digestion of the material. The final solution was boiled till the final volume was reduced to near dryness, and then diluted to a total of 25 mL. For each set of measurement, a blank that had gone through the same procedure as the sample was also analyzed to assess the matrix effects. Figure 1 is the presentation of microwave digestion of sludge samples for heavy metals.

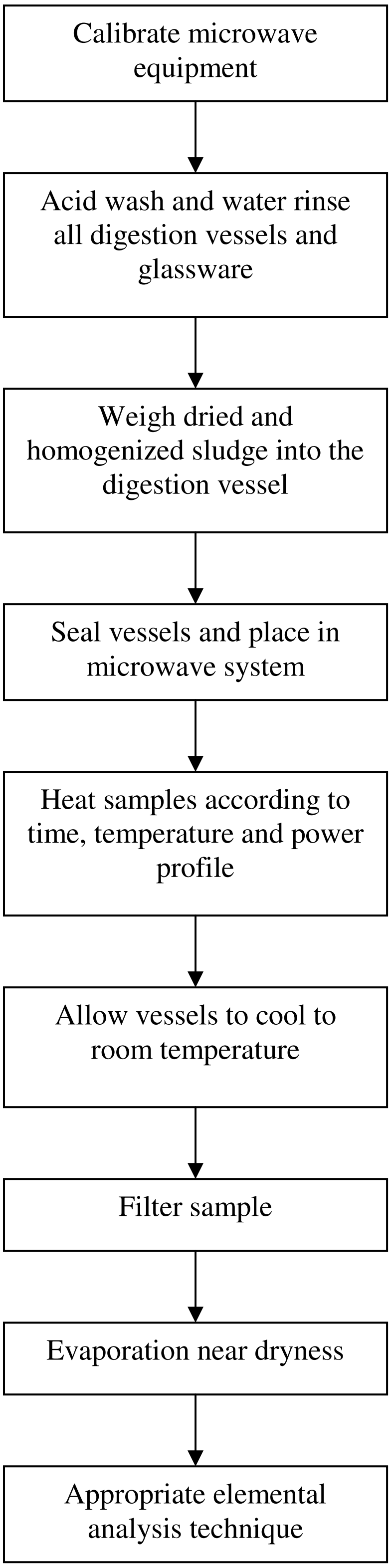

Figure 1. Overall digestion procedure

3.3. Microbiological Analysis

3.3.1. Pretreatment and Preparation of Solid Samples for Microbiological Analysis

Sludge samples to be analyzed for microbiological parameters fecal coliform, fecal streptococcus and *Salmonella* in accordance with SM 9222 D, SM 9230 C and ISO 6579:2002, respectively require dilution prior to analysis. Detection of microorganisms mentioned above in undiluted samples could exceed the detection limits therefore the following procedure was applied (Standard Methods, 1995).

Sterile buffered dilution water used is the stock phosphate buffer solution prepared according to SM 9050 C (U.S. EPA, 1999). A serial dilution procedure for sludge samples as suggested by Control of Pathogens and Vector Attraction in Sewage Sludge EPA/625/R-92/013 document was as follows:

1) In a sterile dish, weigh out 30 g of well mixed sludge sample.
2) Transfer to a blender for well mixing.
3) Use 270 mL of sterile phosphate buffer dilution water to rinse any remaining part in the blender.
4) Cover and blend for well mixing.
5) 1 mL of this sample contains 10^{-1} g of the original sample.
6) Use a sterile pipette to transfer 11 mL of the blender content to a beaker containing 99 mL of phosphate buffer dilution water and mix. 1 mL of this sample contains 10^{-2} g of the original sample (this is dilution ‘’A’’).
7) Transfer 11 mL of dilution A to a second beaker containing 99 mL of sterile buffered dilution water and mix carefully. 1 mL of this sample contains 10^{-3} g of the original sample (this is dilution ‘’B’’).

8) Transfer 11 mL of dilution B to other beaker containing 99 mL of sterile buffered dilution water and mix carefully. 1 mL of this sample contains 10^{-4} g of the original sample (this is dilution ''C'').
9) Transfer 11 mL of dilution C to other beaker containing 99 mL of sterile buffered dilution water and mix carefully. 1 mL of this sample contains 10^{-5} g of the original sample (this is dilution ''D'').
10) Transfer 11 mL of dilution B to a second beaker containing 99 mL of sterile buffered dilution water and mix carefully. 1 mL of this sample contains 10^{-6} g of the original sample (this is dilution ''E'').

3.3.2. Fecal Coliform Determination in Sludge Samples

Fecal coliform bacterial densities from sludge samples were determined by the Membrane Filter (MF) procedure (SM 9222 D) as explained in Standard Methods (1995). The fecal coliform MF procedure uses M-FC medium with rosolic acid. In this study, Millipore commercially prepared media in liquid form was used. The temperature interval for M-FC medium is 44.5±0.5 ^{o}C over a 24 h period (Standard Methods, 1995).

Fecal coliform quantification in accordance with SM 9222 D, as mentioned above, requires dilution prior to analysis. Therefore the dilution procedure explained in Section 3.3.1 was applied to the samples. After the sample preparation given in part 3.3.1, the filtration process of each 110 mL samples was conducted. Sterile membrane filters were placed onto the surface of the sterile petri dishes which consisted of M-FC medium. Finally sludge samples were incubated at 44.5±0.5 ^{o}C for 24 hours.

To determine colony counts on membrane filters, a cool white fluorescent light sourced colony counter device was used. The typical coliform colonies formed on M-FC medium are various shades of blue. The desired range of fecal coliform colonies is 20-60 fecal coliform colonies. If the colonies are not discrete and appear to be growing together results should be reported as “too numerous to count” (TNTC). The densities of fecal coliforms were recorded per 100 mL (Standard Methods, 1995).

To compute the number of colonies per grams dry weight of sewage sludge, the following Equation 1 was used for the membrane filters with 20-60 fecal coliform colonies and not more than 200 colonies per membrane.

During the experimental study, seven samples of sewage sludge were prepared for the analysis as expressed above as suggested by EPA/625/R-92/013. Dry solids content of the sample was determined. Numbers of fecal coliform colonies per grams dry weight of sewage sludge are calculated using Equation 1 and the whole result is the geometric average of those seven samples. In Appendix B, sample computation is available.

$$\text{Fecal coliforms/g dry weight}= \frac{\text{coliform colonies counted}\times 100}{\text{dilution chosen}\times \%drysolids} \text{......(Equation 1)}$$

3.3.3. Fecal Streptococcus Determination in Sludge Samples

The determination of the fecal streptococcus group consisting of various numbers of species of the genus was done according to the Standard Methods for the Examination of Water and Wastewater (SM 9230 C). For counting, KF Streptococcus Agar Dehydrated provided from Millipore was used. The incubation temperature for fecal streptococcus is 35 ^{o}C for 48 hours. Sludge samples analyzed for fecal streptococcus should be prepared for the test by the dilution scheme explained in Section 3.3.1.

After filtering 110 mL of samples through a 0.45 μm membrane filter, sterile membrane filters were placed onto the surface of the sterile petri dishes and incubated for 48 hours at 35 °C

To count the number of colonies, a fluorescent light sourced colony counter device was used. The colony appearance on KF Streptococcus Agar after the incubation period is red or pink. The desired density of the fecal streptococcus appear on membrane filter is within the range of 20-60 fecal streptococcus colonies. If the colonies are not discrete and appear to be growing together results should be reported as "too numerous to count" (TNTC). The densities of fecal streptococcus were recorded per 100 mL (Standard Methods, 1995).

To compute the number of colonies, the following Equation 2 was used for the membrane filters with 20-60 fecal streptococcus colonies and not more than 200 colonies per membrane (Standard Methods, 1995).

During the experimental study, seven samples of sewage sludge were prepared for the analysis as expressed above. Numbers of fecal streptococcus colonies are calculated using Equation 2 and whole result is the geometric average of those seven samples. In Appendix B, sample computation is presented.

$$\text{Fecal streptococcus/g dry weight} = \frac{\text{coliform colonies counted} \times 100}{\text{dilution chosen} \times \%drysolids} \quad \text{(Equation 2)}$$

3.3.4. Detection of *Salmonella* in Sludges

The analytical method suitable for the detection of *Salmonella* sp. was based on ISO 6579:2002. The analytical method consists of sample preparation, pre-enrichment, secondary enrichment, isolation and confirmation steps. The medium utilized and application of these medium and the method to isolate *Salmonella* from sludges were showing similarities in the use of same selective agars between the studies conducted by the following researchers M.A. Morinigo et al., 1986, Venglovsky et al., 2002, Sahlström et al., 2004, Espigares et al., 2006.

The sludge sample preparation analyzed for *Salmonella* species was done in line with the document EPA/625/R-92/013 expressed in Section 3.3.1.

Three series of five tubes should be used for this MPN procedure. Each series represents the prepared dilutions expressed as dilution A, dilution B and dilution C (EPA/625/R-92/013). The MPN value of salmonella per 100 mL was obtained from probability tables available in Standard Methods (1995).

In the pre-enrichment step of salmonella analysis, 1 mL of sample dilution as described in Section 3.3.1 was directly inoculated into 9 mL of buffered peptone water to enhance the recovery of salmonella. All tubes were incubated at 37^{o}C for 24 hours. At the end of 24 hours of incubation time in pre-enrichment period, 0.1 mL of samples from each of the buffered peptone water tubes were inoculated into selective enrichment broth of Rappaport Vassiliadis Soy broth and incubated at 41.5 ^{o}C for 24±3 hours. After incubation for 24 hours, a loopful from each of the selective broth tubes was plated on both Salmonella and Shigella (SS) and Xylose Lysine Deoxycholate (XLD) selective isolation agars at 35-37 ^{o}C for 48 hours (ISO 6579:2002).

SS agar is a selective agar for the isolation of *Salmonella* and *Shigella*. It enables the detection of colonies which ferment lactose and reduce thiosulphate. After the incubation time, non lactose fermenting colonies are colorless. Lactose fermenting colonies, such as coliforms, are pink or red. H_2S production from thiosulphate, is identified by black colonies, which, depending on the strains, may appear after 24-48 hours. In this agar, colorless colonies with a black centre could be Salmonella. Colorless colonies could be non H_2S producing shigella or salmonella. But the possible formations of some *Shigella sonnei* and *Salmonella arizonea* mean the formation of non H_2S producing colonies (Biomerieux 08544 B-GB-01/2001). XLD agar is a selective medium recommended for the isolation of enteric pathogens, especially Salmonella and Shigella. XLD agar provides primary identification of enterobacteria with the following biochemical criteria (Biomerieux 09324B-12/98);

- Fermentation of xylose, lactose and sucrose, detected by a yellow color in the presence of phenol red.
- H_2S production in a medium that is not too acid (colonies with black centre).

Table 5 is the diagnosis for the possible colonies grown on XLD agar (Biomerieux 09324B-12/98). The identification of possible isolated salmonella were confirmed with API 20E System which is suitable for the identifcation of Enterobacteriacea and other Gram negative rods.

Table 5. Appearance of colonies and grown microorganisms on XLD agar

Yellow colonies	-Escherichia -Citrobacter -Enterobacter -Proteus -Serratia -Klebsiella
Red colonies	-Shigella -Providencia -Salmonella $H_2S(-)$ (Para A, gallinarum-pullorum, cholerasius)
Red colonies with black center	-Salmonella -Edwardsiella

3.3.5. Detection of *Giardia* Cysts, *Cryptosporidium* Oocysts and *Helminth* Eggs in Sludges

Giardia cysts, *Cryptosporidium* oocysts and *Helminth* eggs determination tests were conducted in the laboratories of Refik Saydam Hıfzısıhha Center (RSCH), Ankara. *Cryptosporidium* Oocysts and *Giardia* analysis were conducted with the ready to use Crypto/Giardia Cel kits obtained from Cellabs. Before the application of ready to use kit to recover the *Giardia* and *Cryptosporidium*, the Formalin-Ether sedimentation technique is widely used for concentrating eggs, larvae, and cysts in fecal specimens. This method is an efficient procedure and is relatively easy to perform (Young et al., 1976).

