Evaluation of the use of treated sewage sludge as fertilizer and in the Bio-and Phyto-remediation of diesel-contaminated soil

Suaad Jaffar Abdul Khaliq

A thesis submitted in partial fulfillment of the requirements for the degree

Doctor of Philosophy

in

Soil and Water Management

Department of Soils, Water and Agricultural Engineering

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DEDICATION

I would like to dedicate this work to Sayida Fatima Al-Zahra the daughter of Prophet Mohammad (pbuh) - who has been a continuous source of inspiration to me.

Also, this research is dedicated to each and every member of my large, supportive and encouraging family, starting from my mother who really has deeply influenced my life by her prayers and encouragement which has helped me to accomplish this study, and to my father's soul who has always been the greatest inspiration in my life, ending with the other members of my family who have given me their support.

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Publications emanating from this thesis

Journal Papers

1. Suaad Jaffar Abdul Khaliq, Mushtaque Ahmed, Malik Al-Wardy, Ahmed Al-Busaidi and B.S. Choudri. (2017). Wastewater and Sludge Management and Research in Oman: An Overview. *Journal of Air & Waste Management Association.* 67(3), 267-278 Journal home page is http://www.tandfonline.Com/loi/uawm20. Published online on 07 Oct 2017.

2. Suaad Jaffar Abdul Khaliq, Ahmed Al-Busaidi, Mushtaque Ahmed, Malik Al-Wardy, Hesham Agrama and B.S. Choudri. (2017). The effect of municipal sewage sludge on the quality of soil and crops. *Int J Recycl Org Waste Agricult*. 6(4), 289-299. DOI 10.1007/s40093-017-0176-4. Published online on 19 September 2017.

Conference Presentation

1. Suaad Jaffar Abdul Khaliq, Prabha Padmavathiamma, Mushtaque Ahmed. (2015). Phytoremediation of total Hydrocarbons (TPH) in diesel-contaminated Soils. Workshop on International Year of Soils. SQU, Muscat.

2. Suaad Jaffar Abdul Khaliq, Prabha Padmavathiamma, Mushtaque Ahmed. (2015). Phytoremediation for Diesel-contaminated soil of Oman. Conference on Nanotechnology for Water Treatment and Solar Energy Applications. Muscat, Oman.

3. Suaad Jaffar Abdul Khaliq, Prabha Padmavathiamma, Mushtaque Ahmed, Hesham Agrama. (2016). Effects of Sewage Sludge Compost in Enhancing the Growth of Grasses in Phytoremediation of Diesel-Contaminated Soil. 13th IWA Specialized Conference on Resource-Oriented Sanitation. Athens, Greece.

4. Mushtaque Ahmed, Mahad Baawain, Salim Ali Al-Jabri, Suaad Jaffar Abdul Khaliq & B.S. Choudri. (2016). Small- Scale Wastewater Treatment Systems and Reuse Studies in Oman. 13th IWA Specialized Conference on Resource- Oriented Sanitation. Athens, Greece.

5. Suaad Jaffar Abdul Khaliq, Mushtaque Ahmed, Ahmed Al-Busaidi Hesham Agrama. (2017). The effect of municipal sewage sludge on the quality of soil and crops. 2nd National Conference on Agricultural and Fisheries Research (Research for Sustainable Development). SQU, Muscat.

Other Publications

Prabha Padmavathiamma, Suaad Jaffar Abdul Khaliq, Mushtaque Ahmed. (2015). Phytoremediation for Diesel-Contaminated Soils of Oman. Horizon. Department of Public Relation and Information. SQU, Muscat.

ABSTRACT

It is well-recognized that management of wastewater and sludge is a critical environmental issue in many countries. A regular and environmentally-safe wastewater treatment and associated sludge management requires the development of realistic and enforceable regulations as well as treatment systems appropriate to local circumstances. Furthermore, treated wastewater and sludge development should encourage the revision of existing standards, regulations, and policies for their management in the Sultanate. Many studies have been conducted on reusing treated wastewater as a beneficial source in Oman, but little research has been carried out on using sewage sludge.

The objectives of this research are to compare the current Omani legislation with international legislations like World Health Organization (WHO) and United States Environmental Protection Agency (US-EPA) in terms of treated wastewater reuse for agricultural purposes and with European Guidelines (EU) and US-EPA in terms of sludge application reuse to recommend any necessary implementation of amendments and modifications to the national regulations. Moreover, the effect of composted sewage sludge (Kala compost) was investigated to reduce hydrocarbons from diesel-contaminated soil by applying phytoremediation and bioremediation methods. Phytoremediation was used by means of Bermuda grass and Ryegrass and bioremediation was carried out using isolated microorganisms. Lastly, the effective application of Kala compost and inorganic (NPK) fertilizers on soil quality and on two crops (Radish and Beans) was studied.

The study revealed that the national regulations are considered to be too general and a number of recommendations have been made to the decision-makers to consider modifying the guidelines in Oman. The remediation of diesel-contaminated soil showed that 77% removal of Total Petroleum Hydrocarbons (TPH) in the phytoremediation method was achieved when 10% of Kala compost was applied in contaminated soil cultivated with Bermuda grass compared to Ryegrass. The isolated strains in the bioremediation method were *Bacillus* genera which belong to degradable diesel strain categories which had shown their capabilities to degrade diesel fuel up to 66% after incubation for one week and 90% after incubation for two weeks, especially when 1% Kala compost was added to the treatment. In addition, their concentration dropped from 87 to 29 mg of alkanes/g soil in the same treatment. These strains are considered as halotolerant and mesophilic, which can grow in coastal sediments where the salinity could reach up to 10% of NaCl concentration, and can survive in the Omani hot summer months where the temperature reaches up to $55^{o}C$. Finally, Kala compost showed its efficiency in producing higher crop yields of Radish and Beans compared to NPK fertilizers in the agricultural experiment. Moreover, chemical analysis of soil and the two crops did not show any risk of heavy metal accumulation.

In the area of treated municipal wastewater and sludge management in the Sultanate of Oman, the experiments showed the efficiency of using municipal composted sewage sludge (Kala compost) for enhancing the remediation of diesel hydrocarbon-contaminated soil and using it as organic fertilizer in agricultural activities.

الخلاصة

شهدت السلطنة في الآونة الأخيرة كغيرها من الدول، نموًا مضطردًا في عدد السكان واكبه نموًا وتطورًا هائلاً في التنمية الزراعية والاجتماعية والاقتصادية، وقد نجم عن ذلك ازدياد في الطلب على المياه النقية والصالحة للشرب لمواكبة مقومات التنمية. ففي ظل هذا التسارع كان من الضروري استخدام بدائل أخرى للمياه النقية خشية استنزافها ونضوبها، كاستخدام مياه الصرف الصحي المعالجة في الكثير من المشاريع التنموية. عليه، وبهذا الخصوص تم تنفيذ مشروع إقامة محطة الصرف الصحي المعالجة في مسقط والتي تنفذها شركة حيا للمياه وفق أسس ومعايير عالمية بغية استخدامها لمشاريع التنمية في البلاد. ولتحقيق الإدارة المتكاملة لاستخدام المياه المعالجة والحمأة الناتجة بعد عمليات المعالجة، كان من الضروري استخدام الضوابط والمعايير المثلى لتطبيقها.

تهدف هذه الدراسة إلى مقارنة الضوابط المعتمدة للسلطنة في إدارة مياه الصرف الصحي والحمأة الناتجة عنها بتلك المعايير المستخدمة في كثير من دول العالم، ومن ثم الاقتراح إذا ما كانت هذه الضوابط صالحة لاستخدامها الآن أو العمل على تطويرها وفق ما يتطلبه الوضع الراهن في التطور الهائل التي تشهده بلدان العالم. كما هدفت الدراسة إلى معرفة صلاحية السماد العضوي (الكلأ) والتي تنتجه شركة حيا للمياه من الحمأة المستخلصة من عمليات معالجة مياه الصرف الصحي في التسارع لمعالجة التربة الملوثة بالزيت بجانب الأعشاب الممتصة للملوثات الهيدروكربونية والكائنات الحية الدقيقة، وأخيرا استخدامه للأغراض الزراعية ومعرفة مدى جودته للحصول على إنتاج الوفرة لمحصولي الفجل الأبيض واللوبيا وتأثيره على التربة الزراعية بمقارنته مع السماد الغير عضوي (NPK) أثناء استخدام ري التربة بمياه الصرف الصحي المعالجة والمياه الجوفية.

ولتحقيق أهداف هذه الدراسة، فقد تم تقسيم العمل فيها إلى قسمين، القسم الأول قسم نظري و تم فيه مقارنة معايير استخدام مياه الصرف الصحي المعالجة بمعايير منظمة الصحة العالمية (WHO) و الوكالة الدولية لحفظ البيئة (US-EPA) بواشنطن.، كما تم مقارنة المعايير المحلية من استخدام الحمأة بمعايير المستخدمة للوكالة الدولية لحفظ البيئة في واشنطن (US-EPA) ومعايير المستخدمة في أوروبا (European Guidelines) أما القسم الثاني فقد أجريت فيه التجارب في المختبرات التابعة لجامعة السلطان قابوس ، وقد ركزت على معالجة التربة الملوثة بالديزل باستخدام سماد الكلأ واستخدامه للأغراض الزراعية،

من أهم النتائج التي توصلت إليها هذه الدراسة، الاقتراح بضرورة تطوير معايير إعادة استخدام مياه الصرف الصحي المعالجة والحمأة الناتجة للأغراض الزراعية من قبل صناع القرار من الهيئات والوحدات الحكومية والخاصة لأجل مواكبة تنامي التسارع في عجلة التنمية الاقتصادية والزراعية.

كما تم التوصل أن السماد الكلأ قد اثبت فاعليته لمعالجة التربة الملوثة بزيت الديزل بنسبة 77% عند استخدامه بنسبة 10% لكل 1,5 كيلو غرام للتربة الملوثة بزيت الديزل مع الأعشاب الممتصة للملوثات الهيدروكربونية Bermuda grasses بالمقارنة مع أعشاب Ryegrass، وبنسبة 90% أثناء استخدامه مع الكائنات الحية الدقيقة حيث إن تركيز زيت الديزل قد انخفض من 87 الى 29 ملغم في عينات الكربون لكل غرام في التربة الملوثة، و أوضحت الدراسة أن هذه الكائنات والمنتمية إلى *Bacillus* genera والتي تم توليدها من نفس التربة الملوثة بالديزل لها خاصية النمو والمعيشة في المحيط التي تبلغ درجة حرارته 55 درجة مؤية وفي المحيط الملحي بنسبة 10% لملح كلوريد الصوديوم.
اما نتائج تحليل التربة وعينات محصولي الفجل و اللوبيا فأشارت إلى خلوها من العناصر الثقيلة السامة، وبالزيادة الملحوظة في وفرة هذين المحصولين عند إضافة سماد الكلأ بالمقارنة مع السماد الغير العضوي.

استنتجت الدراسة إن سماد الكلأ قد اثبت فاعليته في معالجة التربة الملوثة بزيت الديزل كما انه يصلح باستخدامه في الأنشطة الزراعية.

TABLE OF CONTENTS

CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

ABBREVIATIONS AND DEFINITIONS

AES: Agricultural Experiments Station at Sultan Qaboos University

ANOVA: Analysis of Variance

Biosolids or sludge: Solid product or end-product generated from municipal wastewater treatment processes

BOD: Biological Oxygen Demand

CCI: Chlorophyll Content Index (chlorophyll measurement unit)

COD: Chemical Oxygen Demand

CRD: Completely Randomized Design

CRBD: Completely Randomized Block Design

EC: Electrical Conductivity

FAO: Food and Agricultural Organization

GC-MS: Gas Chromatography Mass Spectrophotometry

GW: Groundwater

HW: Haya Water Company

IC: Ion Chromatography

ICP: Inductively-Coupled Plasma

Kala compost: Organic fertilizer is produced from the municipal sewage treated wastewater (Kala is the commercial name of the product sold in the market

MAF: Ministry of Agriculture and Fisheries

MAR: Managed Aquifer Recharge

MBR: Membrane Bio-Reactor

MECA: Ministry of Environment and Climate Affairs

Mm^3: Million cubic meters

MM: Minimal Media

MCM: Million cubic meters

MOH: Ministry of Health

MPN: Most probable number of pathogens

MRMWR: Ministry of Regional Municipalities and Water Resources

NA: Nutrient Agar

PFU: Plaque Forming Unit

Preliminary treatment: Removal of large solids and grit particles

Primary treatment: Removal of suspended solids

RC wastewater: Reclaimed wastewater

RD (115/2001): Royal Decree No.115 issued in 2001
The law on protection of Sources of Drinking Water from Pollution

RPM: Revolution Per Minutes

RO: Reverse Osmosis

Secondary Treatment: Biological treatment and removal of common biodegradable organic pollutants such as Biological Oxygen Demand (BOD)

SS: Suspended Solids

STP: Sewage treatment plant

TE: Treated effluent

Tertiary and advanced treatment: Removal of specific pollutants, such as nitrogen or phosphorous, color, odor, etc.