All dewatered sludge samples were concentrated by the Formalin-Ether procedure used in RSHC laboratories. The detailed explanation of the method is as follows:

10 mL of amount of 10% Formalin is added to 1-1.5 g of dewatered sludge in a plastic cup with filter apparatus and holding for 30 minutes to ensure that the fixation and mixing is completely maintained. The filtered suspension is transfered to centrifuge tube with a capacity of 15 mL and then 0.85% Saline solution is added to the centrifuge tubes. The tube was stoppered by finger and shaken in an inverted position for 30 seconds and then the solution is centrifuged for 2 minutes at 400-500G. After centrifugation, 1-2 mL of 10% Formalin solution is added to precipitate and shaken carefully. At the end of this process, 10% formalin solution is added to the sample to complete the total volume of 10 mL. Again, 3 mL of ethyl acetate is put into the sample and shaken for 30 seconds. Then the sample is centrifuged for 2-3 minutes at 400-500G. At the end of the centrifugation, the usual four layers are formed as; solvent ethyl asetate, a plug of debris, formalin and sediment. The plug of debris was loosened by ringing with an applicator stick, and the top three layers were decanted. Unstained and

iodine-stained mounts were prepared to scan the specimen using a microscope initially at X10 magnification for *Helminth* egg, *Giardia* and *Cryptosporidium.* After the precipitation step, 50 µL of sludge specimens preserved in 10% formalin are diluted in phosphate buffered solution with 0.1% sodium azide and are mixed thoroughly to disperse the specimen using an applicator stick. 20 µL of the sludge specimen is placed from water onto a microscope slide. Then the specimens are allowed for completely air dry. The slides are fixed in acetone for 5 minutes and then are allowed for air dry. Then 25 µL of Crypto/Giardia cel reagent is added to the fixed specimen. Sludge specimens are incubated at 37 ^{o}C in a humid chamber for 30 minutes and then rinsed in a bath of phosphate buffered solution for one minute. Slide is drained and excess moisture around well is removed with tissue. Then a drop of mounting fluid is added to the slide well and a coverslip is placed on the top of the drop to remove air bubbles. Finally, the entire specimen is scanned for *Giardia* and *Cryptosporidium* using a fluorescence microscope initially at 200 magnification then at 400 and 1000 magnification for confirmation. Results are expressed as positive for the presence of *Giardia* and *Cryptosporidium* and negative for the absence of *Giardia* and *Cryptosporidium.*

CHAPTER 4

RESULTS AND DISCUSSION

4.1. Optimization of Digestion Method

Four different microwave programmes, five different attemtps in microwave digester and conventional digestion method SM 3030 D in Standard Method (1995) over the hot plate and various acid combinations were used to optimize the digestion of certified reference sludge and reference sediment. Among all the attempts for the digestion of the certified reference sludge, three programmes labeled as Program C, Program D and Program E have higher and most efficient recoveries for the extraction of heavy metals. The results for these three programmes C, D, and E are presented and compared in terms of the highest recoveries for the extraction of heavy metals. Program A and Program B which are applied to the reference sediment were considered to give inaccurate results in the instrumental analysis part because after the digestion procedure, sample still had sediment particles which were not completely digested. Therefore, the analytical determination of sediment material was not carried out in flame absorbtion spectrometry.

In developing this part of the study, microwave oven's manufacturer's recommendations and similar studies done by other scientists were taken as references. From all the programs, program D was developed from the study done by Sandroni et al. (2002). In the program A, B, C and E, microwave digestion of sewage sludge and applied acids was selected under the guidance provided by the manufacturer. In addition to optimization trials in microwave digester, reference sludge was tested with a standard method which was given in SM 3030 D by heating and digesting the reference material with a combination of various acids over the hot plate. The detailed information on applied programs and acid combinations are given in Table 3 in Materials and Methods part of the study.

Two microwave heating programs initially tested for reference sediment material and labeled Program A and Program B results were not successful for the digestion procedure, because samples were not digested completely and there were precipitates of sediment material which was observed visually after the application of the method. As a result, after the application of both Program A and Program B, instrumental analyses of digested reference sediment material were not conducted so the results are not presented here. Therefore program C, D and E were developed and the results obtained after the heavy metal analysis are presented. Program C was first tested for the digestion of certified reference sludge by using 5 ml HNO_3 acid and 5 ml HF acid. The recoveries of each metal were found according to the values represented for the certified reference sludge material. Reference values listed in the certificate of the sewage sludge product are average results with standard deviations. Standard deviations in certification of the reference materials were developed from multilaboratory analysis. Data obtained are subjected to a robust statistical analysis. Therefore, the certificate shows the mean value and standard deviation from the mean. The mean values of the recoveries and the standard deviations for the microwave heating programme C is shown in the Table 6.

Table 6. Metal concentrations and % recoveries obtained after Program C with acid combinations nitric/hydrofluoric

Metal	Certified reference concentration (mg/kg)	Concentration (mg/kg)	% recovery
Cu	665±42.2	316.6±44.7	47.6
Pb	277±31.7	99.8±12.9	36.0
Cd	537±74.8	227.1±62.8	42.3
Ni	150±17.1	100.9±14.9	67.3
Zn	847±117	682±214	80.5
Cr	325±29.9	319.5±35.2	98.3

As shown in the Table 6, by the Program C, the highest metal recoveries obtained after the digestion of the reference sludge are 80.5 % Zn and 98.3 % Cr, respectively. The extraction of other metals from the sludge are very low, therefore to find a better extraction method, Program D was tried. Lower recoveries obtained with Program C can be due to the lower power settings applied to the microwave. The power range of microwave digester is between 40%-90% of 1000 W. Program C was operated in the minimum power for 2 stages of the program. In the literature, it was stated that for microwave digestion system without pressure control, the power and the digestion time could be critical variables. By setting power and the digestion time, the decomposition of samples could be carried out at elevated pressures and temperatures. However, the relationship between the program setting and the result of sample digestion was purely empirical. By increasing the power, a shorter time is needed for the pressure and temperature to reach the maximum. The effect of power is limited by other variables such as the maximum pressure setting, mixed acids and digestion (Sandroni et al., 2003).

In Program D, two different acid combinations were tested. The first choice was 6 ml of HNO_3 and the second one is the combination of 5 ml of HNO_3 with 5 ml of HF. Table 7 represents the metal recoveries obtained after the heating Program D with two different acid alternatives.

Table 7. Metal concentrations and % recoveries obtained using Program D with acid combinations nitric and nitric/hydrofluoric

		Concentration (mg/kg)		**Recovery (%)**	
Metal	**Certified reference concentration (mg/kg)**	**6 ml HNO_3**	**5 ml HNO_3 + 5 ml HF**	**6 ml HNO_3**	**5 ml HNO_3 + 5 ml HF**
Cu	665±42.2	615.6±2.3	592.6±63	92.6	89.1
Pb	277±31.7	106.6±2.3	73.6±15.7	38.5	26.6
Cd	537±74.8	252.9±9.7	270.9±9.7	47.1	50.5
Ni	150±17.1	107.6±5.6	96.9±5.3	71.8	64.6
Zn	847±117	1140.7±156.8	1096.7±37.8	134.7	129.5
Cr	325±29.9	542.4±25.6	431.4±48.5	166.9	132.8

If we examine the metal concentrations obtained after the extraction with Program D, we can see that metal recovery values obtained after the digestion with nitric acid are better for Cu, Pb, Ni, Zn and Cr than the digestion with the acid combinations nitric and hydrofluoric. Pb and Cd recovery values are 38.5% and 47.1% with the use of nitric acid only, respectively, however the extraction of other metals except Pb and Cd exhibit higher efficiencies. Zn and Cr recoveries are 134.7% and 166.9%, respectively. These high recoveries more than 100% could be due to the quantification of the elements in flame atomic absorbtion spectrometry. Because of lower recoveries obtained from the extraction for Pb and Cd with Program D, Program E which was recommended by manufacturer for the digestion of sludge was performed for the extraction of heavy metals from certified reference sludge material.

The results for the extraction of the reference sludge with Program E having acid combinations of nitric, hydrofluoric, hydrochloric and boric acids were shown in the Table 8.

During Program E, acid combinations of 2 ml HNO_3, 6 ml HCl, 0.5 ml HF, and 5 ml H_3BO_3 were applied to the reference sludge. As seen from the Table 8, metal extraction recoveries obtained from Program E is higher than Program C and Program D. The recoveries of Cd was 42.3 % in the application of Program C and 50% while using Program D whereas the extraction of Cd using Program E is nearly the same as that expressed in the catalog of certified reference sludge. The Program E that was set with these operational conditions represents high recoveries within the case of volatile Hg and Cd, as well as Pb and Cu with recoveries 110.6%, 107.6%, 125.7% and 103%, respectively. The recoveries obtained that exceed 100% can be due to the previously explained reasons. The use of various acid combinations with adequate time programming yield in high recoveries obtained for all heavy metals.

Table 8. Metal concentrations and % recoveries obtained using Program E with acid combinations nitric/hydrofluoric/hydrochloric/boric

Metal	Certified reference concentration (mg/kg)	Concentration(mg/kg)	% recovery
Cu	665±42.2	685±20	103
Hg	4.17+1.13	4.61±0.14	110.6
Pb	277±31.7	348.3±2.7	125.7
Cd	537±74.8	578±32	107.6
Ni	150±17.1	150.1±1.6	100
Zn	847±117	850±49	100.4
Cr	325±29.9	267.3±11.1	82.2

For comparative purposes, the conventional digestion method over the hot plate was also tested. The experimental data obtained with conventional digestion procedure SM 3030 D and the certified sludge is tabulated in Table 9. The agreement between experimental and certified value was good for heavy metals Cu, Cd, Ni. The recoveries of all elements given in Table 9 were ranged from 17% to 144%. The lowest recovery value 17.21% was found for Cr. The highest but not an acceptable recovery was obtained as 144.63% for Zn. This result for Zn is due to the analytical determination in spectrometry. The recovery for Pb was also very poor, approximately 30%. Metal extraction recoveries obtaining by Program E is higher than SM 3030 D digestion procedure for heavy metals Cu, Pb, Cd and Cr. Therefore, heavy metal extraction procedure with microwave digester is preferred rather than applying hot plate digestion procedure.

Table 9. Metal concentrations and % recoveries obtained after SM 3030 D digestion

Metal	Certified reference concentration (mg/kg)	Concentration(mg/kg)	% recovery
Cu	665±42.2	518.33±6.53	77.94
Pb	277±31.7	82.25±61.13	29.69
Cd	537±74.8	483.83±15.63	90.09
Ni	150±17.1	161.38±9.70	107.59
Zn	847±117	1225±35	144.63
Cr	325±29.9	55.92±3.15	17.21

As stated by other researchers; the reasons for the widespread preference for microwave technology relate to its clear advantages over more traditional technologies are (Florian et al., 1998); a shorter acid digestion time; while conventional sample digestion can take several hours or even days, it can be carried out in a few minutes by microwave digestion (Bordera et al., 1996); a supposed better recovery of volatile elements and compounds; lower risk of external contamination levels due to the exsitence of closed pressurized vessels (Bordera et al., 1996); minimal volumes of reagents are required, more reproducible procedures; and a better working environment (Melaku et al., 2005; Sastre et al., 2002; Agazzi and Pirola, 2000; Bettinelli et al., 2000; Veschetti et al., 2000; Lavilla et al., 1998); handling of large samples that can generate a huge amount of gas mainly when working with organic materials; use of various types of materials to construct reaction vessels, such as borosilicate glass, quartz, and PTFE; programmable addition of reagents at any time during the digestion which occured during the operation of Program E of the microwave digester (Nobrega et al., 2002). Possibly all these factors caused the higher, rapid and reproducible heavy metal extraction recoveries obtained in the Program E of the microwave digester. The developed methodology was used to extract heavy metals from sewage sludge samples during the rest of the study.

4.2. Heavy Metal Analyses Results of Sewage Sludges from Selected Wastewater Treatment Plants

Table 10 summarizes the content of heavy metals in the investigated sludges with respect to different months of the year and represents values of limits for agricultural use of heavy metals with respect to heavy metals. The data included the heavy metal concentrations with standard deviations at each treatment plant. Standard deviations of heavy metal concentrations are the represantitive of deviations from the mean value for each sampling. For Ankara and İzmir wastewater treatment plant (WWTP), five investigations with 3 or 5 replicates for each sampling at different months of the year were conducted. Three surveys with 3 or 5 replicates and 2 surveys with 4 or 5 replicates for each survey were done for the sludge samples from Kayseri and Tekirova WWTP, respectively. Detailed information on sampling strategy is given in Section 3.1.

4.2.1. Ankara Wastewater Treatment Plant

Heavy metal concentrations measured for dewatered sludges of Ankara WWTP are found to be acceptable with respect to maximum heavy metal concentrations permitted for agricultural use of sludges as shown in Table 10. Heavy metal concentrations analyzed demonstrate that this sludge is well suited for Cu, Pb, Cd, Ni, Cr and Hg in agriculture use for all samples taken during the study. However the relatively high amounts of Zn concentration were observed in two samples taken for Ankara WWTP. Zn concentration of 4065 mg/kg was obtained in sample taken in 14.07.2005. This value exceeded the limit value of 4000 mg/kg set for Zn. Relatively high or similar concentrations for Zn were obtained in the rest of the sampling period in Ankara WWTP compared to the value given in SPCR shown in Appendix A, Table A12, showing that Zn concentrations in Ankara sludge makes it inappropriate for agricultural use from time to time.

Treatment plant receives about 15% industrial inputs generated from Şaşmaz, Ostim, and İvedik Organized Industrial Zone. Main industrial activities within the area are from automative, building and construction, chemicals, construction machines, electric and electronics, food, health, machine and machine equipments, metal and metal treatment, textile and leather, rubber plastics industries , urban furnitures and landscape. Zn compounds are mainly used in industries to make paint, rubber, dye and wood preservatives (N.C. Department of Agriculture and Consumer Services, 2005). Therefore high concentrations may be due to the low pretreatment applied to wastewater from those industries within the region.

4.2.2. İzmir Wastewater Treatment Plant

Summary of the results obtained from heavy metal analysis in the dewatered sludge of İzmir WWTP are represented in Table 10. For all samples, heavy metal concentrations found in the dewatered sludges lay within the range of values set for the agricultural use of sewage sludges in Turkey. During the sampling period, Pb and Cd concentrations were not detected in three of the sampling of dewatered sludges.

The likely cause of the relatively lower heay metal concentrations for sludges samples from İzmir WWTP has been shown to be the effect of the efficient pretreatment applied to the industrial sources coming into the treatment plant. Although treatment plant receives about 20% industrial inputs mainly generated from food preparation and processing, the leather and textile industries, metallurgy, marbel production, alcoholic beverage productions, heavy metal concentrations of sludges seem to be fit for agricultural use.

4.2.3. Kayseri Wastewater Treatment Plant

Heavy metal concentrations analyzed demonstrate that Kayseri wastewater sludge is well suited for Cu, Pb, Cd, Zn, Cr and Hg in agriculture use for all samples taken during the study. Cu, Pb, Cd, Zn and Cr were below the limit values set by SPCR in Turkey given in Appendix A, Table A12. In general, the quality of sewage sludges from Kayseri WWTP has been shown to be suitable for agricultural use in two of the three sampling period however Ni concentrations were prone to violate the maximum permissible Ni value given in SPCR. Ni concentration of 529.88 mg/kg was obtained in the sample taken in 24.05.2006. Relatively higher Ni concentrations were observed during the rest of the study. Ni concentrations tend to exceed the limit values given in SPCR.

Kayseri treatment plant receives about 15% industrial inputs generated mainly from wood processing and furniture production and textile industry. Although wastewaters from those industries mentioned above contain Cr, Cu and Zn in their origin, heavy metals emphasized were below SPCR limits in dewatered sludge samples from Kayseri WWTP. Therefore the reason for high Ni concentrations may be due to the different processes applied in the exisiting industries in that region.

4.2.4. Tekirova Wastewater Treatment Plant

Summary of the results obtained from heavy metal analysis in the dewatered sludge of Tekirova WWTP are represented in Table 10. For all samples, heavy metal concentrations found in the dewatered sludges lay within the range of values set for the agricultural use of sewage sludges in Turkey. During the sampling period, Pb and Cd concentrations were not detected in any samples.

The reason for the relatively lower heavy metal concentrations for sludges samples from Tekirova WWTP has been shown to be the effect of the absence of the industrial sources coming into the treatment plant.

Table 10. Heavy metal concentrations of dewatered sludges of Ankara, İzmir, Kayseri and Tekirova and comparison with SPCR of Turkey (nm: not measured)

Sampling Date	**Cu (mg/kg)**	**Pb (mg/kg)**	**Cd (mg/kg)**	**Ni (mg/kg)**	**Zn (mg/kg)**	**Cr (mg/kg)**	**Hg (mg/kg)**
Ankara							
04.01.2006	184.00±10.81	94.75±3.38	4.51±0.66	43.48±3.36	2863.00±208.10	304.80±14.99	1.400±0.008
17.11.2005	357.33±37.46	73.04±3.79	10.07±0.58	59.80±5.47	3195.00±342.53	232.10±24.41	nm
19.10.2005	166.83±5.53	62.15±3.59	5.85±0.17	43.22±9.78	1695.00±201.68	279.90±21.88	nm
14.07.2005	364.17±15.50	112.87±8.47	7.52±0.43	94.18±5.46	4065.00±78.10	310.33±54.29	nm
13.05.2005	238.50±18.36	126.50±18.46	6.57±0.50	71.15±3.53	2143.33±116.44	261.17±52.12	nm
İzmir							
04.07.2006	255.10±25.08	0.00	0.00	94.79±1.29	1319.00±187.56	154.75±25.20	0.511±0.019
28.02.2006	136.30±7.38	0.00	0.00	54.47±5.89	968.00±49.82	312.80±17.67	nm
26.12.2005	229.20±9.07	59.78±8.21	0.00	42.47±2.71	602.00±41.92	181.30±8.87	nm
30.05.2005	246.50±25.93	0.00	2.47±0.48	79.82±4.56	1496.67±205.93	199.33±7.15	nm
31.03.2005	264.23±3.41	100.35±2.61	1.90±0.51	50.87±3.14	561.00±51.40	195.67±19.83	nm
Kayseri							
24.05.2006	301.60±50.99	196.02±7.56	0.00	529.88±100.03	1146.00±167.23	577.80±28.21	2.026±0.185
06.12.2005	762.00±51.83	184.70±21.25	0.00	324.50±78.35	881.00±196.67	715.10±37.09	nm
19.04.2005	526.17±20.59	138.47±26.78	4.45±0.15	355.00±22.11	1276.67±85.78	734.33±33.93	nm
Tekirova							
27.06.2006	95.13±6.37	0.00	0.00	51.11±8.89	452.40±49.31	34.48±11.18	0.493±0.033
31.01.2006	171.63±10.94	0.00	0.00	86.05±10.44	726.25±31.98	60.81±1.07	nm
SPCR	1750	1200	40	400	4000	1200	25

4.2.5. Comparative discussion of heavy metal contents of the sludges from plants

All analysed sewage sludge samples from Ankara, İzmir, Kayseri and Tekirova wastewater treatment plants contained Zn as the dominant metal. The lowest concentration was observed with Cd for all sewage sludge samples. From Table 10 it can be seen that there are variations among the four plants in terms of the levels of heavy metals as well as the types of most abundant heavy metals. The contents of Zn and Cr are the highest followed by Cu, Ni, Pb, Cd (Zn>Cr>Cu>Pb>Ni>Cd) in the sludges of Ankara wastewater treatment plant and Zn and Cu are the highest followed by Cr, Ni, Pb, Cd (Zn>Cu>Cr>Pb>Ni>Cd) in the lime stabilized dewatered sludges of İzmir wastewater treatment plant. The concentration distribution of metals in the sewage sludge samples of Kayseri wastewater treatment plant show that Zn and Cr are the highest followed by Cu, Ni, Pb, and Cd (Zn>Cr>Cu>Ni>Pb>Cd). The abundance of heavy metals in the dewatered sludges of Tekirova wastewater treatment plant can be presented in the decreasing order of Zn>Cu>Ni>Cr>Pb=Cd. From Table 12, it can be seen that minimum and maximum range of heavy metals in different treatment plants given in literature show relatively high variations which could be affected by the industrial input of wastewaters.

The concentrations of heavy metals in sewage sludges of four wastewater treatment plants are given in Table 10. When comparing the results of heavy metals in sludges from four wastewater treatment plants with each other, one must notice that heavy metal contents of sludges differ from plant to plant as well as in one single plant, they differ from time to time. The mean concentrations of Cu, Pb, Cr and Ni in Kayseri dewatered sludges are higher than other plants where Cd and Zn concentrations of dewatered sewage sludges in Ankara plant are higher than the others. Cu, Pb, Cd, Zn and Cr concentrations in Tekirova dewatered sludges are relatively lower than the dewatered sludge samples from other treatment plants. From all the heavy metals listed in Table 10, Pb and Cd were not detected in any of the fresh dewatered sewage sludge samples analysed for Tekirova wastewater treatment plant.

Heavy metal distributions of sewage sludges obtained in different studies are presented in Table 11. Similar order of heavy metals distribution are seen in the study of Bodzek et al. (1997) and Zufiaurre et al. (1998) with the results obtained for dewatered sludges of Ankara wastewater treatment plant and Kayseri wastewater treatment plant, respectively. Alvarez et al. (2002) emphasized in the results of the analysis for dewatered sludges from five different wastewater treatment plants that the highest heavy metal concentration was obtained for Zn where Cu, Cr and Pb comprised the group of metals measured in decreasing order after Zn. As stated by Aulicino et al. (1998) Zn and Cu are predominant heavy metals found in dewatered sludges from İzmir and Tekirova wastewater treatment plants. Karvelas et al. (2003) and Mantis et al. (2005) indicated in the reference study that Zn appeared to be the most abundant metal whereas Cd exhibited the lowest abundance same as the data obtained for sludges from Ankara, İzmir, Kayseri and Tekirova wastewater treatment plants.

Table 11. The order of heavy metals found in several sewage sludges

Reference study	Heavy metals distribution of sludges
Alvarez et al. (2002)	Zn>Cu, Cr, Pb
Bodzek et al. (1997)	Zn>Cu>Pb>Ni>Cd
Bright and Healey (2003)	Cu>Zn>Pb>Cr>Ni>Cd
Dinel et al. (2000)	Zn>Cu>Pb>Ni>Cr
Düring and Gath (2002)	Zn>Cu>Pb>Cr>Ni>Cd>Hg
Jensen and Jepsen (2005)	Zn>Cu>Pb>Cr>Ni>Cd
Wang et al. (2005)	Cu, Zn>Cr, Ni, Pb, Cd
Zufiaurre et al. (1998)	Zn>Cr>Cu>Ni>Pb>Cd

By comparison, the mean heavy metal concentration in sewage sludges from other studies and heavy metal concentration limits for the land application of sewage sludges are shown in Table 12 and Table A9, respectively in Appendix A. As seen from these tables, the sludge generated in four wastewater treatment plants investigated in this study violate the limits set for Cu, Ni and Zn in Austria, Belgium, Denmark, Finland, France, Germany, Luxembourg, Netherlands but meet the requirements given in USA. Dewatered sludges from Tekirova WWTP meet the requirements for Cr and Pb in the above mentioned countries and as well in USA. Cd levels in İzmir and Tekirova WWTP dewatered sludges meet the levels set in EU countries mentioned above and USA.

Cu content of dewatered sewage sludges from Ankara, İzmir, Kayseri and Tekirova wastewater treatment plants given in Table 10 are lower than the result reported by Al-Enezi et al. (2004), Alvarez et al. (2002), Fuentes et al. (2004), Debosz et al. (2002), Wang et al. (2005), Joshua et al. (1998) and Wong and Su (1996) whereas 1.5-6 times higher than the results reported by Goi et al. (2006), Jensen and Jepsen (2005) given in Table 12. The mean concentration of Pb in dewatered sludges reported by Al-Enezi et al. (2004), Alvarez et al. (2002), Joshua et al. (1998), Nunez-Delgado (2002) given in Table 12 were approximately 2-10 times higher than the results obtained for all sludges in our study, the results reported by Fuentes et al. (2004), Navas et al. (1998), Petersen et al. (2003) and Wang et al. (2005) are 2-5 times higher than Pb concentration observed for Ankara, İzmir and Tekirova dewatered sludges. Pb concentration reported by Wang et al. (2005), Petersen et al. (2003), Navas et al. (1998) given in Table 12 are lower than the results for Kayseri dewatered sludges given in Table 10. Pb concentration in dewatered sludges declared by Jensen and Jepsen (2005) are higher than Pb levels in dewatered sludges from Ankara and Tekirova, lower than the concentration obtained for İzmir and Kayseri dewatered sludges. The abundance of Cd in sewage sludges from the studies conducted by Al-Enezi et al. (2004), Joshua et al. (1998), Wang et al. (2005) and Wong and Su (1996) given in

Table 12 are extremely higher than our survey for four treatment plants given in Table 10. Approximately same Cd concentrations are presented in the studies of Petersen et al. (2003), Navas et al. (1998), Jensen and Jepsen (2005) and Goi et al. (2006) compared to our survey given in Table 10 and Table 12. Debosz et al. (2002), Fuentes et al. (2004) reported nearly 3 times higher Cd concentrations given in Table 12 than the results obtained for sludges from four plants given in Table 10. The sludge contents of Ni found in the investigations of Fuentes et al. (2004), Goi et al. (2005), Jensen and Jepsen (2006) and Petersen et al. (2003) are lower than the concentration that is presented in our study. Al-Enezi et al. (2004) and Joshua et al. (1998) presented much higher Ni concentrations for sewage sludges than the results shown in dewatered sludges from Ankara, İzmir and Tekirova wastewater treatment plants. Wang et al. (2005) reported nearly the same concentrations with our investigation for Ankara, İzmir and Tekirova dewatered sludges. Zn concentrations analyzed in sewage sludges from reference studies show similar trends except for the results presented in the studies of Joshua et al. (1998) and Wang et al. (2005). They found much higher mean Zn concentrations represented in Table 12. From the results of Petersen et al. (2003), Navas et al. (1998), Wang et al. (2005), Jensen and Jepsen (2005), Goi et al. (2005), Debosz et al. (2002) and Al-Enezi et al. (2004) represented for heavy metal Cr, metal levels are lower than the analytes of four treatment plants of this study. Cr concentrations in dewatered sludges of Tekirova are lower than for almost all reference studies expressed in Table 12.