TN: Total Nitrogen

TOC: Total Organic Carbon which is found in soil when organic matters are decomposed

TPH: Total Petroleum Hydrocarbon

TSE: Treated Sewage Effluent

TWW: Treated Wastewater

SBR: Sequencing Batch Reactor

Unrestricted irrigation: Irrigation by TWW, e.g. vegetables or fruits irrigated by TWW can be eaten raw, or TWW used to irrigate sports fields, etc.

US-EPA: United States Environmental Protection Agency

Waste stabilization pond system: A series of anaerobic, facultative, and maturation ponds, in which anaerobic and facultative ponds remove biological oxygen demand (BOD) and a maturation pond removes pathogens

WHO: World Health Organization

GLOSSARY

Agronomic rate is the whole sludge application rate (dry weight basis) designed:

(1) To provide the amount of nitrogen needed by the food crop, feed crop, fiber crop, cover crop, or vegetation grown on the land; and

(2) To minimize the amount of nitrogen in the sewage sludge that passes below the root zone of the crop or vegetation grown on the land to the ground water

Annual pollutant loading rate is the maximum amount of a pollutant that can be applied to a unit area of land during a 365 day period.

Annual whole sludge application rate is the maximum amount of sewage sludge (dry weight basis) that can be applied to a unit area of land during a 365 day period.

Bagged biosolids: are sold or given a way in a bag or other container.

Bulk sewage sludge is sewage sludge that is not sold or given away in a bag or other container for application to the land.

Cumulative pollutant loading rate is the maximum amount of an inorganic pollutant that can be applied to an area of land.

Ceiling Concentration limits for all bio-solids applied to land.

Land application is the spraying or spreading of sewage sludge onto the land surface; the injection of sewage sludge below the land surface; or the incorporation of sewage sludge into the soil so that the sewage sludge can either condition the soil or fertilize crops or vegetation grown in the soil.

Monthly average is the arithmetic mean of all measurements taken during the month.

Pasture is land on which animals feed directly on feed crops such as legumes, grasses, grain stubble, or Stover.

Public contact site is land with a high potential for contact by the public. This includes, but is not limited to, public parks, ball fields, cemeteries, plant nurseries, turf farms, and golf courses.

Range land is open land with indigenous vegetation.

Reclamation site is drastically disturbed land that is reclaimed using sewage sludge. This includes, but is not limited to, strip mines and construction sites.

Source: Anon (1992)

CHPTER ONE

INTRODUCTION

1.1 General

As the world population growth accelerates, more water will be required to satisfy our basic and social needs, as well as cultural and economic demands. Most developing countries will be facing problems of wastewater and sludge production in the coming decades due to the increase of demands of water and land use resulting from population growth and economic development (Fulazzaky, 2009). Strategies are set regularly for institutional structures, economic policies, technological and other choices that affect our use of such resources to achieve specific goals. Therefore, decisions, policies, and legal controls are needed to minimize health and environmental risks associated with the reuse of municipal treated water as well as sludge management to maximize the beneficial reuse of both resources.

The government of the Sultanate of Oman requires environmentally, technically and economically sound wastewater facilities and sludge management at the least possible cost, to meet the present and future needs of the Sultanates' governorates. However, there are regulatory conditions for treated sewage wastewater and sludge management in the Sultanate such as inspection, control and legislation, to ensure compliance with national and international standards/regulations in order to sustain the best levels of protection while at the same time benefiting from the reuse of these resources (Kamizoulis, 2003).

1.2 Project justification

Treated wastewater (TWW) and sludge management are important issues for Oman. The increase of wastewater and amount of sludge products via wastewater treatment plants are rising from year to year due to the large pressure of population on one hand and economic growth on the other. Oman's population is expected to increase from 3,041,460 individuals in 2010 to 5,572,149 in 2025 Anon (2018a). The total population in the Muscat governorate for both Omani and non-Omani individuals was recorded at around 775,878 individuals in 2010 Anon (2018b) and this will rise to around 1 million individuals in 2025 Anon (2018c) putting more pressure on water resources, thus increasing production of wastewater and generating sewage sludge.

Treated wastewater reuse becomes necessary for conserving and managing the available water supplies in the country. The Sultanate of Oman has future plans and strategies for the utilization of TWW as an alternative source to meet its future needs. Therefore, the government of the Sultanate aims to use TWW in its water planning. Haya Water Company (HW) in this respect is implementing modern wastewater technologies and facilities to serve all the Wilayats of Muscat Governorate and these include maintaining, operating, and managing the wastewater network. Haya sewage treatment plants generated an average volume of 84,144 m^3/day of treated effluent in 2011 (Al Muselhi, 2011), which will rise to 327,853 m^3/day by 2025 when the wastewater infrastructure is completed (Al Muselhi, 2014).

Sludge, has very great value in the Sultanate; it acts as a renewable resource, such as fertilizers. Several potential sources of sludge result from different activities such as domestic and industrial wastewater, solid waste disposal and sludge of water and wastewater (Fulazzaky, 2009). However, the main source of sludge in the Sultanate is the result of the treatment of sewage water in Sewage Treatment Plants (STPs) high quality techniques, the total dewatered sludge produced in Muscat Governorate was 159,065 kg/day in 2011 and will rise to 281,790 kg/day in 2025 (OWSC, 2005).

HW in 2010 established a project to introduce an organic agricultural fertilizer called Kala compost, achieving Class A of 1993 US-EPA guidelines; it is an end-product of the wastewater treatment process. The composting plant is located in Al-Amerat (Al-Maltaqa) in the Muscat Governorate with an area of 60,000 square meters.

Kala was used in some experiments which were conducted at SQU. It was revealed by Al-Busaidi et al. (2015a) in their experiment of applying Kala on soil and plant productivity (cucumber, tomato, cabbage, lettuce and carrot) that, this fertilizer improved soil physiochemical characteristics, and has no accumulation of metal concentrations in soil and plants.

In Oman, the management of effluent and sludge from municipal wastewater treatment is currently controlled under Ministerial Decision No 145/93 (MRMWR, 1993). The Ministry of Environment and Climate Affairs (MECA) has the responsibility for the implementation of these regulations. However, these regulations should be revised and up graded to international standards in accordance with the development of the country.

On the other hand, arid and semi-arid regions occupy one third to one fourth of total lands of our planet (Padmavathiamma et al., 2014). These regions are subjected to several environmental problems, such as drought and salinity of water and soils. Beside these problems, organic contamination nowadays can contribute in polluting the environment. It is estimated that between 1.7 and 8.8 million metric tons of oil are released into the world's water and soil every year (Dadrsina and Agamuthu, 2013a). Hydrocarbon (crude oil) contamination of water and soil can occur in many ways; this include accidents by oil tankers, spilling of oil from production wells, leakage from underground storage tanks, leaching from gas work sites during coke production and from a range of other sources, these will contribute in polluting the environment. Therefore, many studies were conducted to introduce a cost-effective and environmentally-attractive technology to diminish the negative impacts of petroleum hydrocarbon pollution in the surroundings (Namkoong, 2006; Dadrasnia, 2013a; 2013b).

Several forms of treatment of petroleum-contaminated soils like diesel, kerosene and crude oil. Phytoremediation and bioremediation, with addition of organic amendments, have shown successful, economic and efficient methods to remove toxic organic compounds from oil-contaminated soil. For instance, Ghanem et al. (2013) reported that, adding compost to petroleum hydrocarbon contaminated soil increased the efficiency of the phytoremediation technique as this enhanced plant growth rate and microbial action. Karamalidis et al. (2010) also showed that biodegradation of hydrocarbon pollutants can be achieved by isolating native microbes from petroleum-contaminated soil.

1.3 Main objective

This project consists of two parts: firstly to study the existing strategies and policies of wastewater and sludge reuse management, which have been adopted in the Sultanate. These strategies were compared to international guidelines prescribed by USEPA, WHO and EU. And secondly to conduct laboratory experiments to study the efficiency of phytoremediation and bioremediation techniques by adding Kala compost (produced using locally available treated sewage sludge) to the diesel-contaminated soil and to study the effect of Kala compost and NPK fertilizer on the growth of Radish and Bean crops in an agricultural field experiment.

1.4 Specific objectives

1. To present an overview of the current national legislations and guidelines of treated sewage wastewater and sludge management in the Sultanate, in order to compare these guidelines to international guidelines and regulations and to recommend whether it is necessary to carry out some amendments and modifications to the Sultanate's guidelines.
2. To investigate the effect of Kala compost on the phytoremediation method on diesel-contaminated soil by means of two plants species (Bermuda grass and Ryegrass).
3. To study the effect of isolated microorganisms from diesel-contaminated soil on the bioremediation of diesel-contaminated soil in the presence or absence of Kala compost.
4. To examine the effects of the application of composted sewage sludge fertilizer (Kala compost) and inorganic (NPK) fertilizers on soil quality and on two crops (Radish and Beans) using groundwater and treated wastewater for irrigation.

1.5 Research questions

1. What is the current status of Omani wastewater treatment plants?
2. What are the roles of Oman's government in the management of wastewater and sludge activities?
3. What are the activities of Omani wastewater companies?

4. What are the plans to recover the cost of reclaimed wastewater and sludge reused in the country?
5. Does Omani legislation comply with international legislations with respect to the rapid growth of Oman's population and infrastructure changes?
6. Do the 1993 legislation and standards in MD 145/93 need to be modified?
7. Are the treatments affecting the uptake of hydrocarbons by plants and microorganisms by adding compost?
8. Does Kala compost have any effect on the quality of soil and crops, as this product is composted sewage sludge comprising heavy metals?

1.6 Research rationale

The rationale for this study is that under proper management and application of national guidelines, the sewage sludge can have many sustainable and beneficial uses such as phytoremediation and bioremediation of diesel-contaminated soil and increase of crop yield and soil quality when used as fertilizer.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

The Sultanate of Oman is located in arid and semi-arid region where many problems of water deficit can be found. Therefore, many countries in these regions use treated wastewater (TWW) to overcome the shortage of water as a useful source for agricultural and industrial purposes (Saffari and Saffari, 2013).

The rapid growth in Oman's population has made the usage of TWW one of the necessary objectives of the country in its infrastructure projects, through managing it in terms of technical, economic, social and environmental aspects.

The source of public wastewater in the Sultanate is sewage treatment plants (STPs) which are spread in various regions of the country to collect household sewage; a number of these plants belong to the government and some to the private sector.

Earlier, all government STPs were owned, managed and run by Muscat Municipality. At the present time, all treated wastewater of Muscat city is administered by Haya water Company (HW) via very modern system. In the report of Arab Water Council (2011) it was recorded that about US$ 4.3 billion was spent for expanding the new net-work system in Muscat city. It was reported by Al Muselhi (2014) that STPs in Muscat will produce about 327,853 m^3/day by 2025 of total TWW.

2.2 International experiences of wastewater and sludge reuse

Municipal water occupies 11% of global water and out of this 3% is consumed mainly by agricultural purposes and 8% is released as wastewaters which can be used for irrigating of 15% of all irrigated lands (UN WATER, 2017).

This section addresses the extent of treated wastewater and sludge reuse practices for a number of countries:

In the south part of Los Angeles in United States, the recycled wastewater was used to recharge ground water over 35 years (US-EPA, 1998). In California, around 434 Mm^3 of domestic wastewater is used and 68% of total recycled wastewater is used for agricultural purposes (Asano et al., 2000). According to the information provided by Nakshbendi (2006), the Washington Suburban Sanitary Commission (WSSC) is the one of the largest STPs in the United States; it serves around 1.6 million residents and produces 634,470 m^3 of water per day and about 82% of the budget is financed through bonds (capital fund) in which a group of banks and investors are sharing in its syndication, so a fixed amount of the savings returns to the investors annually and at the same time it benefits the country in terms of agricultural usage.

As regards Mediterranean countries, in the occupied land of Palestine, municipal sewers collect 92% of the wastewater where 72% is used for irrigation and 42% for aquifers recharge (Angelakis et al., 1999). In Lebanon in 1991, the total amount volume of industrial and domestic wastewater was around 165 MCM where 130 MCM was from urban wastewater and the rest from industrial wastewater. In 2002, only 2 m^3 of TWW was reused for irrigation purposes and the rest was dumped in the marine environment (Kamizoulis, 2003). In Tunisia, the report by Arab water Council (2011) mentioned that the total volume of treated wastewater in Tunisia will reach 500 MCM by 2021 compared with 240 MCM in 2010. Treated wastewater is reused for golf courses, agricultural activities and recharging groundwater aquifers, the estimated cost of operation and maintenance of TWW of Ben Sargo STP in Agadir city in Morocco produces 750 m^3/day, in addition, TWW and sewage sludge are used for agriculture purposes taking into consideration their composition, soil types, chemical and biological analyses before applying them to the lands. In Jordan, As-Samra and Zarqa are the two largest wastewater treatment plants in the country serving 2.27 million people. These plants have a capacity design ranging from 68,000 m^3/day to 268,000 m^3/day (Arab Water Council, 2011). The quantity of TWW was about 74 MCM for the year 2003 (Ulimat, 2004).

Table 2.1, shows the amount volume of wastewater in the Mediterranean countries (produce by domestic and industrial sector).