Table 12. The concentration of heavy metals in reference studies in literature and permitted heavy metal concentrations for land application in different countries

Reference study	Cu (mg/kg)	Pb (mg/kg)	Cd (mg/kg)	Ni (mg/kg)	Zn (mg/kg)	Cr (mg/kg)	Hg (mg/kg)
Al-Enezi et al. (2004)	700	337	21	111	2002	80	58
Alvarez et al. (2002)	326	223			1600	439	
Debosz et al.(2002)	360		2.40			32.50	3.50
Fuentes et al. (2004)	337	167	18.30	29	871	3.81	
Goi et al. (2005)	105.80	48.40	<2	26.20	404.10	32.10	<0.10
Jensen and Jepsen (2005)	243	50	1.30	20	700	21	1.10
Joshua et al. (1998)	1257	323	13	166	3060	308	
Navas et al. (1998)		132.50	4.28	77.78	1126	4.13	
Nunez-Delgado (2002)	200-840	200-300	<DL	<DL	320-780	0.00-430	0.10-1.82
Petersen et al. (2003)		106	2.20	20	977	30	2.60
Wang et al. (2005)	581.60	93.73	112.03	59.76	6718.87	108.54	
Wong and Su (1996)	979		13.70		1268		
EU Limits (NRC, 2002)	1750	1200	40	400	4000		25
USA Limits (NRC, 2002)	4300	840	83	420	7500		
Netherlands Limits (NRC, 2002)	75	100	1.25	30	300	75	0.75

The distinct differences observed from the results between treatment plants can be the result of the environmental impact connected to industrial sources. Industrial flow received by Ankara and Kayseri wastewater treatment plant is about 15 % of the total influent flow rate. The industrial effluents receive considerable pretreatment before coming into Ankara wastewater treatment plant, whereas, no pretreatment is applied in the case of Kayseri wastewater treatment plant during the analyses period. Although the industrial input is 20% in İzmir wastewater treatment plant, the wastewater and sludge quality is considerably higher than that of the other plants when the heavy metals are considered, this could be attributed to the efficient pretreatment of industrial sources. Industrial effluents are the predominant source of Cd, Hg, Cr and Ni, while Cu and Zn are mainly of domestic origin, and the major source of Pb may be both surface runoff and domestic wastewater (Wang et al., 2005). Data given in Table 10 is in compliance with this information; the lowest Cd, Ni, Zn, Cr and Hg concentrations occur in sludges from Tekirova wastewater treatment plant for which the industrial contribution does not exist. The most abundant metal found in the sludges of three wastewater treatment plants is Zn and again Hg exhibited the lowest abundance as also referred in literature (Karvelas et al., 2003).

Comparison of heavy metal concentrations in sludges with the standards set for the use of sludges in agriculture show that for all the heavy metals the average values are below the required limit values when EU and Turkish sludge regulations are considered. Therefore, these results indicate that sludge is within permissible levels to be land applied in Turkey. However, Zn and Ni concentrations need to be given special attention even with the Turkish regulations in consideration to sewage sludges from Ankara wastewater treatment plant and Kayseri wastewater treatment plant, respectively. This means that the sludge should be used carefully considering Zn concentration in Ankara wastewater treatment plant sludge. Ni concentration in Kayseri wastewater treatment plant sludge shows that special attention should be paid. Almost all sludge samples taken from İzmir and Tekirova treatment plants comply with the limit values given in SPCR of Turkey.

The fate of the metals in wastewater treatment plants are the important issue in the accumulation of heavy metals in final sludges. The factors affecting the abundance of heavy metals are the weight loss of fresh sludge during anaerobic digestion. Cu, Cd, Pb and Zn contents in digested sludge were 50-99% higher than their contents in undigested sludge (Karvelas et al., 2003). In Ankara and Kayseri WWTPs, anaerobic stabilization are applied to raw sludges therefore heavy metal concentrations obtained for those plants are higher than the results obtained for İzmir and Tekirova WWTPs due to higher weight losses.

Determination of extractable trace metal contents is crutial in defining bioavailiability, toxicity and fate of heavy metals during treatment. From the literature study mentioned in section 2.4.5 came into conclusion that less available fractions of oxidizable and residual fractions show a clear rise along the sludge treatment. Higher degree of mineralisation and stabilisation of sludges showed a lower metal availability index since all the heavy metals in sludges were associated to the oxidisable and residual fractions, which are the least mobile. Unstabilised sludge contained the highest accumulations of heavy metals in the most easily assimilable fractions (exchangeable and reducible) Fuentes et al. (2004). Our study did not cover the heavy metal speciation therefore differences in the treatment technologies applied were not discovered.

4.2.6. Potential for land application

Results in Table 13 are the representative values for the potential land application of sludges with respect to application areas. Calculations were done by using measured heavy metal concentrations of sludges in mg/kg from four treatment plants representing in Table 10 and limit values for amounts of heavy metals in g/da/y that may be added annually to soil given in Table A13. In order to calculate the potential for application with respect to application areas for each metal, daily sludge production quantities in ton/day were used in the calculation. During the calculation, application area for each metal was defined with the assumption of the use of sludge in one year basis.

Table 13. Land application potential of sludges from four treatment plants with respect to permitted land application areas

Cu Area (da)	Pb Area (da)	Cd Area (da)	Ni Area (da)	Zn Area (da)	Cr Area (da)	Hg Area (da)
Ankara						
17544	5025	36960	16694	74739	14864	11242
İzmir						
48177	13639	37303	54915	84258	35560	13056
Kayseri						
8382	2190	5631	25504	6967	8550	3845
Tekirova						
365			751	645	104	161

The required application area for the management strategy of sewage sludges is presented in Table 13. Calculated land application areas are the ones to make sure that sludges from four wastewater treatment plants can be safely applied. Application area calculations according to the annual pollutant loading rate limitations, sludge quantity and contaminant concentrations yielded in different area values for each heavy metal. These results are the representative of minimum application areas required for the application of sludges produced in each treatment plant in a year. To clarify the discussion, to be able to prevent the toxic effects of heavy metals in soil, minimum application areas presented in Table 13 must be met. In short, sludges must be spread not lower than the calculated values. For Ankara, İzmir and Kayseri WWTPs, sewage sludges should be spread by considering Zn whereas in Tekirova, Ni was the rate limiting heavy metal.

Another discussion on the land application of sewage sludges can be conducted by using the limit values set for the heavy metal concentrations in soil. In this calculation, the aim is to find the amount of sludge that can be applied to soil by using the permitted heavy metal concentrations in soil (Appendix A Table A10) and the average heavy metal concentrations found after the analyses of heavy metals. The assumptions in this calculation were the complete transfer of heavy metals from sludge into the soil media. Table 14 is the summarizing the amount of sludges that can be applied to soil with respect to maximum heavy metal limits in the soil. To be able to stay on the safe side, soil characteristics were assumed to be acidic.

Table 14. Maximum sludge quantities applicable with respect to limit values for heavy metals in soil

Cu Quantity (kg)	Pb Quantity (kg)	Cd Quantity (kg)	Ni Quantity (kg)	Zn Quantity (kg)	Cr Quantity (kg)	Hg Quantity (kg)
Ankara						
0.19	0.53	0.14	0.48	0.05	0.36	0.71
İzmir						
0.22	0.62	0.46	0.47	0.15	0.48	1.96
Kayseri						
0.09	0.29	0.22	0.07	0.14	0.15	0.49
Tekirova						
0.37			0.44	0.25	2.10	2.04

From Table 14, it can be said that the land application of sludge with respect to heavy metal standards in soil was limited by heavy metal concentrations in sludges. The higher the heavy metal concentration in sludge, the lower the quantity of sludge can be applied to the soil with the assumptions given below. For all wasterwater treatment plants, Zn limits the application of sludges. Minimum 0.05 kg, 0.15 kg, 0,14 kg and 0.25 kg of sewage sludges can be applied to soil per decar area in Ankara, İzmir, Kayseri and Tekirova WWTPs, respectively.

4.3. Microbiological Properties of Sewage Sludges

Microbiological quality of the sludge samples taken from the four treatment plants was analyzed and results are discussed in this section.

Table 15 through Table 24 represents fecal coliform, fecal streptococci and salmonella densities in Ankara, İzmir, Kayseri and Tekirova wastewater treatment plants. The results are expresssed as Colony Forming Units per gram Total Solids (CFU/g TS). During the experimental studies of bacteria, seven samples of a dewatered sludge were analyzed with membrane filter (MF) procedure. Samples were prepared in accordance to the MF procedure given in materials and methods part of this study. This yielded 21 individual membrane filters for a single dewatered sludge sample taken from wastewater treatment plants. The average densities were obtained from the geometric mean of these 21 individual membrane filters. The densities of bacteria in dewatered sludges from Ankara were analyzed for five different times. Dewatered sludge samples from İzmir and Tekirova wastewater treatment plants were analyzed for three times and samples from Kayseri wastewater treatment plant was analyzed for two different times. The densities of salmonella were expressed as Most Probable Numbers per 4 grams of Total Solids (MPN/4 g TS). Giardia and Cryptosporidium existence of dewatered sewage sludges from wastewater treatment plants were expressed as positive for the existence and negative for the absence of the microorganisms. The densities of fecal coliform, fecal streptococci, *Salmonella*, *Giardia* and *Cryptosporidium* are given and compared by the results of similar studies in literature. Mean concentrations of each bacteria in samples are used to compare with EPA Part 503 regulation.

The sludge samples taken from Ankara and Kayseri wastewater treatment plants are anaerobically digested and dewatered sludges. The sludges from İzmir wastewater treatment plant are stabilized by lime stabilization method. Dewatered sludges from Tekirova wastewater treatment plant do not receive any stabilization to reduce pathogens.

4.3.1. Fecal coliforms

Tables 15 to 18 show the fecal coliform densities measured in samples taken from Ankara, İzmir, Kayseri and Tekirova wastewater treatment plants, respectively. The fecal coliform densities in the effluent of the anaerobically digested and dewatered sludge samples from Ankara wastewater treatment plant ranged from 9.12×10^5 to 1.70×10^7 CFU/g TS. The fecal coliform numbers in the effluent of the dewatered sludge from Tekirova wastewater treatment plant were between 6.94×10^7-2.26×10^9 CFU/g TS. Fecal coliform densities varied from 4.55×10^5 CFU/g TS to 9.55×10^6 CFU/g TS for dewatered sludge samples from İzmir wastewater treatment plant. The abundance of fecal coliforms in anaerobically digested/dewatered sludges from Kayseri wastewater treatment plant are in the level of 9.32×10^6-3.89×10^8 CFU/g TS. The fecal coliform levels of dewatered sludges from Ankara and İzmir wastewater treatment plants were much lower than the values obtained for Kayseri and Tekirova dewatered sludges.

Table 15. Fecal coliform densities in dewatered sludges from Ankara wastewater treatment plant

Sampling Date	**Fecal Coliform Densities (CFU/ g TS)**		
	Sample no.	**Coliform density in each sampling**	**Average**
18.04.2006	1	1.48×10^{7}	1.23×10^{7}
	2	6.52×10^{6}	
	3	7.39×10^{6}	
	4	1.58×10^{7}	
	5	1.50×10^{7}	
	6	1.49×10^{7}	
	7	1.70×10^{7}	
04.01.2006			9.12×10^{5}
17.11.2005			7.86×10^{6}
19.10.2005			1.54×10^{6}

Table 16. Fecal coliform densities in dewatered sludges from İzmir wastewater treatment plant

Sampling Date	**Fecal Coliform Densities (CFU/ g TS)**		
	Sample no.	**Coliform density in each sampling**	**Average**
04.07.2006	1	no growth	1.28×10^{6}
	2	no growth	
	3	no growth	
	4	no growth	
	5	9.55×10^{6}	
04.07.2006	6	4.55×10^{5}	1.28×10^{6}
	7	4.55×10^{5}	
28.02.2006			9.50×10^{6}
26.12.2005			no growth

Table 17. Fecal coliform densities in dewatered sludges from Kayseri wastewater treatment plant

Sampling Date	**Fecal Coliform Densities (CFU/ g TS)**		
	Sample no.	**Coliform density in each sampling**	**Average**
24.05.2006	1	3.54×10^{7}	1.57×10^{8}
	2	3.34×10^{8}	
	3	1.67×10^{8}	
	4	no growth	
	5	1.95×10^{8}	
	6	3.89×10^{8}	
	7	1.00×10^{8}	
06.12.2005			9.32×10^{6}

Table 18. Fecal coliform densities in dewatered sludges from Tekirova wastewater treatment plant

Sampling Date	**Fecal Coliform Densities (CFU/ g TS)**		
	Sample no.	**Coliform density in each sampling**	**Average**
27.06.2006	1	3.32×10^{8}	6.63×10^{8}
	2	4.35×10^{8}	
	3	3.48×10^{8}	
27.06.2006	4	6.25×10^{8}	6.63×10^{8}
	5	2.26×10^{9}	
	6	4.35×10^{8}	
	7	1.83×10^{9}	
12.04.2006	1	no growth	3.72×10^{8}
	2	1.11×10^{9}	
	3	1.73×10^{8}	
	4	7.43×10^{8}	
	5	6.94×10^{7}	
	6	7.15×10^{8}	
	7	not reliable	
31.01.2006			8.00×10^{7}

The existence of fecal coliforms has been surveyed by several investigators. Some of the results given for fecal coliform densities in sewage sludges are 2.0×10^{2}-7.0×10^{6} MPN/g TS for anaerobic digested sludges evaluated by Aulicino et al. (1998). The fecal coliform densities observed from the analysis for dewatered sludges from Kayseri and Tekirova wastewater treatment plants are higher than the results reported by Aulicino et al. (1998). The fecal coliform densities expressed for dewatered sludges from İzmir and Ankara wastewater treatment plants are within the range of values reported by Aulicino et al. (1998). The mean level of fecal coliforms reported from the survey of Sahlström et al. (2004) for 8 Swedish treatment plants was 5.00×10^{4} CFU/g TS, whereas fecal coliform data reported by Hong et al. (2004) was 1.78×10^{5} CFU/g TS and reported fecal coliform level for digested sludge in the study of Watanabe et al. (1997) was 10^{3} MPN/g TS. Dewatered sludge samples from four treatment plants in our study show higher concentration for fecal coliforms. George et al. (2002) emphasized that classical treatment reduce fecal coliform densities by 1-3 orders of magnitude which accounts for 10^{3}-10^{7} CFU/100 mL. Straub et al. (1993) reported that the fecal coliforms levels in anaerobic digested sludges gave a range between 10^{2}-10^{6}/g TS. The fecal coliform densities found in our study typically in the range of levels reported by George et al. (2002), but data for fecal coliforms in dewatered sludges from Kayseri and Tekirova wastewater treatment plants are sometimes exceeding these reported ranges. Berg and Bergman (1980) reported fecal coliform concentration in mesophilic anaerobic digested sludge samples of the City of Los Angeles. Fecal coliform levels varied from 1.5×10^{6} CFU/100 mL to 1.0×10^{7} CFU/100 mL. Dahab et al. (1996) determined the range of fecal coliform densities of aerobic digested sewage sludges and reported fecal coliform levels were from 5×10^{4} MPN/g TS to 4×10^{6} MPN/g TS with an average density of 1.7×10^{6} MPN/g TS. The fecal coliform densities reported for Kayseri and Tekirova dewatered sludges are higher than most of the reported values whereas Ankara and İzmir dewatered sludges are within the ranges reported from above studies. The geometric mean of fecal coliform densities found for dewatered sludges from Ankara, Kayseri and Tekirova wastewater treatment plants do not meet the EPA Part 503 requirements for the land application of sewage sludges for agricultural use. However mean concentration of fecal coliform found for İzmir lime stabilized dewatered sludges clearly meet the requirement for two months with the average value of 1.28×10^6 CFU/g TS where this result is lower than the Class B limit value of 2.00×10^6 CFU/g TS. One sample showed no growth for the fecal coliform in İzmir dewatered sludge.

Advanced biological treatment techniques are applied both in İzmir and Kayseri WWTPs. In İzmir WWTP, lime stabilization is used as a stabilization technique to reduce pathogens whereas in Kayseri WWTP, mesophilic anaerobic digesters are used for stabilization. From the analyses of dewatered sludges, we can come into conclusion that lime stabilization is more efficient in reducing the number of fecal coliforms. It is clear that the use of which stabilization techniques for sludge treatment reduces fecal coliform levels. Results obtaining dewatered sludges from Tekirova WWTP show higher densities than the rest of the sampling sites. In literature it was stated that anaerobic digestion produces Class B type biosolids having fecal coliform level of 2×10^6 CFU/g TS at mesophilic temperatures whereas lime stabilization technique application is available to achieve Class A type biosolids having fecal coliform level of 1,000 MPN/g TS.

4.3.2. Fecal streptococci

The results of fecal streptococci levels in selected wastewater treatment plants are presented in Table 19 through 22. From Table 19, the fecal streptococci densities in the effluent of the anaerobically digested and dewatered sludge samples from Ankara wastewater treatment plant range from 4.88×10^6 to 2.13×10^8 CFU/g TS. Fecal streptococci densities varied from no growth to 3.31×10^8 CFU/g TS for lime stabilized dewatered sludge samples from İzmir wastewater treatment plant as shown in Table 20. From Table 21, the levels of fecal streptococci in anaerobically digested/dewatered sludges from Kayseri wastewater treatment plant are in the range of 5.15×10^{8}-3.22×10^{9} CFU/g TS. The fecal streptococci numbers in the effluent of the dewatered sludge from Tekirova wastewater treatment plant are between 1.04×10^{8}-3.30×10^{9} CFU/g TS. The fecal streptococci levels of dewatered sludges from Ankara and İzmir wastewater treatment plants were much lower than the values obtained for Kayseri and Tekirova dewatered sludges.

Table 19. Fecal streptococci densities in dewatered sludges from Ankara wastewater treatment plant (TNTC: too numerous to count)

Sampling Date	**Fecal Streptococci Densities (CFU/ g TS)**		
	Sample no.	**Coliform density in each sampling**	**Average**
18.04.2006	1	1.17×10^{8}	1.38×10^{8}
	2	1.09×10^{8}	
	3	2.13×10^{8}	
	4	TNTC	
	5	1.48×10^{8}	
18.04.2006	6	1.61×10^{8}	1.38×10^{8}
	7	1.09×10^{8}	
04.01.2006			1.93×10^{7}
17.11.2005			2.88×10^{7}
19.10.2005			1.69×10^{7}
14.07.2005			4.88×10^{6}

Table 20. Fecal streptococci densities in lime stabilized dewatered sludges from İzmir wastewater treatment plant

Sampling date	Fecal Streptococci Densities (CFU/g TS)
04.07.2006	no growth
28.02.2006	3.31×10^8
26.12.2005	no growth

Table 21. Fecal streptococci densities in dewatered sludges from Kayseri wastewater treatment plant

Sampling Date	Fecal Streptococci Densities (CFU/ g TS)		
	Sample no.	**Coliform density in each sampling**	**average**
24.05.2006	1	3.22×10^9	2.77×10^9
	2	2.95×10^9	
	3	2.67×10^9	
	4	2.39×10^9	
	5	2.95×10^9	
	6	2.78×10^9	
	7	2.50×10^9	
06.12.2005			5.15×10^8

Table 22. Fecal streptococci densities in dewatered sludges from Tekirova wastewater treatment plant

Sampling Date	Fecal Streptococci Densities (CFU/ g TS)		
	Sample no.	**Coliform density in each sampling**	**Average**
27.06.2006	1	3.30×10^9	1.45×10^9
	2	1.45×10^9	
	3	7.59×10^8	
	4	not reliable	
	5	9.80×10^8	
	6	1.74×10^9	
	7	1.65×10^9	
12.04.2006	1	1.32×10^8	1.45×10^8
	2	1.53×10^8	
	3	2.22×10^8	
	4	not reliable	
	5	1.37×10^8	
	6	1.04×10^8	
	7	1.37×10^8	
31.01.2006			4.35×10^8

The results related with the fecal streptococci level of dewatered sludges from four treatment plants are much higher than the results obtained by Berg and Bergman (1980), Dahab et al. (1996) and Lucero-Ramirez (2000). Fecal streptococci levels in mesophilic anaerobic digested sludge reported by Berg and Bergman (1980) were between 1.50×10^6-4.00×10^6 CFU/g TS. Dahab et al. (1996) reported the average fecal streptococci in digested sludges from four wastewater treatment plants as 8.50×10^5 CFU/g TS. Lucero-Ramirez (2000) evaluated the effectiveness of belt-thickening, anaerobic digestion, air drying and composting methods in the reduction of bacteria. The results for the field study show that fecal streptococci levels in anaerobic digestion were between 324-4.00×10^6 MPN/g TS. The differences between results may be due to the different recovery techniques applied in each study. The existence of different commercial medium and recovery techniques such as multiple tube fermentation techniques and membrane filter technique proposed by Standard Methods for the growth of fecal streptococci may led to get different levels for fecal streptococci.

Fecal coliform levels obtained for four wastewater treatment plants; in general; give lower results than the levels obtained for fecal streptococci. Lucero-Ramirez (2000) had similar findings for the presence of fecal streptococci and concluded the study that fecal streptococci are more resistant to inactivation than fecal coliforms. Therefore pathogen survival monitoring during wastewater treatment process can be conducted with the parameter fecal streptococci.

4.3.3. *Salmonella*

The method that is used for the detection of the *Salmonella* in our study was first verified with a sample which is known to have Salmonella in its content. The recoveries of Salmonella from XLD agar were 88.8% and SS agar was 95.1%. Figure 2 and Figure 3 are the representative photographs of the Salmonella culture that was grown on specific agars.

The results of *Salmonella* analysis for all of the treatment plants' sludge samples are given in Table 23. After the analysis period, *Salmonella* were not detected in some samples. Those values are indicated as <2 MPN/4g TS as stated in Standard Methods (1995) The *Salmonella* densities in the effluent of the anaerobically digested and dewatered sludge samples from Ankara wastewater treatment plant ranged from none to 1.22 MPN/4g TS. *Salmonella* numbers in the dewatered sludge from Tekirova wastewater treatment plant were between none to 3.48 MPN/4g TS. *Salmonella* densities varied from none to 2.55 MPN/4g TS for dewatered sludge samples from İzmir wastewater treatment plant. The abundance of *Salmonella* in anaerobically digested and dewatered sludges from Kayseri wastewater treatment plant are in the range of none to 5.34 MPN/4g TS.

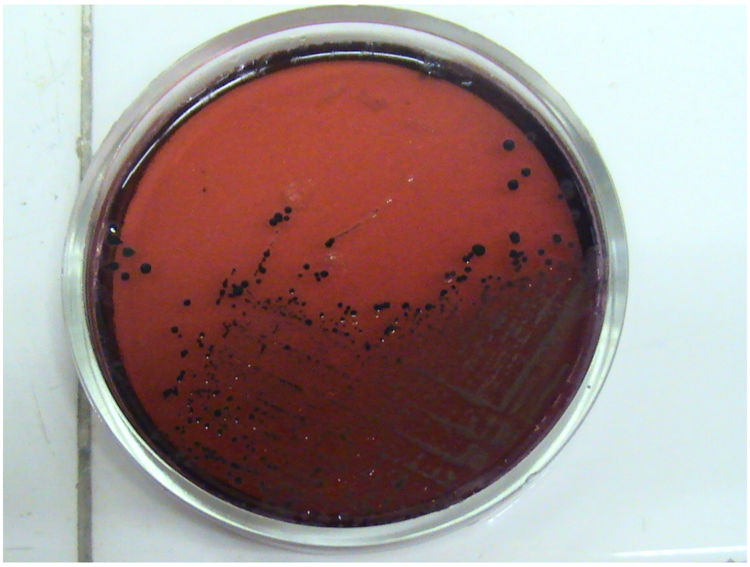

Figure 2. *Salmonella* on XLD agar

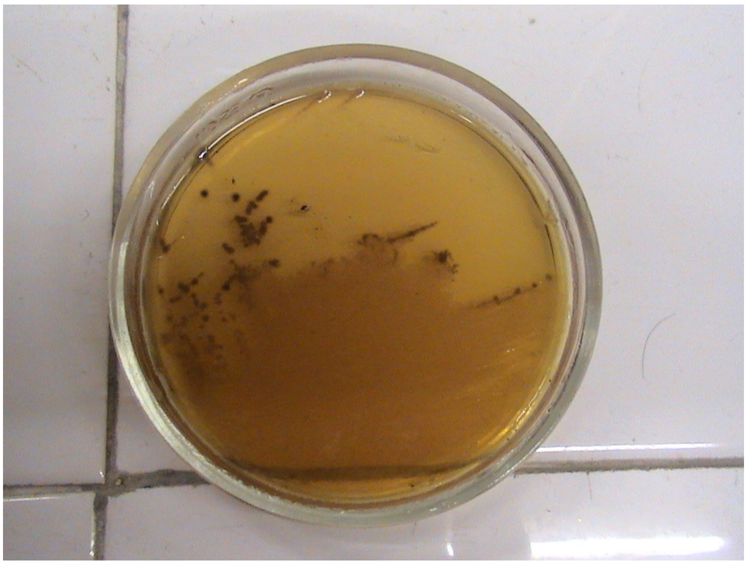

Figure 3. *Salmonella* on SS agar

The study conducted by Aulicino et al. (1998) on the evaluation of the abundance of *Salmonella* levels in anaerobic digested sludges show that only 2 samples out of 10 samples present *Salmonella* existence. Sahlström et al. (2004) found that 38 samples out of 69 anaerobic digested sludge samples that account for 55% of samples show *Salmonella* existence. In our study, *Salmonella* was found in 4 samples out of 13 samples from four wastewater treatment plants. Straub et al. (1993) reported about the influence of aerobic and anaerobic digestion on pathogen reduction and concluded that *Salmonella* concentration in anaerobic digested sludge varied from 3 to 10^3/g TS. In comparison, dewatered sewage sludge samples taken in 27.06.2006 and 24.05.2006 from Tekirova and Kayseri have 3.48 MPN/4g TS and 5.34 MPN/4g TS of *Salmonella,* respectively. Watanebe et al. (1997) reported that *Salmonella* level in digested sludge was 10^3 MPN/g TS much higher than our results and number of salmonella in mesophilic anaerobic digested sludge was ranged from 1.8 to 30 MPN/g TS. These are similar results compared to the ones obtained in our study in terms of *Salmonella*. Dahab et al. (1996) reported that anaerobic sludge samples from two of the four wastewater treatment plants had densities of 80-82 MPN/4g TS, and the other two had densities of 2340-3840 MPN/4g. Dewatered sewage sludge samples from four wastewater treatment plants in our study have lower concentrations than the results obtained from the study of Dahab et al. (1996). Salmonella detection survey conducted by Köy Hizmetleri Genel Müdürlüğü Ankara Araştırma Enstitüsü on sewage sludge samples of Ankara WWTP show trends similar to our study. During 7 months of 2000, dewatered sludge samples show that no trace of salmonella was detected.

EPA Part 503 requirements Class A biosolids for the land application of sewage sludges for agricultural use related with *Salmonella* concentration emphasize that sewage sludges must meet the limit *Salmonella* density<3 MPN/4g TS. The *Salmonella* concentration in the anaerobic digested and dewatered sludges from Kayseri and untreated sludge samples from Tekirova are above the limit concentration set for *Salmonella* in USA regulation. Therefore, attention should be paid in the land application of sewage sludges from Kayseri and Tekirova wastewater treatment plants. Other treatment plants meet the pathogen requirement for biosolids.