Table 2.1 Total water withdrawals, municipal wastewater produced and treated wastewater in Mediterranean Arab Countries in (BCM*/year)

Country	Total water withdrawals	Municipal wastewater produced	Volume of treated wastewater
Algeria	2.723 (2001)	0.820 (2012)	n.a
Egypt	68.300	7.078 (2012)	3.711 (2012)
Jordan	0.941 (2005)	0.180 (2002)	0.111 (2010)
Lebanon	1.310 (2005)	0.310 (2011)	0.056 (2001)
Libya	4.326 (2000)	0.546 (1999)	0.040 (2008)
Morocco	10.430(2010)	0.700 (2010)	0.124 (2010)
Palestine	0.418 (2005)	0.071 (2001)	n.a
Syria	16.760	1.370 (2012)	0.550 (2012)
Tunisia	2.850 (2001)	0.287 (2009)	0.226 (2010)

BCM = billion cubic meters / ** Occupied Territory of Palestine / n.a. = data not available Source: SWIM SUSTAIN WATER (2016) adopted from FAO AQUASTAT Database (last accessed on February 2015). All data derived from FAO's source and are deemed accurate as such).

In the GCC, the cost of secondary treatment for a wastewater treatment plant in Bahrain was estimated at US$0.164/$m^3$, while its tertiary treatment was US $ 0.317/m^3 (Al-Zubari, 1998). However, the cost of tertiary-treated effluent in Kuwait is less than US$0.3 for 1000 gallons (Alhumoud et al., 2003). As reported by the Arab Water Council (2011), a volume of 550,000 m^3/d of raw wastewater is produced by residential areas of Abu Dhabi city and is treated through 20 STPs, but with the rapid acceleration of urban growth, the government has planned to construct a 40 km wastewater passage way to increase the flow rate of municipal wastewater. The GCC countries reuse around 43% of the treated effluent for irrigation purposes and industrial uses (Al-Zubari, 2000). Table 2.2 shows the TWW capacity in GCC countries.

Table 2.2 TWW capacity in GCC countries in (m^3/day)

Country	Existing capacity (2010)	Additional capacity planned by 2015
Bahrain	221,000	280,000
Kuwait	679,000	795,000
Oman	106,000	230,000
Qatar	285,000	437,000
Saudi Arabia	1,952,000	2,224,000
Abu Dhabi	414,000	875,000
Dubai	260,000	400,000
Northern Emirates	291,000	332,000

Source: Alkhamisi and Ahmed (2014) adapted from MEED Insight (2010)

As mentioned by UN WATER (2017), all wastewater in GCC are treated using tertiary wastewater treatment, and around 44% of their total treated wastewater volume is used for agricultural purpose.

On the other hand**,** sludge is the end product of wastewater treatment processes; municipal sewage sludge can be applied on land as organic fertilizer. In Europe in 1998, sewage sludge was prohibited to be disposed into the marine environment. Thus about 38% of total sewage sludge production was used for land application (OWSC, 2005). Germany, UK, France and Italy generate 70% of total EU sludge production Anon (2017) and the cost of 75 Euros per tons of sludge was raised from 1998 to 400 Euros per tons in 2005 (OWSC, 2005). Sludge is treated by several methods and the mesophilic anaerobic digestion method is the most accepted method in the EU and can produce 50% of treated sludge. However 20% is produced using the aerobic digestion method. The thermal drying method is the current sludge treatment technology which achieves Class A of pathogen destruction Anon (2017).

In the U.S.A, the report by US-EPA (1999) mentioned that about 6.9 million dry tons of sludge was generated in 1998 of which 60% was used for landfill cover and 40% was disposed into the marine environment. The report estimated an increase of sludge production from 6.9 million US dry tons in 1998 to 7.1 million US dry tons in 2000 and to 8.2 million US dry tons in 2010. The thermal drying method is used in the U.S for producing treated sewage sludge (OWSC, 2005).

PURE (2012) reported that the amount of sludge which is generated in some countries in the Baltic Sea area is about 3.5 million tons of dry solids annually and may increase to 4 million tons by 2020. Table 2.3 illustrates the amount of the sludge in these countries.

Table 2.3 Total sludge volumes in tons of dry solids per year (tDS/a) of different countries of the Baltic Sea Region

Country	2005/2006	2010	2020
Belarus*	50,000	50,000	70,000
Denmark	140,021	140,000	140,000
Estonia	n.a	33,000	33,000
Finland	147,000	155,000	155,000
Germany	2059,351	2000,000	2000,000
Latvia	23,942	25,000	50,000
Lithuania	71,252	80,000	80,000
Poland	523,674	520,000	950,000
Russia*	180,000	180,000	200,000
Sweden	210,000	250,000	250,000
Total	3,045,240	3,433,000	3,928,000

n.a. = data not available

Source: PURE (2012)

2.3 International standards and guidelines of wastewater and sludge reuse

Several international regulatory practices in term of reusing TWW for irrigation purposes have been adopted in many developing countries to fulfill the national guidelines, For example, Mara & Cairncross (1989) mentioned the guidelines of 1989WHO and the revised 2006 WHO guidelines, these reports provide all pathogenic vectors and their survival times in crops and soil which are adopted in many Arab countries like Kuwait, Oman, Lebanon, Iraq Tunisia Jordan and Yemen. In addition, FAO guidelines of (1992; 1999) mentioned all kinds of vegetables which can be irrigated with TWW using suitable techniques. US-EPA guidelines of 1992, 1993, 1997, 2004 and 2012 for reuse of TWW in agricultural aspects discuss important issues and practices during the application of such guidelines. Furthermore, the Australian guidelines for water recycling, managing health and environmental risks were published in 2006, these guidelines provide regulations for recycling wastewater such as storm water, sewage effluent and greywater, and focusing mainly on the risk management frameworks, managing health risks, managing environmental risks, monitoring sites and provide effective consultations and communications with stakeholders.

Many studies have been conducted to focus on important aspects when reusing TWW for agricultural purpose, Al Salim (2000) mentioned some pathogens (Bacteria, Enteric Viruses, Protozoa and Helminth Worms) which can be found in municipal effluent which can be removed by appropriate feasible treatment methods such as conventional secondary treatment followed by slow sand filtration and waste stabilization ponds with detention time not less than 14 days. However, he suggested a sludge pasteurization method for sludge treatment, as this method is used in Switzerland and Germany at 70^{o}C for 30 minutes.

A review of wastewater reuse guidelines in Mediterranean region is prepared by Kamizoulis et al. (2003) as shown in Table 2.4.

Table 2.4 Wastewater reuse guidelines in the Mediterranean countries

Country	Existence of Legislation	Contemplating legislation	No legislation at all
Albania			X
Algeria		X	
Bosnia and Herzegovina			X
Croatia			
Cyprus	X		
Egypt		X[a]	
France	X		
Greece		X[b]	
Israel	X		
Italy	X		
Lebanon		X	
Libya		X	
Malta		X	
Monaco			X
Morocco		X	
Slovenia			X
Spain	X[c]		
Syria		X	
Tunisia	X		
Turkey	X		

a: programme – strategy, b: under the form of sanitary regulation, c: in some regions of Spain

Source: SWIM-SUSTAIN WATER MED (2016) adapted from Kamizoulis et al. (2003)

Saskatchewan Environment Agency (2004) outlined some important information and instructions for consultants and clients before starting their work; these include obtaining permits to purify sewage wastewater, monitoring reports of water quality of TWW and land control instructions among other factors. The working group of MED-EUWI (2007) identified the objectives of TWW policy in Europe and Mediterranean countries. The group worked on investigating the problems of implementing such a policy and tried to find solutions to overcome those obstacles which maybe faced during application of the policy. The report discussed the benefits and risks of reusing TWW on the environment and its economics, health and social aspects. It also highlighted water stress and the adaptation aspects toward the climate change of involved countries, and finally gave some conclusions for using TWW in the future. Fulazzaky (2009) addressed the management of re-using TWW and sludge management and emphasized involving the government and private agencies in addition to stakeholders to build a comprehensive management of sludge and to reuse all types of wastewater.

The most important guidelines for reusing sewage sludge on agricultural land are 1986 European guidelines through implementing Directive 86/278/EEC. This directive is adopted in many European countries such as Greece, Ireland, Italy, Luxembourg, Portugal, Spain, the United Kingdom, Estonia and Latvia, whereas Denmark, Finland, Sweden, Netherlands, Austria, Belgium and Germany have set some regulations which are more stringent than 86/278/EEC regulations (Inglezakis et al., 2014).

The second important guidelines regarding usage of sewage sludge for agricultural purposes are the 1993 US-EPA guidelines through implementing 40 CFR part 503 regulations. Some states in the United States have suggested revising the current regulations and some have already set their own regulations more stringent than Federal guidelines, like New York State (Harrison et al., 1999).

2.4 Studies on reusing treated wastewater and sludge in the Sultanate

It is essential that strategies, regulations and guidelines about the reuse of TWW and land application of sewage sludge are based on research carried out in the environmental conditions of the Sultanate. Wastewater research needs to be related to agricultural, biological and industrial purposes, impact on soil, aquifer recharge using TWW and other applicable issues (Abdelrahman et al., 2011; Alkhamisi and Ahmed, 2014; Al-Busaidi and Ahmed, 2014; Alkhamisi et al., 2015). Sludge studies looked at its quality and likely use in crop production and remediation of contaminated sites according to its types (; Padmavathiamma et al., 2014; Al-Busaidi, 2014b; Al-Busaidi et al., 2015a). The sections below highlight briefly the studies which were using TWW and different types of sludges.

The section below highlights the extensive amount of research which has been conducted in Oman by various researchers on wastewater. However, only a few studies have been conducted about sludge under environmental conditions concomitant to Oman. Therefore, it is essential to identify the existing examples of case studies and projects of the uses and land application of sewage sludge and reusing of TWW in the Sultanate.

One study was conducted with the objective of maximizing the use of TWW supplemented by groundwater, by identifying short season crop, and changing the area under cultivation of such crops. Field studies were conducted to assess yield components of wheat, cowpea and maize crop rotation grown with reclaimed water for irrigation. Results showed that by using treated wastewater combined with groundwater (assuming irrigation salinity of 1dS/m and TWW availability of 38,267 m3/day) cropping area increased from 695 ha to 2245 ha of wheat, from 313 to 782 ha (250% increase) of cowpea and from 346 to 754 ha (318% increase) of maize. Of the total irrigation requirement of 24.24 Mm3, 57.6% was to be met with TWW and 42.4% was to be met with groundwater. Field studies confirmed that the TWW irrigation increased the yield parameters of wheat, cowpea and maize crops without any adverse effect (Alkhamisi et al., 2011; Alkhamisi et al., 2013). The study by Al-Wahibi (2017) was conducted using the Excel-based decision support system (DSS) to analyze the cost/benefit of using TWW alone and blending with GW, which was based on three crop rotations: rotation 1: wheat, cowpea and cucumber, rotation 2: potato, cowpea and maize, rotation 3: sweet melon, millet and lettuce, irrigated with different water salinities (control, 1.0, 2.0, and 4.0 dS/m). The performances of crop rotations were evaluated in terms of cultivated area changes. DSS showed clear simulated results when the cost of TWW is unsubsidized by government and equals the treatment cost (0.800 O.R/m3), where the results showed that total profits decreased because of increasing price of TWW and at salinity level of 4 dS/m which indicated no profits for growing crops in three rotations. Profits for the farmers are much higher when the TWW is given free and all of the TWW is utilized in conjunction with GW.

In the second study, TWW was used to compare 2 methods of irrigation water application drip and raised furrow bed. The objective of this study was to modify the furrow system to a furrow bed system and evaluate its water-use efficiency in comparison to the drip irrigation system. The tested crop was wheat which is cultivated as a forage crop for livestock and grain production in Oman. Each plot had either a drip irrigation system or a furrow bed of 60 cm width. The plots were divided randomly using a complete block design with two treatments (water source: freshwater and TWW; irrigation method: furrow bed and drip) and three replications. Wheat was sown and all the required parameters for soil and plants were measured. Plants were irrigated daily by drippers or five days a week by furrows based on crop evapo-transpiration values. From soil salinity data, it was found that both methods added some salts to the root zone, with less salts found in the furrow bed method, due to the heavy leaching process that occurred during irrigation. However, the general data didn't show a significant difference ($p<0.05$) in soil salinity between both irrigation methods. Since TWW has some extra nutrients compared to freshwater, therefore plant growth was better with TWW and almost all the growth parameters were higher with TWW compared to freshwater. Generally, all measured data collected from both irrigation methods didn't show any significant difference ($p>0.05$). Water productivity data gave better results with furrow bed compared to the drip method. This indicates the higher efficiency of the furrow bed compared to the old method of furrow irrigation. However, drip irrigation could be better in reducing water evaporation whereas furrow bed is an easy method in getting good yields with lower cost and higher productivity (Al-Busaidi and Ahmed, 2014; Al-Busaidi et al., 2014).

Abdelrahman et al. (2011) examined the chemical composition of forage maize by using water quality (freshwater and municipal TWW) and water quantity according to the reference of evaporation at (1.4 ETC, 1.0 ETC, 0.6 ETC) and the interaction between them, the results indicated that, crops were grown faster in TWW than those grown in fresh water because TWW contain dissolved organic matter that allowed more nutrients to penetrate the soil. Sodium adsorption ratio reduced by 74% when TWW was used compared to 68% by freshwater but all macro and micro elements uptake in the crops were not significant between treated and fresh water.