As stated previously, advanced biological treatment techniques are applied both in İzmir and Kayseri WWTPs. In İzmir WWTP, lime stabilization is used as a stabilization technique to reduce pathogens whereas in Kayseri WWTP, mesophilic anaerobic digesters are used for stabilization. From the analyses of dewatered sludges for *Salmonella*, we can come into conclusion that lime stabilization applied in İzmir WWTP is more efficient in reducing the number of *Salmonella* than anaerobic digestion. Conventional activated sludge treatment techniques are applied both in Ankara and Tekirova WWTPs. In Ankara WWTP, anaerobic digesters are used as stabilization technique to reduce pathogens whereas in Tekirova WWTP, sludges are not stabilized for pathogen reduction. From Table 23, Salmonella levels in dewatered sludges from Ankara WWTP is between none to 1.22/4 g TS whereas in dewatered sludge samples from Tekirova, *Salmonella* levels are between none to 3.48/4 g TS. Advanced biological treatment is applied to wastewaters in both İzmir and Kayseri WWTP. The difference between two treatment plants is the presence of anaerobic sludge digestion in Kayseri WWTP. From Table 23, the reason for higher *Salmonella* concentrations in Kayseri WWTP could be the typical characteristics of those bacteria. *Salmonella* are facultative anaerobic bacteria therefore anaerobic conditions in Kayseri WWTP during the advanced biological treatment phase could have enhanced the level of bacteria.

Table 23. Salmonella densities in Ankara, İzmir, Kayseri and Tekirova wastewater treatment plants

Sampling Place	**Sampling Date**	***Salmonella* Densities (*Salmonella* spp./4 g TS)**
Ankara	18.04.2006	1.22
	04.01.2006	<2
	17.11.2005	<2
	19.10.2005	<2
	14.07.2005	<2
İzmir	04.07.2006	2.55
	28.02.2006	<2
	26.12.2005	<2
Kayseri	24.05.2006	5.34
	06.12.2005	<2
Tekirova	27.06.2006	3.48
	31.01.2006	<2

4.3.4. *Cryptosporidium* and *Giardia*

Table 24 is the represantation of *Cryptosporidium, Giardia* and *Helminth Eggs'* existence after the sampling and analyses of related parameters. The findings of the study are given as positive for presence and negative for absence of microorganisms.

EPA Part 503 requirements Class A biosolids for the land application of sewage sludges for agricultural use related to helminth eggs concentration emphasize that sewage sludges must meet the limit helminth egg density<1 viable ovum/4g TS. Helminth eggs were not found in all of the dewatered sludges from four wastewater treatment plants. Therefore, all dewatered sludges can be classified as Class A biosolids in relation to helminth eggs existence. Attention should be paid in the case of *Giardia* for the land application of sewage sludges from Ankara and

Kayseri WWTPs, because all the dewatered sludge samples taken were positive for *Giardia*. In Ankara and Kayseri WWTPs, anaerobic digesters are used for stabilization of sludges. From the study of Ramirez (2000), a factor that may account for such presence of *Giardia* in anaerobic digested sludges is due to the concentration of solids in the digested sludges. The destruction of volatile solids may release more oocysts from the sludge matrix. Lime stabilized dewatered sludge samples from İzmir WWTP do not show *Giardia* existence. In Tekirova treatment plant, stabilization methods are not applied to raw sludges therefore the existence of Giardia is related with this property. All sludge samples from four different treatment plants are negative for *Cryptosporidium* and helminth eggs.

The absence of *Cryptosporidium* in all sewage sludge samples may be due to the detection method applied during the study. The presence of giardia in almost all sampling was the indicator for the presence of *Cryptosporidium* species. Different extraction methods such as PCR amplification and immuno-magnetic separation techniques can be used for the detection of *Cryptosporidium.*

Table 24. *Cryptosporidium, Giardia* and *Helminth Eggs* in Ankara, İzmir, Kayseri and Tekirova wastewater treatment plants

Sampling Place	Sampling Date	Cryptosporidium	Giardia	Helminth Eggs
Ankara	20.07.2005	-	+	-
	19.10.2005	-	+	-
	17.11.2005	-	+	-
	18.04.2006	-	+	-
İzmir	04.07.2006	-	-	-
	28.02.2006	-	-	-
Kayseri	24.05.2006	-	+	-
	06.12.2005	-	+	-
Tekirova	27.06.2006	-	+	-
	31.01.2006	-	-	-

CHAPTER 5

CONCLUSION

In this thesis it is aimed to present the agricultural utilization potential of wastewater sludges from Ankara, İzmir, Kayseri and Tekirova wastewater treatment plants.

For measuring the heavy metals, an extraction procedure assisted with microwave digestion and conventional digestion were studied in a comparative manner. Microwave digestion system was optimized with 5 programs labeling as Program A, Program B, Program C, Program D and Program E. All programmes were optimized with certified reference sludge material and different acid combinations. Among all program attempts, Programme E consisting of 2 steps and 5 stages yielded in higher results. Time settings are as follows; Step 1 stage1; 5 min, stage 2; 5 min and stage 3; 20 min. and Step 2 stage 1; 15 min, stage 2; 15 min. Temperature settings; Step 1 stage 1; 140 ^{o}C, stage 2; 160 ^{o}C, stage 3; 175 ^{o}C and Step 2 stage 1; 160 ^{o}C, stage 2; 100 ^{o}C. Power settings; Step 1 stage 1; 750 W, stage 2; 850 W, stage 3; 900 W and Step 2 stage 1; 800 W, stage 2; 400 W. The Program E that was set with these operational conditions represents high recoveries within the case of volatile Hg and Cd, as well as Pb and Cu with recoveries 110.6%, 107.6%, 125.7% and 103%, respectively. Zn, Cr and Ni recoveries were 100.4%, 82.2% and 100%, respectively. Therefore, Program E was used to extract heavy metals from sewage sludge samples.

Levels of the measured heavy metals differed between the different plants due to variable industrial inputs to treatment plants, applied pretreatment processes prior to the wastewater reach the plants and differences in the treatment scheme. The mean concentrations of Cu, Pb, Cr and Ni in Kayseri dewatered sludges are higher than other plants where Cd and Zn concentrations of dewatered sewage sludges in Ankara plant are higher than the others. Cu, Pb, Cd, Zn and Cr concentrations in dewatered sludges of Tekirova WWTPs which has no industrial input are relatively lower than the dewatered sludge samples from other treatment plants. Both Ankara and Kayseri WWTPs receive 15% industrial inputs and higher concentrations obtained are due to the lower pretreatment applied to wastewaters. The results were evaluated with respect to Soil Pollution Control Regulation of Turkey. They show that all wastewater sludges meet the limits set in the regulation. However, attention should be paid to zinc and nickel content of Ankara and Kayseri sludges, to be able to use these them for agricultural purposes.

Pathogen and indicator microorganisms in selected wastewater treatment plants were analyzed according to methods given by Standard Methods, ISO Standards and EPA. Indicator microorganisms and pathogen levels in all sludges (Ankara, İzmir, Kayseri and Tekirova dewatered sludges) showed variations during a year. Mean concentration of fecal coliform found for İzmir lime stabilized dewatered sludges clearly meet the requirement for two months with the average value of 1.28×10^6 CFU/g TS where this result is lower than the Class B limit value of 2.00×10^6 CFU/g TS. Dewatered sludges from Ankara, Kayseri and Tekirova wastewater treatment plants do not meet the EPA Part 503 requirements for the land application of sewage sludges for agricultural use. In conclusion, lime stabilization which is applied in sludges from İzmir WWTP is more efficient in reducing the number of fecal coliforms from the results obtained. Relatively high fecal coliform levels for Tekirova WWTP are due to absence of any stabilization technique applied.

Fecal streptococci showed higher levels than fecal coliform for almost all wastewater treatment plants. Fecal streptococci is thought to be more resistant to inactivation than fecal coliforms during processing of the raw sludge. Therefore, fecal streptococci may be a better indicator than fecal coliform for monitoring pathogen survival during conventional sludge treatment processes.

The results for *Salmonella* densities in four treatment plants show that *Salmonella* concentration of 5.34 MPN/4g TS in the anaerobic digested and dewatered sludges from Kayseri and 3.48 MPN/4g TS in untreated sludge samples from Tekirova are above the limit concentration of 3 MPN/4g TS set for *Salmonella* in USA regulation, respectively. Again, lime stabilization is more efficient in destructing the pathogens.

Attention should be paid in the case of *Giardia* for the land application of sewage sludges from Ankara and Kayseri WWTPs, because all the dewatered sludge samples taken were positive for *Giardia*. Dewatered sludge samples from four wastewater treatment plants do not show *Cryptosporidium* and helminth eggs existence. The presence of *Giardia* in anaerobic digested sludge samples are the result of destruction of volatile solids therefore oocysts are released during anaerobic digestion.

In summary, wastewater sludges from İzmir and Tekirova WWTPs can be safely applied to land for agricultural purposes with consideration of heavy metal limitations in SPCR. In addition, lime stabilized dewatered sludges of İzmir WWTP clearly meet fecal coliform and salmonella requirements given in US EPA regulation. Therefore, we can come into conclusion that lime stabilized wastewater sludges from İzmir WWTP are the most appropriate for land application with the consideration of heavy metals standards in SPCR as well as fecal coliforms and salmonella limitations in US EPA Regulation.

Considering the pathogen levels measured in four wastewater treatment plants, one can concluded that the existence of pathogens and indicator microorganisms in considerable amounts in the sludge samples to be applied should be controlled by setting microbiological limits in SPCR of Turkey.

CHAPTER 6

RECOMMENDATIONS FOR FUTURE STUDY

In this study the possibility of the utilization of wastewater sludges from four wastewater treatment plants in Turkey regarding heavy metals and indicator microorganisms and pathogen levels were evaluated. Analyses were conducted on dewatered sludge samples. In further studies, in order to evaluate the accumulation and removal of heavy metals and microbiological parameters during the treatment process, raw wastewater samples and sludge samples collected from each treatment step of two or more wastewater treatment plants can be investigated. A better understanding of each treatment unit in reducing heavy metals and microbiological parameters can be achieved by this methodology. In addition knowledge of raw wastewater characteristics may give a chance to make a comparison between treatment plants.

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APPENDIX A

POLLUTANT CONCENTRATION LIMITS IN USA, EU AND TURKEY

Table A1. Pollutant Concentration Limits and Loading Rates for Land Application in the United States (NRC, 2002)

Pollutant	(1) Ceiling concentration limit (mg/kg)[a]	(2) Cumulative loading rate limit (kg/ha)[a]	(3) Pollutant concentration limit (mg/kg)[a]	(4) Annual pollutant loading rate for distributed biosolids exceeding column (3) (kg/ha/y)[a]
Ar	75	41	41	2.0
Cd	85	39	39	1.9
Cu	4,300	1,500	1,500	75
Pb	840	300	300	15
Hg	57	17	17	0.85
Mo	75	-	-	-
Ni	420	420	420	21
Se	100	100	100	5
Zn	7500	2,800	2,800	140
Applies to:	All sewage sludges that are land applied	Bulk solids	Bulk or bagged sewage sludges[b]	Bagged[b] sewage sludges where at least one element does not meet column (3)

[a] dry weight basis

[b] Bagged biosolids are sold or given away in a bag or container containing less than 1 metric ton (MT)

Table A2. Treatment Alternatives Required for Class A Biosolids (Iranpour, 2004)

Alternatives	Treatment Specific Requirements	Pathogen Reduction Level
1	*One of the four time-temperature regimens*	
2	*Alkaline tretment-high pH,high temperature process*	
3[a]	*Process monitoring*	Helminth ova<1 ovum/4gTS Enteric viruses<1 PFU/4gTS
4[b]	*Undefined process*	Helminth ova<1 ovum/4gTS Enteric viruses<1 PFU/4gTS
5	*Processes to further reduce pathogens(PFRP)* Composting Heat drying Heat treatment Thermophilic aerobic digestion Beta ray irradiation Gamma ray irradiation Pasteurization	
6	*Processes equivalent to further reduce pathogens(PFRP)*	

[a] Helminth ova and enteric viruses to be determined before and after pathogen treatment.
[b] Helminth ova and enteric viruses to be determined for each sale or given away.

Table A3. Processes to Significantly Reduce Pathogens for Class B (Biosolids Management Handbook)

Alternatives	*Processes to Significantly Reduce Pathogens (PSRP)*
1	Aerobic Digestion: 40 days at 20^oC and 60 days at 15^oC
2	Air Drying: minimum 3 months, average ambient temperature is above 0^oC during two of the three months
3	Anaerobic Digestion: 50 days at 35-55^oC and 60 days at 20^oC
4	Composting: The temperature is raised to 40^oC or higher for five days. The temperature in the compost pile exceeds 55^oC for 4 h. during 5 days.
5	Lime Stabilization: pH=12 after 2 hours of contact.