Another study was done to see the feasibility of managed aquifer recharge (MAR) using treated wastewater. Data showed that TWW volumes will increase from 7.6 Mm3 in 2003 to 70.9 Mm3 in 2035. HYDRUS 3D simulations show that areas with sandy loam soils are suited for infiltration ponds. Numerical simulations with MODFLOW (in combination with PEST and GWM) showed that injection wells can be used for recharge without causing undue water ponding. Numerical simulations also showed that in order to maximize the amount of water injected into aquifers, MAR was subjected to the constraints of limited groundwater mound below 5 meters and a maximum allowable injection rate of 1000 m3/day. Results showed that 68 injection wells, with a total injection rate of 62,205 m3/d were found to be a feasible option. There would be a discharge of a maximum of 7,500 m3/day towards the sea and the injection rate of the wells ranges from 200 to 1000 m3/d. Preliminary financial analysis has shown that a cost of USD 0.353/m3 to 0.550/m3 will be incurred for further Reverse Osmosis (RO) membrane treatment and injection (Zekri et al., 2014; Ebrahim et al., 2015).

The study by Zekri et al. (2014), estimated the cost of MAR (managed aquifer recharge) using surplus volumes of treated wastewater in Muscat as an alternative to disposal into sea. The injection into the aquifer of a volume 85,000 m3/day of treated wastewater by the Reverse Osmosis (RO) processes was considered to achieve drinking water standards as it is the most recommended technology in some countries such as Namibia and California. The study met an obstacle for reaching to its objectives, due to the fears of users regarding potential health risk. The above study was conducted when the recharging of groundwater aquifers using treated wastewater was practiced in Salalah (the southern region of Oman) along the coastal areas in 2005 by injecting 5.48 Mm3/year of treated wastewater through the wells, to constitute a barrier against sweater intrusion, but unfortunately the injecting was stopped due to the technical and mechanical problems.

Al-Busaidi et al. (2015) conducted research to evaluate the suitability of treated wastewater for irrigating date palms and monitoring the partitioning of some heavy metals (e.g., Cu, Cr, Cd, Pb, Mn, Fe, Zn etc.) in soil, plants and fruit. Results showed that the concentrations of heavy metals in both groundwater and treated waste water were within the international standard levels. There were significant variations in heavy metal concentrations in soil at studied locations. In most cases, the concentrations of heavy metals were relatively higher in soils irrigated with treated waste water compared to the soils irrigated with groundwater. Generally, the concentrations of heavy metals in date palm leaves were not significantly different in plants irrigated with treated waste-water or groundwater. However, there were significant differences in the concentrations of heavy metals in date fruit irrigated with different sources of water. The concentrations of some metals (Fe, Zn, and Ni) in date fruit were higher in waste water irrigated plants whereas other metals (Cu, Cd, Pb, and B) were higher in groundwater-treated plants. In all cases the concentrations were within the permissible limits. Thus, the long-term effects of treated waste water did not indicate any adverse effects of irrigation using groundwater and waste water on fruit mineral composition, including heavy metals.

Another study aimed to identify means/tools to optimize treated wastewater reuse in conjunction with other available water resources, by taking into consideration their quantity and quality, in addition to the agronomic, environmental, and economic components. The study was done in an open field at Sultan Qaboos University, Oman. Three types of crops (radish, okra and eggplant) were grown and irrigated by four types of water (A: 50% groundwater and 50% treated wastewater, B: 100% groundwater, C: 75% treated wastewater and 25% groundwater, and D: 100% treated wastewater). Soil physicochemical properties did not show significant differences with treated wastewater irrigation as compared to groundwater. On other hand, some chemical properties significantly increased when treated wastewater was applied, such as total carbon and some major elements (N, P, K). Crop physical analysis showed significant increases in plant productivity when plants were irrigated with treated wastewater with insignificant changes in heavy metals between treatments and no biological contamination in crop yield was recorded (Al-Busaidi and Ahmed, 2015).

The growth of bio-fuel plants was evaluated under TWW irrigation. It was found that Jatropha plants irrigated with treated wastewater gave the best growth in term of plant height and green yield compared to groundwater (Al-Busaidi, 2014).

Lot of research has been conducted in Oman regarding the reuse of treated greywater. Greywater can be defined as any water which is generated from kitchens, laundries, and bathrooms of households except toilet water (Ahmed et al., 2003). In this regard, the study by Prathapar et al. (2004) aimed at measuring the quality and quantity of greywater at two mosques over six months. Results showed that pH, EC and TDS were in the range of the regulations (145/93) whereas; E. coli, TSS and BOD5 were high. The quantity of greywater in both mosques was variable, therefore it was suggested that constructing simple treatment systems with a chlorination tank and sand filter would be an effective solution to use greywater for irrigation purposes. Moreover, Ahmed et al. (2005) suggested guidelines for reusing greywater in Oman, as no standards or regulations exist (Ahmed et al., 2012). The paper revised several greywater guidelines of different countries and came up with the Australian and US states guidelines as the appropriate and best regulations for Omani communities in terms of the Omani environment, price, religious and health risk aspects (Ahmed et al., 2003). A study by Jamrah et al. (2004) aimed to measure fresh water consumption and generation of greywater from five areas of Muscat governorate (169 houses and 1,365 people). The greywater quality was analyzed in order to assess its acceptance by people to reuse it. The results showed that from the total freshwater consumption, about 80 to 83% greywater production was generated from showers, 28 to 33% from kitchens, 6 to 9% from laundries and 5 to 7% from sinks. The quality analysis resulted in high levels of SS, TOC, COD and BOD, Coliform and E. coli bacteria. 76% of people accepted to reuse greywater for irrigation purposes, 53% for washing vehicles and 66% in toilet flushing. The study by Prathapar et al. (2005) indicated many constraints for reusing treated greywater such as its quantity and quality as well as social, financial and legal constraints. The study suggested the construction of a simple system for treating greywater in new houses, mosques and schools to reuse it for irrigation purposes. Other studies revealed that greywater can be utilized in Oman taking into consideration the technical, economic and environmental aspects (Ahmed et al., 2004; Ahmed et al., 2008; Ahmed et al., 2012).

Greywater should be separated from black water in mosques, especially for the woudhu (ablution) water. Woudhu water was analyzed in the Al Hail south mosque over

8 weeks and the analysis showed that pH, EC, TDS and COD were in the range of Omani 145/93 regulations, whereas BOD5, Coliform and E.coli bacteria were at a high level. Therefore, the study which was carried by Prathapar et al. (2006) pointed to the design and construction of a cost-effective unit to separate the two types of water. This unit was composed of a sand trap, chlorination unit, treatment tank, filtration unit and a woudhu tank. Analysis of woudhu water in this unit showed that most parameters were insignificant and within the range of Omani regulations, except for some parameters like EC, TDS, Na, Mg and Ca because of using beach sand as filtration. Also, Jamrah et al. (2008) conducted a study to remove organics from greywater was a sequencing batch reactor (SBR). The results showed that the reactor removed about 50 to 83% of COD and 90 to 100% of SS, pH was at 6.9 to 7.8, and DO was in the range of 2 to 5.1 mg/l.

A number of studies regarding biological contamination in sewage TWW were conducted by Al-Bahry et al. (2009) who studied the presence of pathogens (antibiotic resistant bacteria) in tertiary TWW of the treatment plant (STP) at SQU. They showed that after chlorination of the STP viable bacteria, coliforms and nitrates were decreased. In addition a total of 336 bacteria from 8 genera revealed that the dominant isolates were Enterobacter spp., Pseudomonas spp., and Aeromonas spp from, 59.8% of bacteria were resistant to several antibiotics. The study concluded with a recommendation to modify the current STP. Al-Bahry et al. (2014) also studied the presence of fecal coliform in 3 different media after 4 weeks of spreading sewage sludge on sludge beds in an open area. They found the least number of bacteria when the soil was mixed with compost after dewatering the sewage sludge. In addition, when the soil was mixed with sewage sludge and irrigated by well water, fecal coliform dropped significantly. However, the highest count of these bacteria was found when soil was mixed with sewage sludge and irrigated with sewage TWW. The study concluded that dried sludge is a good practice for use as fertilizer, especially in the hot summer months.

Baawain et al. (2014b) studied characteristics of domestic water (physicochemical and microbiological properties) from 3 regions: Sohar, Salalah and Muscat. The study was a comparison between raw sewage effluent and treated sewage effluent from STP over 1 year. The research revealed that raw sewage effluent parameters in all STPs exceeded the allowable Omani regulations especially for ammonia, except in the regions of Sohar and Salalah whereas Treated Sewage Effluent (TSE) samples were in the range of Omani regulations, except nitrate, total suspended solids and E-coli bacteria.

For biosolid applications in Oman, HW has developed its pioneering Kala Composting Plant to enable the efficient reuse of sewage biosolids and green waste, enabling their conversion to a compost product that can be used for agriculture, landscaping and for individual gardens. However, a high application of sewage biosolids could result in heavy metal accumulation and many health problems. Therefore, sewage biosolids applied to agricultural land must be treated and tested and so meet governmental quality standards. The objective of this study was to evaluate the effect of different fertilizers especially Kala compost on the quality of soil and crops. The study was conducted at Sultan Qaboos University, College of Agricultural & Marine Sciences, in the Agricultural Experiments Station (AES) in an open field with six commercial crops (cucumber, tomato, cabbage, lettuce, carrot and potato). Kala application improved the soil's physiochemical properties by holding relatively more water, reducing soil bulk density and adding mix nutrients compared to NPK fertilizer. Good plant growth was observed with higher plant production and better water productivity in Kala compared to NPK treatments. Generally, it can be concluded that Kala compost was a good medium for plant growth, supporting plants with many elements needed for higher production. Chemical analysis did not show any problem of heavy metal accumulation, either in soil or plant samples. Biologically, all crops grown in this study were free from any harmful bacteria that could affect human health. Using Kala compost as a fertilizer will support organic farming practices but farmers should evaluate its applicability to long run applications (Al-Busaidi, 2014; Al-Busaidi et al., 2015).

Another aspect of sewage sludge research has been the improvement of soil properties and it investigates heavy metals concentrations both in sludge and amended soils. Although land application of sewage sludge has been proven to be an effective disposal method, mainly because it is rich in organic and inorganic plant nutrients, trace metals in sewage sludge are of particular concern in regard to their effects on human and animal health. Bioavailability of trace metals depends to a large extent on soil properties such as soil pH, redox potential, clay content, iron and manganese oxides, organic matter (Rieuwerts et al., 1998) length and rate of sludge application. Another concern is the environmental and health risks posed by organic chemicals present in sewage sludge (Harrison et al., 2006).

Soils in Oman are characterized by high pH and high contents of Ca and Mg carbonates (MAF, 1990). Mobility and bioavailability of most metals is decreased in alkaline soils (Sherene, 2010). Metals can also precipitate with OH- from soil solutions and form metal hydroxides (Basta and Tabatabai, 1992). Carbonates are identified as very effective adsorbents in removing metals from soil solutions (Madrid and Diaz-Barrientos, 1992; Ahmad et al., 2012).

Omani standards for wastewater reuse and discharge were adapted from the Food and Agricultural Organization (FAO) guidelines for trace metals in irrigation water (MRMWR, 1993). However, it is imperative that policies, standards, and regulations about land application of sewage sludge rely on research projects conducted in environmental conditions suitable to Omani conditions. Therefore, it is essential to identify the existing examples of case studies and projects of the uses and land application of sewage sludge in Oman. One major concern about trace metals is their solubility in soils and hence their bioavailability. Therefore, projects on the speciation of trace or heavy metals in calcareous soils after the application of different rates of sewage sludge are important to determine their solubility and mobility. Al-Dughaishi (2009) and Al-Saadi (2016) have carried out research projects on the effects of sewage sludge application on heavy metal speciation, movement, and bioavailability in calcareous soils of Oman. As types of wastewater treatment plants and technologies for processing sewage sludge vary in Oman (Al-Saadi et al., 2012), it was important to characterize these sludges and make recommendations for their use accordingly (Baawain et al., 2014a; 2014b; 2015).

The studies of contamination by heavy metals from their industrial origin to the environment and to the groundwater in Oman by sewage sludge or industerial TWW have been conducted by many researchers. Al-Musharafi et al. (2013a) studied the pollution of heavy metals of industrial TWW around the pond of an industrial site. The samples from TWW, sediments, snails, and grass roots were analyzed weekly over 3 months, the results showed that the majority of heavy metals of the 3 sources (sediments, snails, and grass roots) exceeded heavy metals of Omani TWW standards. The study concluded that if no action is taken for finding suitable solution, then pollution causing health risks could occur.

Furthermore, Al-Musharafi et al. (2013a) studied the infiltration of heavy metals into the soil and into tomato plants when irrigated by the TWW of industrial origin. The samples of soil, plant tissues and the TWW were analyzed for heavy metals weekly for two months and compared to Omani standards. The results showed that the highest range of Al and Zn were found in the TWW. Cu and Fe were higher in soil samples compared to other elements and Zn in plant tissues was more dominant than other elements. In the TWW, all elements of heavy metals exceeded the minimum permissible level except B, Ba, Hg, and Mn. Whereas; most heavy metals in soil samples were higher than the minimum permissible level of Omani standards. Cd and Pb were higher in root tissues and Cr in leaf parts; these exceeded the minimum permissible level. The study showed that there was an infiltration of heavy metals from TWW to the soil.

Moreover, Al-Musharafi et al. (2013c) studied the concentration of heavy metals in marine fish as extra TWW was dumped there. The results showed that the TWW had the highest concentration of Ni then of Cu, Mn, Fe, Co, Pb and Zn. Although high ranges of heavy metals of Ni, Cu, Pb and Zn were found in fish tissue, they were within the limits of Omani standards.

In addition, Al-Musharafi et al. (2014) conducted research at industrial sites where the STP was used to treat the raw wastewater. Samples of TWW, sludge, soil landfill and groundwater were analyzed twice over 6 months. The results showed that heavy metals in the TWW were lower than in the sludge and soil land- fills but higher than in the groundwater.

2.5 Phytoremediation

Phytoremediation technique can be defined as the elimination of hydrocarbon contamination in polluted environments such as soils and water resources by means of plant species, which have the ability to offer the best environmental conditions for microbial production in the root zone, thus enhancing the biodegradation of organic waste (Chaudhry et al., 2005; Gómez-Sagasti et al., 2012; Phillips et al., 2012). Petroleum hydrocarbon contamination threatens the ecosystem and the environment in many ways which include fuel tankers accidents, transportation fuel, gasworks sites, fuel sites, mining, shipping, refining and breaking of oil pipelines (Saadoun and Al-Ghazawi, 2005; Chakrabarti and Ghosh, 2010). Petroleum hydrocarbons include gasoline, diesel and motor oil (Padmavathiamma et al., 2014). Diesel fuel can negatively influence soil microorganisms, vegetation and groundwater (Hong et al., 2005; Zhang et al., 2014). Straight and branched alkanes, such as cycloalkanes, monaromatics and polyaromatics, are the main contents of diesel fuel (Adam and Duncan, 1999; Komilis et al., 2010; Das and Tiwary, 2013).

Several efficient methods were investigated to remediate hydrocarbon polluted sites (Dott et al., 1995; Gaskin and Bentham, 2010; Ibraham et al., 2013) using the phytoremediation method by means of some plant species (Mariano et al., 2007; Gaskin and Bentham, 2010) or through both remediation methods of phytoremediation and bioremediation by adding microbes and nutrients to degrade hydrocarbons from contaminated soils (Speight and Arjoon, 2012; Al-Hinai, 2013; Ghanem et al., 2013; Dadrasnia and Agamuthu, 2013a).

2.5.1 Plant species used in phytoremediation and their effectiveness in TPH degradation

Many plant species were examined to investigate their efficiency to degrade petroleum hydrocarbons. For example, Soleimani et al. (2010) reported that *Festuca arundinacea* Schreb and *Festuca pratensis* Huds are effective in phytoremediation to reduce from 80-84% and 64-72% TPH in the soil by the plants respectively within 7 months. Furthermore, Dadrasina and Agamutu (2013b) used *Podocarpus polystachyus* and *Dracaena reflexa* in their study; they observed the removal by 90 to 99% and 52 to 62% of diesel fuel contaminated soil by these plants respectively over 270 days. Shahsavari et al. (2013) used *Medicago sativa, arrow Trifolium vesiculosum, Trifolium alexandrinum, Ornithopus sativus, Zea mays Pisum sativum, Trifolium resupinatum, Austrodanthonia* *richardsonii* and *Elymus scaber*. The study showed that 66 to 72% of TPH was removed from hydrocarbon-contaminated soil by using maize and wheat compared to a much smaller amount of removal with other plant species.

Two plant species were described in many studies that showed their efficiency to degrade soil hydrocarbon contamination through the phytoremediation technique. These are ryegrass (*Lolium perenne*) and Bermuda grass (*Cynodon dactylon*). Günther et al. (1996) showed that 76% of n-decane and 13% of n-tetradecan were lost by using ryegrass. Also, Kirk et al. (2005) used ryegrass and alfalfa to examine the population of microorganisms in the rhizosphere in these plants, and they concluded that alfalfa can reduce complex hydrocarbon contaminants more than ryegrass, but both of them have capabilities to increase the number of microorganisms. Furthermore, Kechavarizi et al. (2007) observed that some plants died and their shoots heights and biomasses were reduced using *Lolium perenne*, and the proportion of up to 74% of the plant had survived in the diesel-contaminated soil compared to 100% survival in the control treatment. In addition, TPH concentration was recorded at 16% and 19% of initial diesel concentration of 25 mg/g, thus showing little dissipation between all treatments over 49 days. Razmjoo and Adavi (2012) also examined Bermuda grass for reducing TPH when using different concentration of oil sludge to contaminate soil of 0, 10, 20, 30 and 40%. They observed that the concentration reduced to 0, 2, 4, 6 and 8% TPH. They also noticed that root biomass went up to 6% of total petroleum hydrocarbon level over six months. Thomas et al. (2014) used Bermuda grass exudates in complex lead in an aqueous medium; they noticed that the plant's exudates can dissolve 60% more lead than deionized water. Besides all of these findings, Ghanem (2013) found that the removal of pyrene after 90 days was better achieved when compost was added to the contaminated soil at a rate of 16 to 26% dissipation of pyrene with three kinds of plant species: *Lolium perenne, Medica sativa and Brassica napus*. Liu and Cole (1996) observed that maximum stimulation of plant growth was found when 20% of mature yard waste compost was mixed with pesticide-contaminated soil, and no stimulation in microbial activity was noticed at <20% compost, the experiment was done when different levels of 0,1,5,10,20 and 40% of compost were mixed with contaminated soil.

2.6 Bioremediation

Many types of pollution contribute in damaging the environment and the surroundings; these can be either organic or inorganic pollution (Ashraf et al., 2010). Petroleum hydrocarbon contamination is one type of organic pollution which is described as a global environmental contaminant (Ganesh and Lin, 2009). Other types of organic contaminants include poly nuclear aromatic hydrocarbons, polychlorinated biphenyls, pesticides and chlorinated solvents (Padmavathiamma et al., 2014).

Many studies have been conducted to introduce a cost-effective and environmentally attractive technology to diminish the negative impacts of petroleum hydrocarbon pollution in the surroundings; one of them is the bioremediation technique. Bioremediation could be defined as the degradation of extremely toxic organic contaminants into less toxic ones by means of microorganisms (Speight and Arjoon, 2010). The success of these microorganisms depends on their nature, or their tolerance to the chemicals present during the remediation process (Dadrsina and Agamuthu, 2013a).

2.6.1 Types of bioremediation techniques

There are two types of bioremediation technique enhancing the degradation of hydrocarbon contaminants: bioaugmentation and biostimultation (Richard and Vogel, 1999; Abed et al., 2014). The addition of microbes to eliminate hydrocarbon contamination is called the bioaugmentation process whereas providing nutrients to these bacteria is called the biostimulation process. Some researchers have recommended using a combination of the two processes in order to speed up the removal rate of such contamination (Wong et al., 2002; Gestel et al., 2003; Karamamalidis et al., 2010).

The degradation of organic contaminants can be enhanced through the addition of organic amendments to polluted soils, such as sewage sludge compost or manures, as these offer a good nutrient resource and supply carbon sources for microorganisms (Namkoong et al., 2002; Ling and Isa, 2006). Furthermore, many researchers recommended supplying some optimizing conditions for microbes in the bioaugmentation process to speed up the biodegradation of organic pollution, for example by adjusting pH, soil aeration, control of temperature and water content (Weid et al. 2007; Al-Hinai, 2010). Moreover, offering nutrients to the microorganisms in the biostimulation processes could accelerate microbial action and their co-metabolism thus breaking toxic compounds into less toxic ones very easily (Sunar et al., 2014).

2.6.2 Different types of soils and microorganisms for enhancing bioremediation

Different types of soils were found to develop more or less biomass of microbial communities and as a result, different reduction rates of hydrocarbon pollution could be achieved (Bundy et al., 2002).

Moreover, different kinds of isolated, identified and effective petroleum hydrocarbon degradable microorganisms could accelerate the bioremediation process. These include *Pseudomonas aeruginosa, Enterobacter and Acinetobacter* (Sadddoun, 2002), *Staphylococcus and Kocuria palustris* (Mariano et al., 2007), gram positive and gram negative strains (Ganesh and Lin, 2009), *Bacillus* (Abed, 2014) and many other genera. In addition, some petroleum degradable strains which have been classified as being able to survive in very hot climates and saline environments (Das and Tiwary, 2012), such as *Bacillus* (Dadrasina and Agamuthu, 2013; Abed et al., 2014), could help in the degradation of petroleum hydrocarbons in saline environments.

2.6.3 Rates of TPH degradation with or without compost by means of degradable strains

Bioremediation showed its success in degrading TPH as reported in many studies. A study which was carried out by Weid et al. (2006) showed that *Dietzia cinnamea* bacteria has the ability to degrade a range of alkanes from C11-C36 of Arabian light and Marlin oils incubated within 10 days at 30^{o}C using Gas Chromatograms (GC) to evaluate the degradation rate of oil. In addition, Mariano et al. (2007) found that the degradation rate of oil using GC was achieved at 45.5% after 55 days of soil contaminated by diesel oil underground storage tank, whereas the rate was calculated at 19.8% through the respirometric method. Both the biostimulation and bioaugmentation processes were used in this study through two bacteria species *Staphylococcus* and *Kocuria palustris*. Also, Karamalidis et al. (2010) used three approaches for degrading petroleum contaminated soil (3.5% dry weight of contaminated soil mixed with 1:1 w/w diluted and clean soil), including the biostimulation process of *Pseudomonas aeruginosa* strains by applying optimized conditions (moisture, nutrients, and aeration). The second approach was a combination of the biostimulation and bioaugmentation processes and the third approach used the second approach but with additional inoculation of capsulated cells of *Pseudomonas aeruginosa* strains in sodium and starch. The results after 191 days showed that TPH was degraded at rates of 94%, 79% and 79% for the three approaches respectively.

Saviozzi et al. (2009) investigated the effect of compost in the removal rate of TPH in diesel-contaminated soil for the six treatments (uncontaminated soil, contaminated soil, sterilized and contaminated soil, 1% compost and contaminated soil, 2% compost and contaminated soil and finally 4% compost and contaminated soil). After 120 days, they observed the degradation rates of hydrocarbons decrease by 40% in weight after 7 days of the first treatment, 56% reduction of TPH for the second treatment, and the successful removal of 66% of TPH was noticed with 4% compost compared with other treatments. This is in line with Ling & Isa (2006) who found the highest amount of compost or sewage sludge results in the highest degradation of TPH. Also, Namkoong et al. (2012) used organic amendments for degrading soil contaminated with diesel. They found that the addition ratio of contaminated soil to sewage sludge of 1: 0.1, 1: 0.3, 1: 0.5 1:1 and sludge only could reduce TPH at the rates of 86.1, 98.1, 98.1, 94.6, and 95.8%, respectively. The addition ratio of contaminated soil to compost of 1: 0.1 1:0.3 1:0.5 1:1 and compost only could degrade TPH at the levels of 67.1, 93.1, 98.4 97.1 and 94.6%, respectively within 30 days. In addition, the combination of compost and a *Pseudomonas* microbial consortium in the contaminated diesel soil in the study which was conducted by Taccari et al. (2012) was found to degrade TPH at 96% within 120 days.

2.7 The effect of municipal sewage sludge on the quality of soil and crops

The management of wastewater and sludge production is a critical environmental issue in many countries which require different approaches to tackle them.

Most countries use sewage sludge as an organic fertilizer (Al-Dughaishi, 2009) which has the benefits of having low capital and service costs with elevated treatment effectiveness (Namkoong et al., 2002; Gestel et al., 2003).

On the other hand, sludge usually comprises pathogens, trace and heavy metals and organic chemicals in its contents (Harrison et al. 2006; Ji et al., 2012; Nogueirol et al., 2013) which may cause adverse impacts on the food chain and later to animal and human health (Rieuwerts et al. 1998; Zhao et al., 2012). Contamination by heavy metals damages physiological and biochemical characteristics of soils and plant yield (Singh and Agrawal, 2011).

2.7.1 Effects of compost on physical-chemical soil properties and on plant growth

Sewage sludge contains a lot of organic matter such as TOC (Zhao et al., 2012; Peña, 2015; Mi 2016) which can benefit the yield and fertility of crops and improve the physicochemical features of soil (Onwudiwe et al., 2014, Antonkiewicz and Pelka, 2014, Al Busaidi et al., 2015). Many studies have been conducted using sewage sludge as organic fertilizers: The study which was carried by Garrido et al. (2005), found that the Bean crops which were grown in soil amended with sewage sludge were bigger, higher, greener and showed significant differences ($p<0.05$) among all treatments, the crop yield was higher three times than the plants which were grown in control soil and all heavy elements were within the permissible level. The study conducted by Angelova et al. (2013) showed that soil properties improved when using organic amendments compared with control soil (no organic amendments). Hydraulic conductivity, macro elements (P, K, Ca, Mg), nutrient availability, total N and organic matter were affected positively in soil conditioned with organic amendments, and the DTPA- extractable levels of heavy metals were decreased. The application of compost on soil, increase plant nutrient (poll et al., 2008), root development (Walker and Bernal, 2008) which release enzymes and lead to increase the biomass of plant shoots (Nardi et al., 2002; Zandonadi et al., 2007).