Table A4. Vector Attraction Reduction Options (Iranpour et al., 2004)

Alternatives	Requirement
1	Minimum 38% mass reduction of volatile solids.
2	For anaerobically digested biosolids not meeting alternative 1, demonstrate vector attraction reduction by bench-scale anaerobic digestion (less than 17% reduction of volatile solids over 40 days at 30–37 ^oC).
3	For aerobically digested biosolids not meeting alternative 1, demonstrate vector attraction reduction by bench-scale aerobic digestion (less than 15% reduction of volatile solids over 30 days at 20 ^oC).
4	For aerobically treated biosolids, the specific oxygen uptake rate should be equal or less than 1.5 mg/h/g DS at 20 ^oC.
5	Aerobic treatment of biosolids at temperatures greater than 40 ^oC (average of 45 ^oC) for 14 days or longer.
6	Increase of the pH to above 12, followed by maintaining the pH at 12 or higher for 2 hours and at 11.5 or higher for an additional 22 hours.
7	Increase of the pH to above 12, followed by maintaining the pH at 12 or higher for 2 hours and at 11.5 or higher for an additional 22 hours.
8	Increase of the pH to above 12, followed by maintaining the pH at 12 or higher for 2 hours and at 11.5 or higher for an additional 22 hours.
9	Injection of biosolids beneath the land surface.
10	Incorporation of biosolids into the soil.

Table A5. Site restriction requirements (Iranpour et al., 2004)

Harvesting/Land use	Restriction
Food and other crops with harvested parts that do not touch the soil surface	No harvesting for 30 days
Food crops with harvested parts that are totally above ground but touch the soil surface	No harvesting for 14 months
Food crops with harvested parts that are below the land surface and where the biosolids remain on the land for longer than 4 months before incorporation into the soil	No harvesting for 20 months
Food crops with harvested parts that are below the land surface and where the biosolids remain on the land for shorter than 4 months before incorporation into the soil	No harvesting for 38 months
Turf used for land with a high potential for public exposure or lawn	No harvesting for 12 months
Grazing land	No grazing for 30 days
Land with high potential for public exposure (e.g. park or ballfield)	Access restricted for 12 months
Land with low potential for public exposure (e.g. private farm land)	Access restricted for 30 days

Table A6. European Union Limit Values for Heavy Metals in Biosolids (mg/kg DM) (NRC, 2002)

	Cd	Cr	Cu	Hg	Ni	Pb	Zn	As	Mo	Co
Directive 86/278/EEC	20-40	---	1,000-1,750	16-25	300-400	750-1,200	2,500-4,000	---	---	---
Austria (Carinthia)	0.7-2.5	70-100	70-300	0.4-2.5	25-80	45-150	200-1,800			
Belgium (Walloon)	100	500	600	10	100	500	2,000		---	---
Denmark	0.8	100	1,000	0.8	30	120	4,000	25		
Finland	3	300	600	2	100	150	1,500	---	---	---
France	20	1,000	1,000	10	200	800	3,000	---	---	---
Germany	10	900	800	8	200	900	2,500	---	---	---
Greece	20-40	50	100-1,750	16-25	300-400	750-1,200	2,500-4,000	---	---	---
Ireland	20	---	1,000	16	300	750	2,500	---	---	---
Italy	20	---	1,000	10	300	750	2,500	---	---	---
Luxembourg	20-40	1,000-1,750	1,000-1,750	16-25	300-400	750-1,200	2,500-4,000	---	---	---
Netherlands	1.25	75	75	0.75	30	100	300	---	---	---
Portugal	20	1,000	1,000	16	300	750	2,500	---	---	---
Sweden	2	100	600	2.5	50	100	800	---	---	---
United Kingdom	---	---	---	---	---	---	---	---	---	---

Table A7. European Union Limit Values for Amounts of Heavy Metals That May Be Added Annually to Soil, Based on a 10-Year Average (National Research Council, 2002)

Pollutant	Limit Values (g/ha/y)	
	Directive 86/278/EEC	Proposed
Cd	150	30
Cr	---	3000
Cu	12,000	3000
Hg	100	30
Ni	3,000	900
Pb	15,000	2250
Zn	30,000	7500

Table A8. European Limit Values for Pathogens Concentration in Biosolids (National Research Council, 2002)

	Salmonella	Other Pathogens
France	8 MPN/10 g of DM	Enterovirus: 3 MPCN/10 g of DM
Italy	1000 MPN/ g of DM	
Luxembourg		Enterobacteria: 100/g No egg of worm likely to be contagious
Poland	Biosolids cannot be used in agriculture if it contains *Salmonella*	"Parasites": 10/kg of DM

Table A9. European Union Limit Values for Heavy Metals in Soil (mg/kg DM) (NRC, 2002)

	Cd	Cr	Cu	Hg	Ni	Pb	Zn	As	Mo	Co
Directive 86/278/EEC (6<pH<7)	1-3	---	50-140	1-1.5	30-75	50-300	150-300	---	---	---
Austria (Carinthia)	0.5-1.5	50-100	40-100	0.2-1	30-70	50-100	10-200			
Belgium (Walloon)	2	100	50	1	50	100	200	---	---	---
Denmark	0.5	30	40	0.5	15	40	100	---	---	---
Finland	0.5	200	100	0.2	60	60	150	---	---	---
France	2	150	100	1	50	100	300	---	---	---
Germany	1.5	100	60	1	50	100	200	---	---	---
Greece	1-3	---	50-140	1-1.5	30-75	50-300	150-300	---	---	---
Ireland	1	---	50	3	30	50	150	---	---	---
Italy	1.5	---	100	1	75	100	300	---	---	---
Luxembourg	1-3	100-200	50-140	1-1.5	30-75	50-300	150-300	---	---	---
Netherlands	0.8	100	36	0.3	35	85	140	---	---	---
Portugal										
Soil pH<5.5	1	50	50	1	30	50	150			
5.5<soil pH<7	3	200	100	1.5	75	300	300	---	---	---
Soil pH>7	4	300	200	2	110	450	450			
Sweden	0.4	60	40	0.3	30	40	100-150	---	---	---
United Kingdom										
5<Soil pH<5.5	3	---	80	1	50	300	200			
5.5<soil pH<6	3	---	100	1	60	300	250	---	---	---
6≤soil pH≤7	3	---	135	1	75	300	300			
Soil pH>7	3	---	200	1	110	300	450			

Table A10. Limit Values for Heavy Metals in Soil (mg/kg DS) (SPCR, 2001)

Heavy metals (Total)	pH: 5- 6 mg/kg DS	pH>6 mg/kg DS
Pb	50	300
Cd	1	3
Cr	100	100
Cu	50	140
Ni	30	75
Zn	150	300
Hg	1	1.5

Table A11. Limit Values for Several Pollutants in Soil (SPCR, 2001)

Pollutants	Limit Values
Cl^- (mg Cl^-/l) (Total)	25
Na (mg Na/l) (Total)	125
Co (mg/kg DS)	20
Ar (mg/kg DS)	20
Mo(mg/kg DS)	10
Sn(mg/kg DS)	20
Ba(mg/kg DS)	200
Fl(mg/kg DS)	200
Free Cyanide(mg/kg DS)	1
Complex Cyanide(mg/kg DS)	5
S(mg/kg DS)	2
Br (mg/kg DS)	20
Benzene (mg/kg DS)	0.05
Butyl benzene(mg/kg DS)	0.05
Toliol(mg/kg DS)	0.05
Xylole(mg/kg DS)	0.05
Phenol(mg/kg DS)	0.05
Se(mg/kg DS)	5
Tl(mg/kg DS)	1
U(mg/kg DS)	5
Polycyclic Hydrocarbons(mg/kg DS)	5
Organochlorinated Compounds(mg/kg DS)	0.5
Tarımsal Mücadele İlaçları –Bireysel(mg/kgDS)	0.5
Tarımsal Mücadele İlaçları –Toplam(mg/kg DS)	2
Polychlorinated Biphenyls(mg/kg DS)	0.5
Hexaklor benzol(mg/kg DS)	0.1
Pentaklor benzol(mg/kg DS)	0.1
Ψ- HCH (lindan) (mg/kg DS)	0.1

Table A12. Limit Values for Heavy Metals in Sewage Sludges (SPCR, 2001)

Heavy Metals	Limit Values (mg/kg DS)
Pb	1200
Cd	40
Cr	1200
Cu	1750
Ni	400
Zn	4000
Hg	25

Table A13. Limit Values for Amounts of Heavy Metals That May Be Added Annually to Soil, Based on a 10-Year Average (SPCR, 2001)

Heavy Metals	Annual Pollutant Loading Rate Limits (g/da/y)
Pb	1500
Cd	15
Cr	1500
Cu	1200
Ni	300
Zn	3000
Hg	10

APPENDIX B

SAMPLE COMPUTATION FOR FECAL COLIFORM AND FECAL STREPTOCOCCUS

Example: Seven sampling of sewage sludge with solids content of 1.31% were analayzed for fecal coliform and fecal streptococcus. Following computation belongs to sample taken from Kemer wastewater treatment plant in 12.04.2006. Results were found to be:

Table B1. Number of fecal coliform colonies on MF plates

Sampling	Dilution: 10^{-5}	Dilution: 10^{-6}	Dilution: 10^{-7}
1	No growth	No growth	No growth
2		14	2
3	19	6	
4	96	11	1
5	9	1	-
6	98	5	1
7	No growth	No growth	No growth

For sampling number 2 the fecal coliform density is:

$$\text{Fecal coliforms/g dry weight} = \frac{(14+2)\times 100}{(0.00001+0.0000001)\times 1.31} = 1.11\times 10^{9}$$

For sampling number 3 the fecal coliform density is:

$$\text{Fecal coliforms/g dry weight=}\ \frac{(19+6)\times 100}{(0.00001+0.000001)\times 1.31}=1.73\times 10^{8}$$

For sampling number 4 the fecal coliform density is:

$$\text{Fecal coliforms/g dry weight=}\ \frac{(96+11+1)\times 100}{(0.00001+0.000001+0.0000001)\times 1.31}=7.43\times 10^{8}$$

For sampling number 5 the fecal coliform density is:

$$\text{Fecal coliforms/g dry weight=}\ \frac{(9+1)\times 100}{(0.00001+0.000001)\times 1.31}=6.94\times 10^{7}$$

For sampling number 6 the fecal coliform density is:

$$\text{Fecal coliforms/g dry weight=}\ \frac{(98+5+1)\times 100}{(0.00001+0.000001+0.0000001)\times 1.31}=7.15\times 10^{8}$$

Coliform densities of all samples were calculated and converted to $\log_{10}$ values to compute a geometric mean. These calculated values are presented in Table B2.

Table B2. Coliform density of sludge samples

Sampling	Coliform density	Log_{10}
1	No growth	
2	1.11×10^9	9.05
3	1.73×10^8	8.24
4	7.43×10^8	8.87
5	6.94×10^7	7.84
6	7.15×10^8	8.85
7	No growth	

The geometric mean for the seven samples is determined by averaging the $\log_{10}$ values of the coliform density and taking the antilog of those values. Sampling number 1 and 7 are excluded from the computation.

Average of $\log_{10}=8.57$
Antilog$=3.72\times10^8$

Therefore the geometric mean fecal coliform density is 3.72×10^8 CFU/g TS.