2.7.2 Effects of compost on biological soil properties

Organic amendments can lead to increase microorganism population such as *Fusarium oxysporum f. sp. lini, Phytophthora cinnamomi and Meloidogyne hapla* (Lozano et al., 2009), earthworms (Cheng and Grewal, 2009) and Gram-positive bacteria (Islam et al., 2009) and their activities to produce various enzymes to consume different types of pollutants in contaminated soils. These in turn recover disturbed soil and increase the fertility of plants (Garcia et al, 2008; Duong, 2013). However, nitrogen content and nutrient concentration may be decreased (Hadas et al., 2004; Dambreville et al., 2006) or may increase in many cases (Poll et al., 2008).

2.8 Conclusions

Most countries use tertiary treatment for treating sewage wastewater such as activated sludge, whereas various types of treatment for treating the generated sludge are adopted in many countries according to the climatic conditions. These are: thermal aerobic digestion, windrow or aerated piles, dewatering and storage.

The beneficial uses of both treated wastewater and sludge are practiced in many countries; some of these uses include fertilizer, remediation of hydrocarbon contaminated soil, soil amendments, recharging groundwater aquifers and for irrigation.

CHAPTER THREE

DATA COLLECTION AND ANALYSIS

This chapter highlights the methodology of institutional work (policy analysis) and the experimental parts which were conducted at the Agricultural Experiments Station (AES) and in the laboratories of SQU in the period from March 2014 to 2016. The policy analysis detailed the current status of wastewater and sludge management in the Sultanate, and the comparison between the national and international regulations in order to suggest some modifications for the current national regulations if necessary. Whereas, the experimental parts show all the methods and materials which were used in three different experiments using composted sewage sludge (Kala compost) to show its efficacy in minimizing hydrocarbon contaminants and enhancing soil qualities and crop yield.

3.1 Analysis of national policies on wastewater and sludge management

With the expanding of urban development in Oman, more treated wastewater will be discharged in the natural streams and reservoirs. Therefore, the management of wastewater and sludge is a critical environmental issue in the country. The updated regulations of reusing TWW are needed regularly to ensure utilizing these resources (TWW and sludge) successfully without causing any adverse impacts to the environment. Wastewater treatment and the sludge production take place under different technical, economic and social contexts, thus requiring different approaches and involving different solutions. In most of cases, a regular and environmentally safe wastewater treatment and associated sludge management requires the development of realistic and enforceable regulations as well as treatment systems appropriate to local circumstances. The main objective of this part is to provide useful information about the current wastewater and sludge treatment, management, regulations and research in Oman, in order to ensure that Omani regulations go parallel with the international regulations.

3.1.1 Methodology

The current status of TWW and sludge management in the Sultanate is provided and detailed in chapter four; this includes projects undertaken and highlighting the costs involved in the wastewater treatment. A summary review of research and development works done in wastewater, greywater treatment and reuse as well as sludge studies done in Oman is also be provided. In order to ensure the updated standards of the treated wastewater and sludge, the national policies, standards, rules and regulations in Oman is outlined and compared to the international standards, World Health Organization (WHO) and US Environmental Protection Agency (EPA) guidelines is considered for comparing them to the national guidelines in term of reusing of treated wastewater. Whereas, US Environmental Protection Agency (EPA) and European guidelines is used to be compared to the national guidelines in term of reusing sludge application and management, these comparisons identify the gaps in the existing Omani regulations, thus leading to recommend and suggest the necessary amendments in the present national regulations.

3.2 Investigating the effect of Kala compost on the phytoremediation method applied to diesel-contaminated soil by means of two plants species

Phytoremediation is a method which contributes to remove or degrade organic contaminants in the soils by means of plant species (see Section 2.5).

Although there are not many studies in the Middle East conducted to investigate this method to remediate such pollution (Padmavathiamma et al., 2014), this study showed the proficiency phytoremediation to reduce hydrocarbons from diesel-contaminated soil using composted sewage sludge (Kala compost) by means of Ryegrass and Bermuda grass.

3.2.1 Experimental methods

A greenhouse pot experiment was conducted at AES in Sultan Qaboos University (SQU) from 31st March 2014 to 7th June 2014. Twenty seven plastic pots (12X14 cm) for 9 treatments and 3 replicates were filled with 2 kg soil after placing a layer of gravel at the bottom of the pots. Twenty seedlings (15 days after sowing seeds) of P1 (Bermuda grass) and P2 (Ryegrass) were transplanted as per the treatments detailed below in Table 3.1. The pots were kept in the greenhouse with a polyethylene transparent covering roof as shown in Figure 3.1. The experiment was conducted in a Completely Randomized Design (CRD).

The plants were irrigated once a day with tap water (200 ml) of pH 6.5, EC 900 µS/cm at levels that there was no run out from the bottom of the pots.

Table 3.1 Treatment details of greenhouse pot experiment

Treatments	**Details**
T1	Contaminated soil alone
T2	Contaminated soil + P1 (Bermuda grass)
T3	Contaminated soil + P2 (Rye grass)
T4	Contaminated soil+10% of compost +P1
T5	Contaminated soil+10% of compost +P2
T6	Contaminated soil+20% of compost +P1
T7	Contaminated soil+20% of compost +P2
T8	Non-contaminated soil (Background soil) + P1
T9	Non- contaminated soil (Background soil) + P2

A. Basic soil analysis

The initial analyses of contaminated and clean soil were carried out at the SQU laboratory as follows:

- **Soil texture**

50 g of soil was placed in a 1L experimental cylinder which was filled with 2/3 distilled water and 10 ml of Calgon, the solution was stirred for 20 minutes. Next, this solution was transferred to another 1L experimental cylinder where distilled water was added to the mark shown in the cylinder. After 40 seconds, the suspension temperature

and hydrometer reading were recorded, and after 2 hours the reading was recorded again. By using calculations mentioned in the hydrometer method by Klute (1986), the percentage of clay, silt and sand were determined. A texture triangle chart by Brady (1984) was used to specify soil texture.

- **pH, EC**

These were determined by saturation paste analysis (Richards, 1954), and was performed by adding 150 ml of distilled water to 300 g of sieved soil. These then were mixed until the mixture became sticky. The mixture was left for 24 hours and then placed under a vacuum to extract water. Finally, pH and EC were measured using pH and EC electrode.

- **Total Organic Carbon (TOC)**

This was performed by the method which is mentioned by Walkley and Black et al. (1934). This was done by transferring 60 mg of soil into ceramic containers placed in the TOC instrument (TOC Analyzer TOC-V CPN from Shimadzu ^{TM}, Japan) where Inorganic Carbon (IC) and Total Carbon (TC) were measured. TOC was calculated by subtracting IC from TC.

- **Total Petroleum Hydrocarbons (TPH)**

This was measured by the method of US-EPA 1664 (2010), in which hydrocarbons were extracted three times from 10 g of soil samples with 10 ml hexane solvent. Hexane was used in this experiment according to US-EPA 1664 (2010); Dadrsina and Agamuthu, (2013b). The solution was placed in an ultrasonic bath for 30 minutes after being dehydrated with Na_2SO_4 in 50 ml glass bottles. The extraction of solution was filtered through nonabsorbent cotton in an experimental funnel. Then the procedure of extraction was repeated twice by adding 10 ml of hexane solvent each time. All the extracts were collected up to 30 ml in a 100 ml weighted round flask and were evaporated completely using rotary evaporation equipment at
40 ^{o}C and 70 rpm (Round per minutes) rotations by providing 140 mbar vacuums. After that, the round flask, which contained the residual oil, was placed in the desiccator for further evaporation of solvent. Then its final weight was recorded and subtracted from its known weight at the beginning of the experiment. The result then was divided by the

original weight of the soil sample to calculate TPH percentage gravimetrically according to Weisman (1998) method.

- **Heavy metals analysis:**

this was performed using Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) instrument.

B. Analysis of soil samples after harvesting

Soil samples after harvesting were kept in the labeled plastic bags, and then kept in the refrigerator to prevent volatilization of hydrocarbons. The soil samples then were ground and passed through a 2 mm sieve for analyzing for TPH and heavy metal analysis as follows:

- **TPH analysis**

The same procedure of determining TPH was performed as mentioned before

- **Heavy metal analysis**

By using digestion method according to Smoly (1992) and Lindsay and Norval (1978) respectively, total and DTPA-extractable metal analysis was performed. The total metal analysis was carried out by digesting each 1 g of soil sample in a combined 4 ml of HNO_3 and 10 ml HCl, the samples then were kept in an oven at 100^{o}C for 2 hours and were shaken for another 2 hours then filtered through filter paper in 100 ml volumetric flasks and diluted with distilled water. The DTPA-extractable metals analysis was carried out by digesting 20 g of each soil sample in 40 ml of diethylene triaminepenta acetic acid solution (0.1M triethanol amine, 0.005M DTPA and 0.01 M Calcium Chloride). The soil samples then were shaken for 2 hours and were filtered through filter paper in 100 ml conical flasks. Finally, all soil samples from two methods of Total extractable metals and DTPA-extractable metals were analyzed for heavy metals using ICP-OES.

C. ***Plant harvesting, sampling and analyses***

Plant tissue samples (both roots and shoots) were placed in separate labeled plastic bags. The samples were washed with tap water to remove soil particles stuck on the roots. Then, they were kept for further dryness in room temperature for about two days. Later, the plant's roots and shoots were cut into small pieces using a scissor and weighed separately. Finally, the roots and shoots were kept in the paper bags and placed in an oven at 60oC for drying. They were ground into powder for analysis of TPH and for heavy metals as follows:

- **TPH analysis**

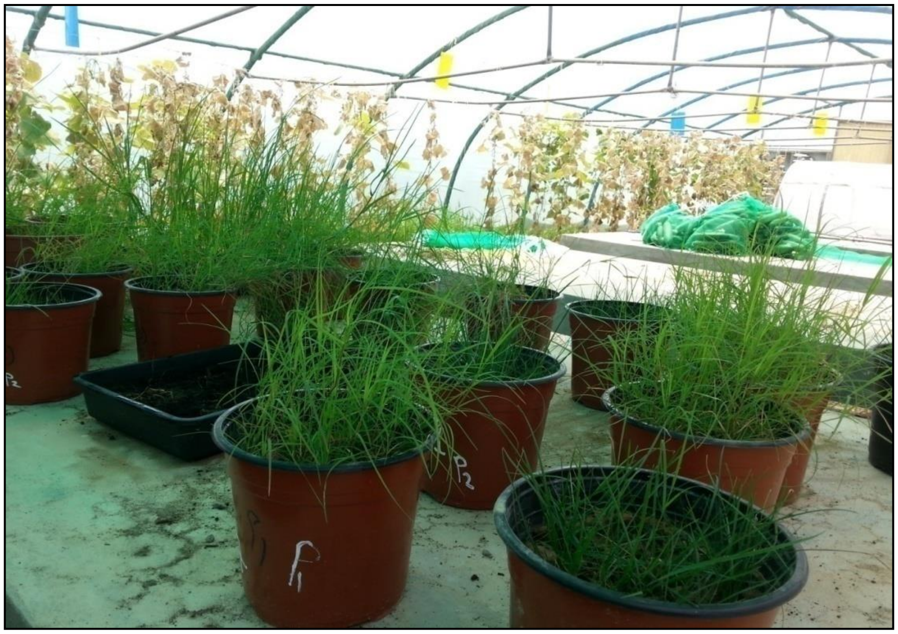
The same procedure for soil analysis of determining the load of hydrocarbons was performed for plant samples as mentioned before.

- **Heavy metal analysis**

This was performed by using the method of Wet Acid Digestion (EPA 3050; AlKhamisi, 2013). In this method, about 1 g of plant sample was digested in 10 ml mixed acids of 500 ml nitric acid, 50 ml of sulfuric acid and 100 ml of perchloric acid at 120^{o}C for 2 hours until a clear solution was appeared. Then the samples were cooled, filtered and diluted in 50 ml volumetric flasks, finally it was analyzed using the ICP instrument.

3.2.2 Statistical analysis

The data for biomasses of roots, shoots, TPH and heavy metals of soil and plants were subjected to Analysis of Variance (ANOVA).



Pots of plants in greenhouse

Bermuda grass **Ryegrass**

Figure 3.1 Greenhouse pot experiment

3.3 Bioremediation of diesel-contaminated soil by means of isolated diesel-degrading bacteria, Kala compost and urea

As mentioned in Section 2.6 the bioremediation includes degradation of extremely toxic organic contaminants into less toxic ones, this study showed the efficiency of composted sewage sludge (Kala compost) to enhance the degradation of hydrocarbons from diesel-contaminated soil by means of diesel degradation microorganisms.

3.3.1 Experimental methods

- **Collection of soil samples and diesel fuel**

Two different types of soils used in this experiment were collected from AES. Stones and pebbles were removed from soils and were sieved through 2.0 mm. Diesel was obtained from the AES and Kala compost was collected from (HW). Determining of soil texture, pH, EC and TOC analyses were performed on the two types of soils as mentioned in Section 3.2.1.

- **Classification of soils**

After the classification of soil types, one type was classified as sandy loam with chemical composition of 70.21% sand, 18.82% silt and 11.01% clay where the other was classified as sandy soil with chemical composition of 90% sand and 10% silt. Sandy loam soil in our experiment was used for isolating diesel-degrading bacteria as the microbial biomass communities are enriched in this type of soil (Bundy et al., 2002).

According to Mariano et al. (2007) who used polluted sandy soil with diesel in their research, sandy soil in our experiment was also used as contaminated soil after spiking it artificially with 1% (by weight) of diesel fuel according to Taccari et al. (2012) who spiked soil with 1% (by weight) of diesel fuel in their experiment.

- **Cultivation of enrichment strains**

One hundred g of sandy loam soil was sieved through a 2 mm mesh sieve, weighted and placed in a plastic disposal glass. To gain a good growth of bacteria the method of Pepper et al. (1995) was carried out in which 15 ml of diesel fuel was added to the soil with the addition of 10 ml of distilled water and 5% of sugar solution. The plastic container was covered with foil paper by punching holes on its top surface to provide sufficient aeration. The container was incubated for 30 days at room temperature and all the treatments were set up in triplicates.

- **Isolation of strains**

In order to isolate strains and to gain an enrichment growth of microorganisms, minimal media (MM) was prepared according to the method used by Richard and Vogel (1999) with the following composites: KCl=0.7 g/l; KH_2PO_4=2 g/l; Na_2HPO_4=3 g/l; NH_4NO_3 = 1 g/l. Trace minor chemicals were added to the above solution containing (per liter) the following concentrations: 4 mg $MgSO_4.7H_2O$; 0.2 mg $FeSO_4.7H_2O$; 0.2 mg $MnCl_2.4H_2O$; 0.2 mg $CaCl_2.2H_2O$. The mixture then was autoclaved for 15 minutes at 120^oC. After that, 18g of Bacteriological Agar and 1% of diesel oil (by weight) as the sole carbon source were mixed and suspended into the MM solution, the solution was adjusted to pH=7 and finally was filled in the petri dishes.

- **Dilution series method**

The dilution plate technique, as described by Pepper et al. (1995), was employed to obtain the growth of microorganisms on the plates. It was performed by filling 3 glass containers (as 3 replicate samples) with 95ml distilled water in addition to 6 test tubes which were also filled with 18ml of distilled water, the solution was diluted up to $10.^{-3}$; 20 µL of previous sequential dilution was spread on MM plates and incubated for two weeks at 28^oC, as the suitable temperature for bioremediation of hydrocarbon process is between 28^oC to 35^oC(Van Gestel, 2003; Sunar et al., 2014) until the transparent zones of bacteria appeared. The colonies then were transferred from MM to Nutrient Agar (NA) dishes and streaked there. Notice that NA media was prepared by suspending 14 g of nutritive agar in 500 ml distilled water and autoclaving it for 15 minutes at 120^oC.

- **Screening of strains**

The isolates (strains) were streaked in three folds and incubated at 28^{o}C for three days. The streaking procedure was repeated several times till separated colonies formed as shown in Figure 3.2 below.

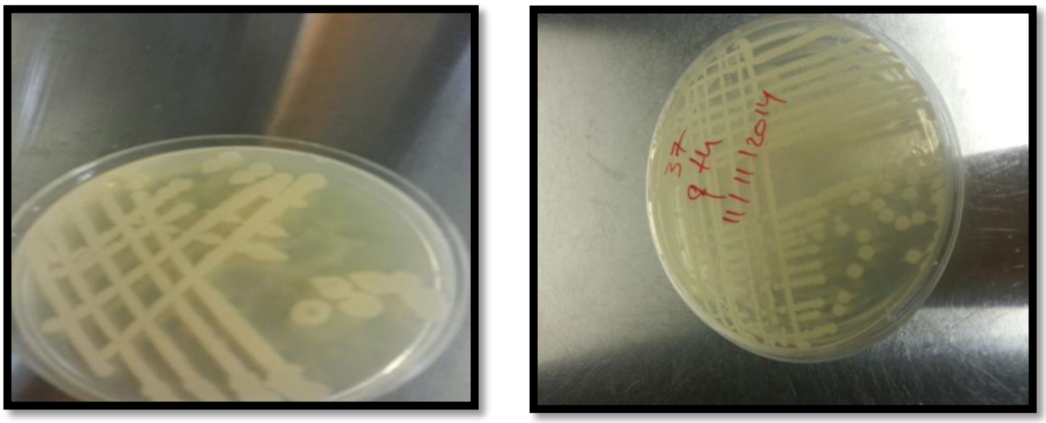


Figure 3.2 Separated colonies of diesel-degrading bacteria

- **Bioremediation of diesel-contaminated soil**

In order to observe the potential of the isolates in the bioremediation process, two approaches were used to remove the load of hydrocarbons from the contaminated soil; the first one is the bioaugmentation method which involved the addition of isolated diesel degradation microorganisms to the artificially contaminated soil. The second one is the biostimulation method in which the isolates were provided with nutrients like urea and sewage sludge compost (Kala).

A. Bioaugmentation method

Out of all strains which were obtained in this study, 17 strains randomly were used to examine their potential in removing diesel fuel through the method of the bioaugmentation; and out of 17 strains, 3 were selected to be used in the biostimulation method. Several tests were carried out as the follows:

- **Preparing of inocula solution**

25 ml autoclaved MM was suspended in the labeled 17 conical flasks (100 ml conical flasks). Then only one colony out of each 17 strains was picked out and transferred individually to its corresponding conical flask. 0.225 ml of diesel fuel was added to each inocula solution and was incubated at 28^{o}C for 3 to 4 days.

- **Preparing experimental pots**

Soil (of the sandy soil) was autoclaved twice at 120^{o}C and spiked artificially with only 1% of diesel oil (by weight). Then 5 ml of inocula solution of each of 17 strains was mixed with the contaminated soil samples individually. A total of 54 autoclaved PVC pots (1 kg of soil per pot with 3 replicates with control samples) were made.

Soil moisture content was maintained at 21% (same amount was found by Taccari et al. (2012)) during the incubation period of 7 days at 28^{o}C. Soil moisture content was determined by oven drying method explained by Richards (1954) in which, some amount of soil sample was weighed in a container, which then was placed in the oven for 24 hours. Finally its weight was re-recorded and subtracted from the pre-weighted sample.

- **TPH analysis during bioaugmentation method**

The same procedure of TPH analysis of soil samples via gravimetric basis was performed as mentioned in Section 3.2.1.1.

B. Biostimulation method

Three selected strains (see Section 3.3.1.7.1) which showed slightly higher degradation rates in the bioaugmentation method were subjected to TPH analysis after supplementing them with urea and compost as described in the experiment below:

- **Preparing experiment pots**

Six different treatments were carried out in this experiment, and all the treatments contained 1 kg of autoclaved soil spiked artificially with 1% of diesel fuel (by weight) per pot as follows:

A) Control soil (neither bacteria nor nutrient addition).

B) Soil inoculated with inocula solution of 3 strains individually.

C) Soil supplemented with 1% of compost according to Ling and Issa (2006).

D) Soil supplemented with 1% Kala compost and inocula solution of 3 strains individually.

E) Soil supplemented with 1% urea according to Kauppi et al. (2011) and,

F) Soil supplemented with urea with addition of inocula solution of 3 strains individually.

All the above treatments were carried in 3 replicates with the same procedure of preparing inocula solution as described in Section 3.3.1.7.1. A total of 36 sterilized pots were prepared and incubated for one week, then for two weeks at 28^{o}C and were watered with distilled water to maintain water content at 21%, the experiment was conducted in a Completely Randomized Design (CRD).

- **TPH analysis of biostimulation method**

TPH analysis was performed using the same experimental procedure which was mentioned before in Section 3.2.1.1, except at the end of the evaporation method, the remaining oil was re-dissolved in 1 ml hexane, transferred to vials and sealed with screw caps to be injected into Gas Chromatogram-Mass Spectrophotometer (GC-MS Shimadzu, Japan, Perkin Elmer Precisely, Clarus 60^{o}C) equipped with a flame ionization detector. The GC-MS was operated by a capillary column 30 m long, 0.25 mm in diameter and 0.25 µm film thickness with the maximum temperature of 350^{o}C. The injected samples volume was 1 µl with a split ratio of 10:1. Helium was used as the carrier gas at a constant flow of 1.0 ml/min. The percentage of TPH removal for each treatment was calculated using GC-MS instrument fitted with Turbo Mass Software; the concentrations of Alkanes (C10-C30) were also determined.

3.3.2 Growth of microorganisms at various salty concentrations and temperatures

To examine the efficiency of degrading bacteria of diesel fuel in a salty media and at high optimum temperatures, three strains which showed higher bioremediation by gravimetric analysis from the bioaugmentation experiment in Section 3.3.1.7.1 were selected and tested on nutrient agar plates mixed with different salty media of 5%, 10% and 15% of NaCl concentration and were incubated for 3 days at 28^{o}C. The same strains were also subjected to different temperatures for the same period on nutrient agar plates at 35^{o}C, 40^{o}C, 45^{o}C, 50^{o}C and 55^{o}C to observe their tolerance to Omani hot summer month temperatures.

3.3.3 Identification of strains

The identification of strains was carried out at the Central Analytical and Applied Research Unit at SQU in the Sultanate of Oman. Bacterial characterization and identification by the Genetic Analyzer method was used to identify the isolated degraded bacteria, this was done by using MO BIO kit to extract DNA, and PCR amplification of 16S rRNA was performed using 27B Forward primer and 1492r Reverse primer. The samples then were overloaded on Agarose gel and finally 534 R was used for the method of sequencing.

3.3.4 Statistical analysis

The Analysis of Variance (ANOVA) in the bioaugmentation method was used to examine the efficiency of bacteria to degrade hydrocarbons, this analysis also was performed in the biostimulation method and for estimating alkanes concentration in order to study the interaction between the factors (contaminated soil, compost and urea) when treating with or without the isolated bacteria for both incubation periods (7 and 14 days).

3.4 The effect of municipal sewage sludge on the quality of soil and crops

This study determines the effect of Kala compost (organic fertilizer) and NPK (inorganic fertilizer) on the quality of soil and crops (green Beans *Phaseolus vulgaris* and white Radish *Raphanus sativus)* using groundwater and TWW for irrigation.

3.4.1 Experimental methods

The research for this study was performed in an open field of AES at SQU as shown in Figure 3.3. There were 6 plots in 3 sites of 3 m width and 3.5 m length, these plots were divided into two rows by 1m buffer zone. According to the study by Al-Busadi and Ahmed (2014) each half plot division was amended with 0.5 kg or 952 kg/ha of NPK and 22 kg or 42,000 kg/ha Kala compost, 5 seedlings (15 days after sowing seeds) of each plant were transplanted as per the experimental details which are illustrated below in Table 3.2. Before growing, all plots were mulched to prevent direct effects of heat on the plants.

The composition of NPK fertilizer was 20% Total Nitrogen (N): 4.6% Nitrate nitrogen, 2.5% Ammonical nitrogen, 12.9% Uric nitrogen, 20% Phosphorus Pentoxide (P_2O_8) = 8.7% P and 20% K_2O = 16.6% K. The composition of Kala compost will be given in Section 5.1.

Each plot was irrigated with 20 L by drip irrigation method with GW and TWW for 15 minutes daily. Municipal sewage treated wastewater was obtained from the sewage treated station plant at SQU, whereas, groundwater was obtained from a well at the AES. Both plants were planted on 3 October 2015. Radish was grown before Beans in a period of 18 days, whereas Beans were grown within a period of 1 month.

The field experimental plots were setup as shown in Figures 3.3 each site had 4 treatments for two crops with 4 replicates of chemical analysis for each treatment, and the chemical analysis data were recorded for the statistical analysis, the experiment was conducted in a Completely Randomized Block Design (CRBD). The treatment details of the experiment are detailed in Table 3.2, and 3.3.

Table 3.2 Experimental details of municipal sewage sludge on the quality of soil and crops

Sites	Details	Irrigation method
1	Radish and Beans +NPK (first site and at right side)	TWW
1	Radish and Beans +Kala (first site and at left side)	TWW
1	Radish and Beans +NPK (first site and at right side)	GW
1	Radish and Beans +Kala (first site and at left side)	GW
2	Radish and Beans +Kala (second site and at right side)	GW
2	Radish and Beans +NPK (second site and at left side)	GW
2	Radish and Beans +Kala (second site and at right side)	TWW
2	Radish and Beans +NPK (second site and at left side)	TWW
3	Radish and Beans +NPK (third site and at right side)	TWW
3	Radish and Beans +Kala (third site and at left side)	TWW
3	Radish and Beans +NPK (third site and at right side)	GW
3	Radish and Beans +Kala (third site and at left side)	GW

The third location was ignored as the crop growth was very poor due to operational problems.