APPENDIX C

FLOW CHARTS OF ANKARA, İZMİR, KAYSERİ AND TEKİROVA WASTEWATER TREATMENT PLANTS

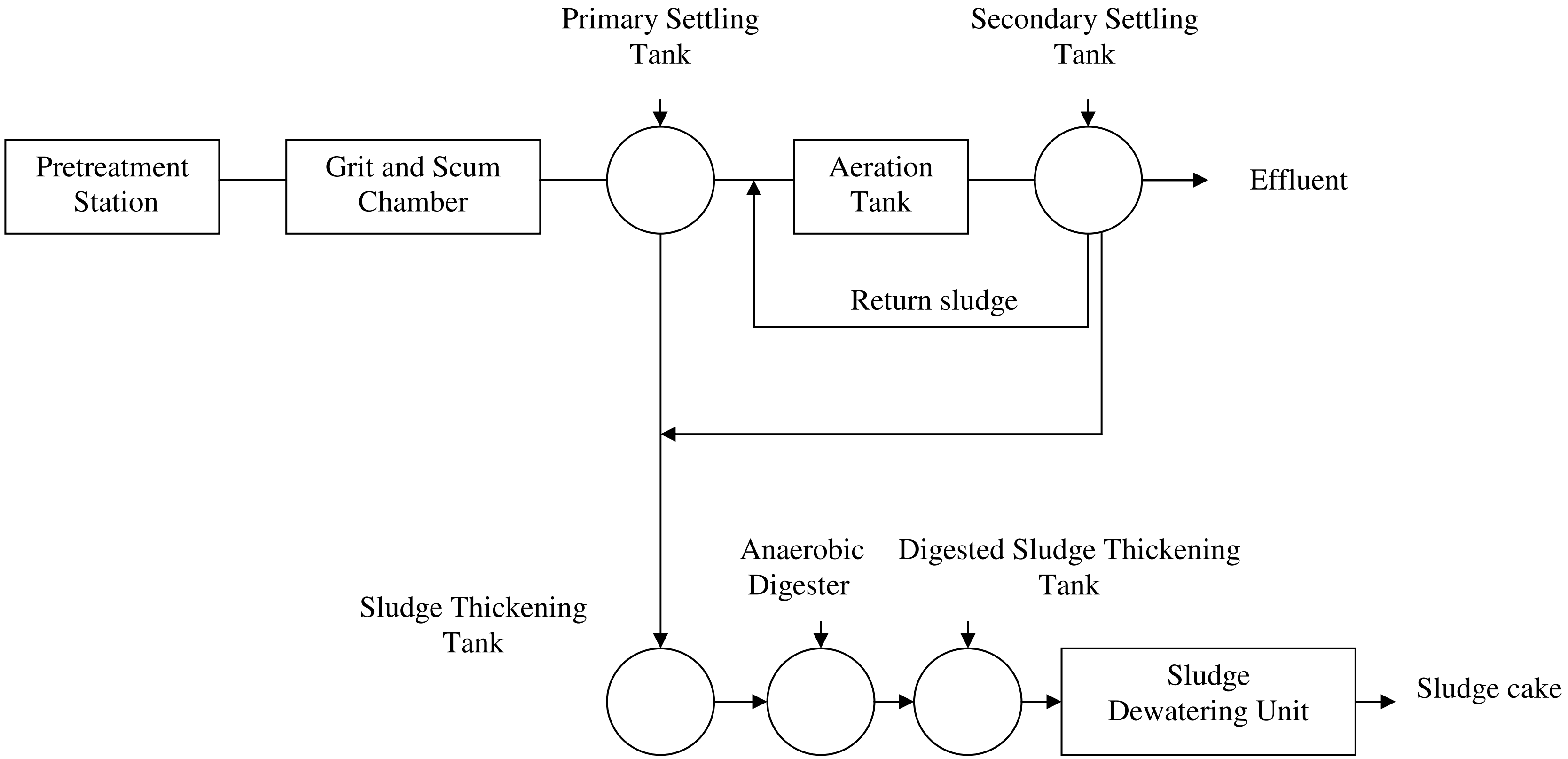

Figure C1. Flow chart of the wastewater treatment plant of Ankara

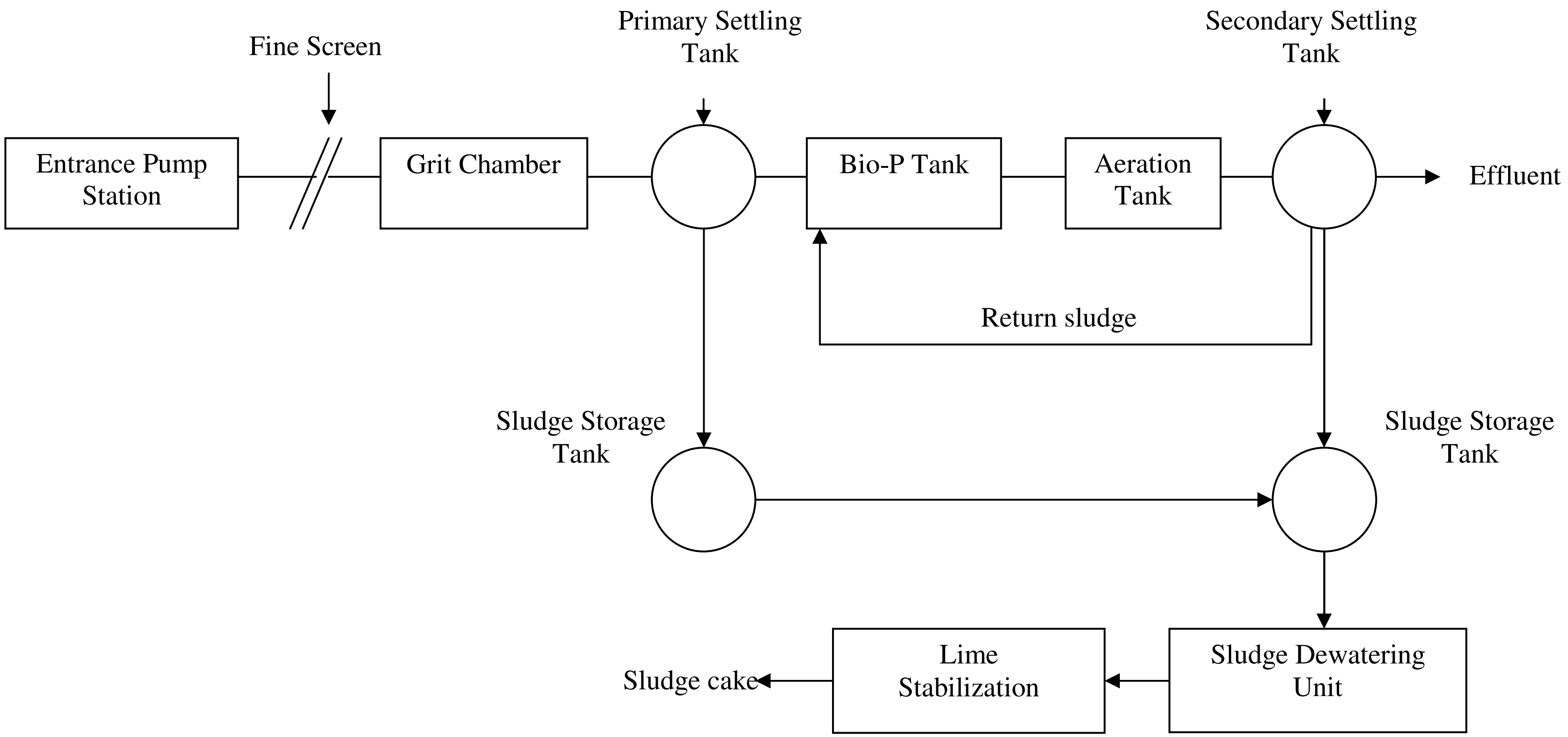

Figure C2. Flow chart of the wastewater treatment plant of İzmir

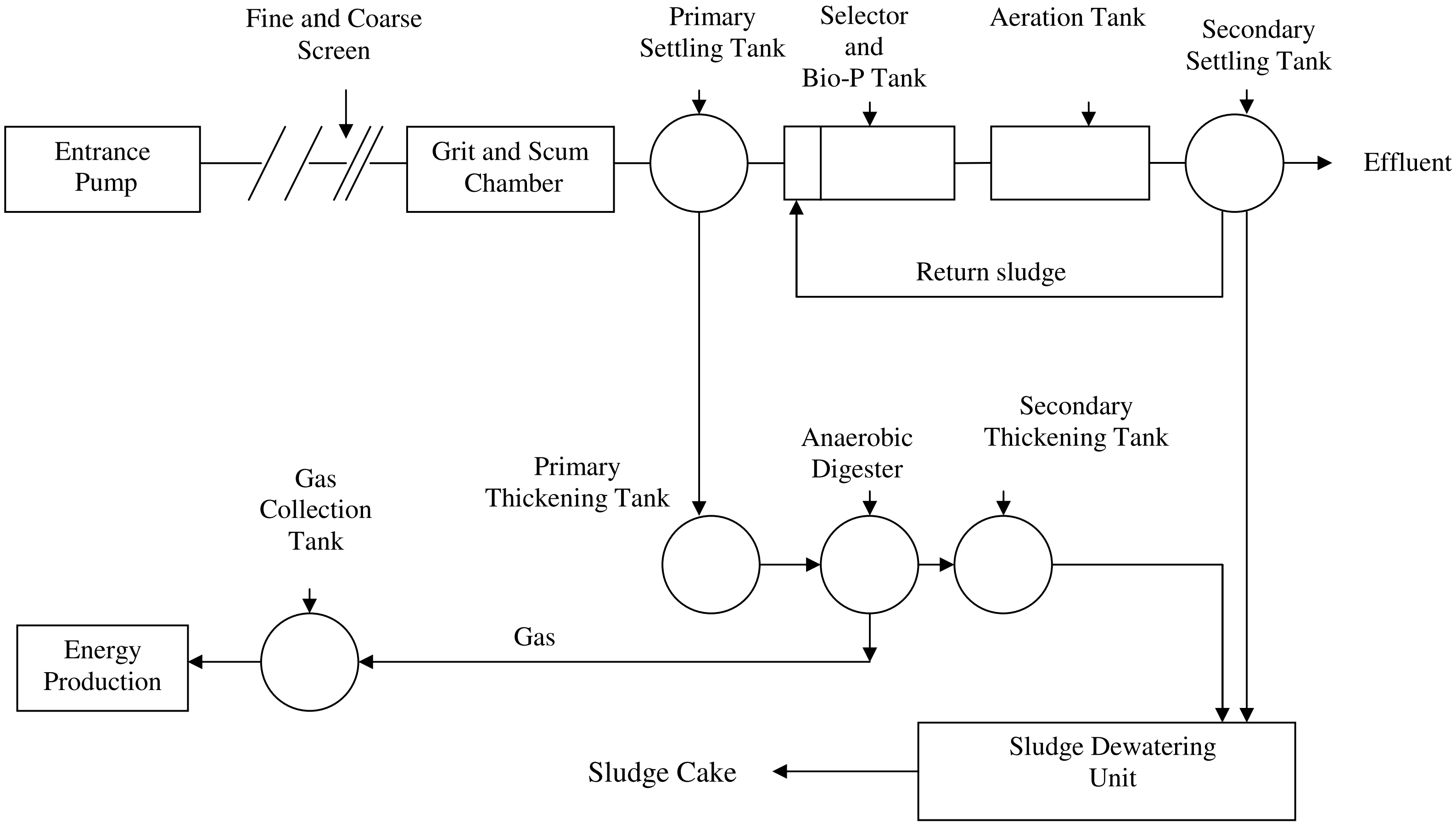

Figure C3. Flow chart of the wastewater treatment plant of Kayseri

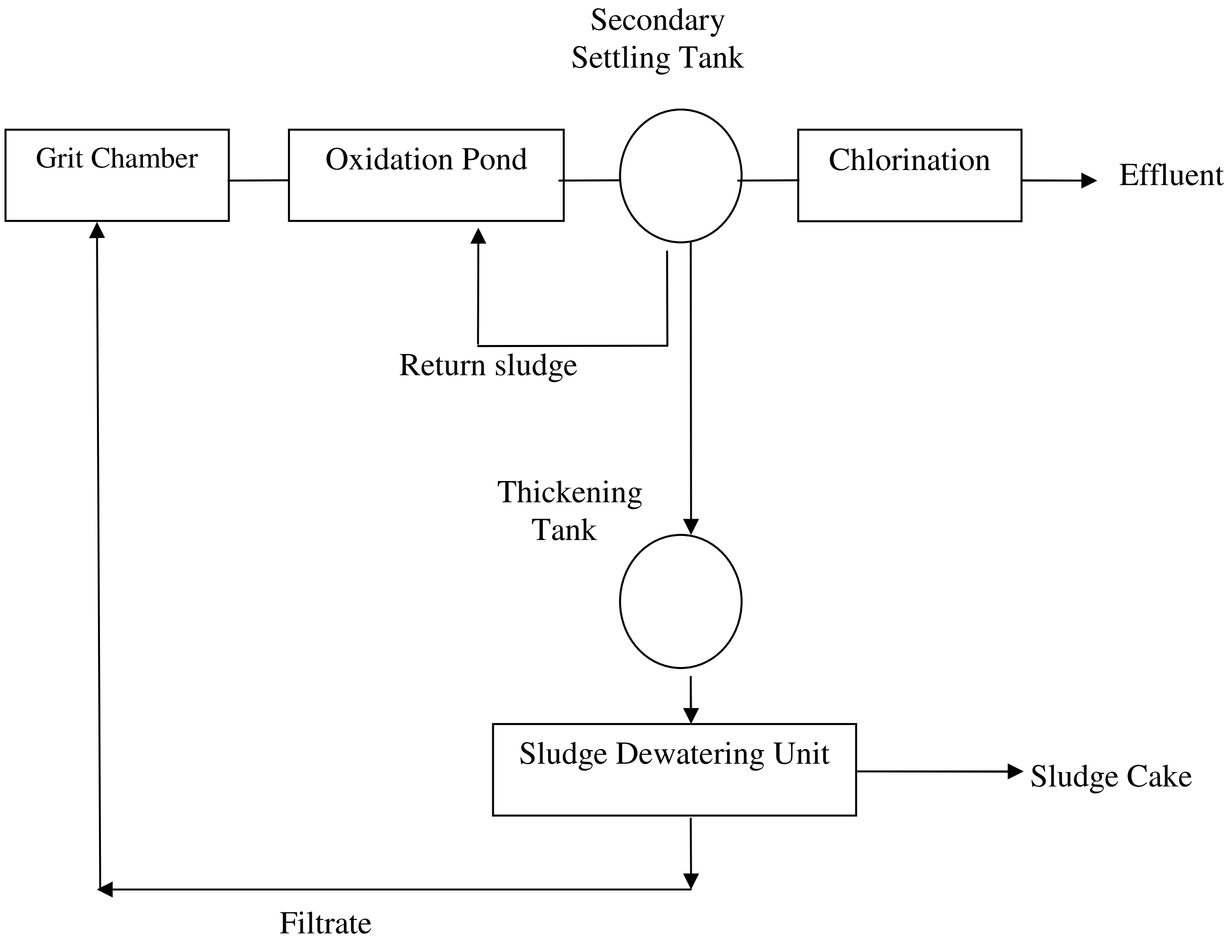

Figure C4. Flow chart of the wastewater treatment plant of Tekirova