Table 3.3 Treatments details for two crops in each site

Treatments	Details
T1	GW+Kala
T2	GW+NPK (control)
T3	TWW+Kala
T4	TWW+NPK

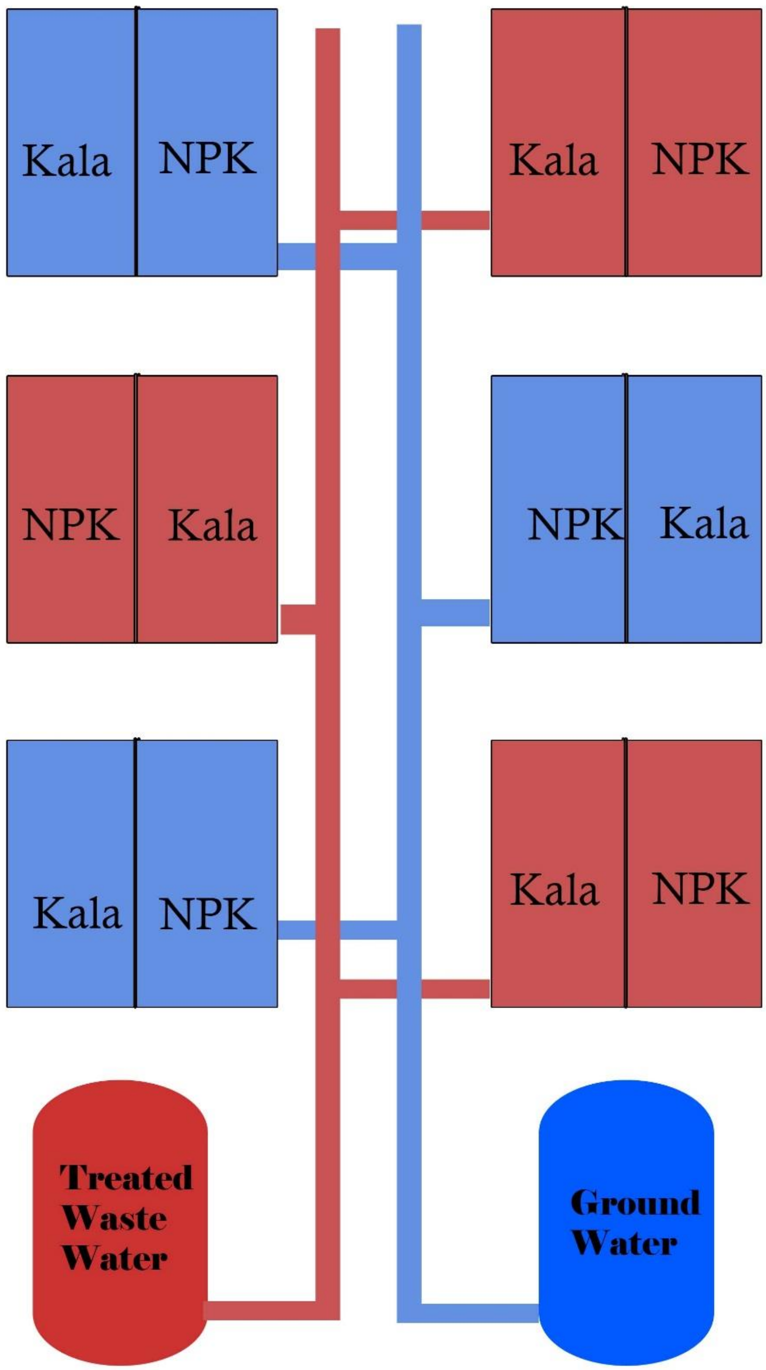


Figure 3.3 The design of the study site

3.4.2 Basic soil analysis

Four soil samples were collected from each site in all plots from a depth of 10 cm. The samples were air dried, sieved through 2 mm sieve and kept in plastic bags. The composition of soil was determined using the hydrometer method (Klute, 1986) and its texture was determined using soil triangle (Brady, 1984). EC and pH were measured using pH and EC electrodes (Thermo Scientific Orion 4-star) after applying the saturated paste method (Richards, 1954). TOC was analyzed by the FORMACSHT TOC/TN ANALYSER model (SKALAR), and the concentrations of heavy metals were performed using ICP instrument after applying the saturated paste method.

3.4.3 Soil analysis after harvesting

Four Soil samples after harvesting were collected from a depth of 10 cm from all plots. These were kept in labeled plastic bags, ground and passed through a 2 mm sieve. EC, pH, TOC and concentrations of heavy metals were determined using the same method as described above in Section 3.2.1, TN was determined by the Kjeldahl method as mentioned by Alkhamisi (2013) and Bremner et al. (1982).

3.4.4 Plant analysis after harvesting

- **Biomass of plants and yield**

The two crops were harvested 2 times weekly and washed to remove sand and dust. For their physical analysis, their weights were recorded and their growth was checked each time (leaf color, surface area).

At the end of each harvesting day, plant samples were kept in plastic bags to be frozen for further chemical analysis.

- **Plant analysis**

In order to determine heavy metals in the plants samples after harvesting period, leaves and roots of the Radish crop were cut as well as the edible part of the Beans crop and the same method of heavy metals analysis as mentioned in Section 3.2.1.3 was performed, 4 replicates per each treatment were analyzed and the chemical analysis data were recorded for the statistical analysis, TN was also determined using the Kjeldhal method and the measurement of chlorophyll contents was also determined using Chlorophyll meter.

3.4.5 Statistical analysis

All data for soil and plant samples were analyzed using the Analysis of Variance (ANOVA).

Data for all three experiments above were analyzed using the computer software JMP (SAS Institute Inc, 2013), and a 5% probability level test was used to determine the significance of the two tests. Tukey's means separation test was also performed to examine the significances of each treatment.

CHAPTER FOUR

EVALUATION OF NATIONAL POLICIES OF WASTEWATER AND SULDGE MANAGEMENT

This chapter details the current status of reusing TWW and sludge in Oman and the researchers conducted in this aspect; also it highlights the methodology for analyzing the national guidelines by comparing it to the international guidelines regarding sludge and TWW management, and finally suggests solutions to the national guidelines.

4.1 Introduction

The quantity of worldwide wastewater has increased rapidly in the last few decades due to rapid population growth and increased use of freshwater. Wastewater, if not properly treated, can cause various harms including threatening public health. Treated wastewater and sludge, which is a byproduct of this treatment, can be considered as resources under certain circumstances.

In the Sultanate of Oman, the majority of cities use septic and holding tanks to collect sewage water from residential areas. Many of these tanks quickly become overloaded due to inadequate construction and maintenance. Sewage from these tanks is transferred by municipal trucks to the closest sewage treatment plants (STPs) or sometimes is discharged to the nearby wadis (dry channel beds). The capital city Muscat is being fully connected to a piped sewer network and the expectation is that large amount of treated wastewater and sludge will be generated as a consequence. Proper planning and management of such resources will help to some extent alleviate the acute water shortage problem in the country.

4.2 Current status of wastewater treatment facilities in Oman

Currently more than 402 STPs in the Sultanate have been recorded in the database of MECA, half of which are in Muscat. Some belong to the government sector and others to private owners such as hotels and industrial estates. In addition to the existing STPs in

Muscat and Dhofar Governorates, the municipalities in these governorates are executing projects to establish and operate integrated networks for collecting, transporting and treating wastewater. In the early 1990s, the budget of wastewater projects for the interior regions in Oman was recorded at 10 million Omani Rials (Mott Macdonald, 1991). That particular year's project budget served nine towns: Khasab, Sur, Al-Buraimi, Al-Rustaq, Nizwa, Ibri, Ibra, Samail and Saham. In 2010, the amount of treated effluent of the Sultanate interior regions was about 22,167 m^3/day (AlKhamisi, 2013) and will be about 39,539 m^3/day in 2016 (Haya, 2016) as mentioned in Appendix1.1.

STPs in the Sultanate produced about 37.446 Mm^3 of TWW in 2000 with individual plant capacities from 8 m^3/day to 15000 m^3/day (MRMEWR, 2002). These produced about 97.8 Mm^3 in 2010 (Al-Omairi et al., 2011) and 100 Mm^3 in 2012. Further, details of projected wastewater flow from domestic and industrial use in Oman is provided in Appendix 1.2.

Wastewater privatization entities, Haya Water (HW) Company in the capital Muscat and Oman Wastewater Services Company in Salalah, now provide centralized sewer systems and treatment to all of the areas of Muscat and Salalah (in Dhofar governorate in the south). The main objectives of these companies are to setup a modern wastewater system to serve the citizens with new technology of operating, maintaining, and managing the wastewater network.

The production capacity of the STP in Salalah was recorded at 27,000 m^3 per day recently (Al Kathiri, 2014), with the plan of building two more stages for increasing the current capacity of the plant. This network served about 120,000 individuals in 2005 and around 200,000 people in 2010 (Al Wahaibi, 2011).

4.3 Infrastructure of wastewater treatment in Oman

TWW reuse plays an important role in the management of water resources and in the environmental, economic and social aspects of a country. The wastewater projects in Oman are considered a significant element of all new development. This section will introduce the activities of Omani Wastewater Services Company which is implementing a new sewage system in the Muscat Governorate. Oman has the capacity to accommodate the sludge generated each year. According to Alkhamisi (2013), agricultural land in Oman under cultivation is recorded at 72,299 ha. Fruits occupied the highest proportion at of 53%, then perennial forages at 30%, followed by vegetables at 11% and finally field crops at 6%. The highest total production in tons is occupied by perennial forages at 58% then by fruits at 23%, vegetables at 17% and grain crops at 2%.

4.3.1 Wastewater management in Muscat

Oman Wastewater Services Company or Haya Water (HW) Company is a joint stock company owned by the government of the Sultanate of Oman. The ministerial decision number 31/2002 established it on 17 December 2002 to design and manage the wastewater collection and treatment system in the Governorate of Muscat. The Ministry of Finance owns the company and started commercial operations from 1 January 2006.

Before the HW, STPs in the Muscat Governorate were owned and operated by Muscat Municipality but now all STPs are operated by HW under a concession agreement with the Government of the Sultanate of Oman. The main objectives of HW are to plan a modern wastewater system to serve all the Wilayats of Muscat Governorate by operating, maintaining and managing the wastewater network in Muscat, as well as building and operating the Bio-Solids Composting Project (composting of sludge). All Muscat’s STPs which belong to this company generated an average volume of 84,144 m^3/day in 2011 (Al Muselhi, 2011) of treated effluent which will rise to an estimated volume of 327,853 m^3/day by 2025 (Al Muselhi, 2014; Haya, 2016). Treated effluent will be used for road landscaping, golf courses, agriculture irrigation, industrial reuse, aquifer recharge and also for potable water. It is estimated that Haya’s treated sewage will serve 290 km of Muscat Governorate (Al Muselhi, 2011).

Currently, HW operates 12 STPs which are located in old Al-Ansab, Darsait, Shati Al Qurm, Madinat Al Sultan Qaboos, Mabella, AlKhoud, AlAmerat, Quriyat, Baushar, Al Manuma, Jibroo and Aynat where all of these plants use tertiary wastewater treatment technology. In addition, some of these plants use Membrane Bioreactor technology (MBR) such as Madinat Al Sultan Qaboos and Shati Al Qurm. Raw wastewater quality of one of the STPs in Al Ansab is provided in Appendix 1.3.

After the completion of HW projects in 2025, the above-mentioned plants will be connected to new five main STPs according to their catchments. Table 4.1 provides the capacities of the existing STPs and five new ones.

Table 4.1 Treatment plant capacity in each catchment

No	Catchment	Existing STPs	Capacity (m^3/d)	New STPs	Capacity (m^3/d)
1	**A' SEEB**	Manuma	140		
		Mabella	1,900		
		Alkhoud	1200		
				AL SEEB	60,000
		Total for A' SEEB	3,240	AL SEEB Ultimate	80,000
2	**BAUSHER**	Al Ansab	14,000		
		Baushar	420		
		Shati Al Qurm	7,500		
		Madinat Al Sultan Qaboos	2,500		
				Al ANSAB (phase1) AL ANSAB (phase2)	55,000 25,000
		Total for Al ANSAB	24,420		80,000
3	**DARSAIT**	Darsait	21,000		
		Aynt	150		
		Jibroo	150		
				DARSAIT	50,000
		Total for DARSAIT	21,300		50,000
4	**Al AMERAT**	Al Amerat	900		
				Al AMERAT	18,000
				AL HAJER	6,000
		Total for Al AMERAT	900		24,000
5	**QURIYAT**	Quriyat	300		
				QURIYAT	6,500
		Total for QURIYAT	300		6,500
6	**Total capacity for existing STPs in (m^3/d)**		1,003,20	**Total capacity for new STPs in (m^3/d)**	461,000

Source: Al Wahaibi (2011)

The new Al Ansab STP is considered the biggest plant in Muscat. The construction of this plant went through two phases in order to expand its capacity for serving all the major towns which are in Muscat. The current production of TWW in phase I was recorded at 57,764 m^3/d (Al Muselhi, 2014). The technology of Membrane Bioreactor (MBR) is used in this plant, and is used to increase the energy efficiency of wastewater treatment plants in order to produce TWW that complies with national standards for reuse in irrigation and for other usage such as recharging of aquifers. The TWW quality of Al Ansab STP is provided in Appendix 1.4.

According to Arab Water council (2011), the Sequence Batch Reactor (SBR) process and Ultra filter (UF) membrane systems are utilized at A'Seeb STP. SBR has been considered as the most effective technology in removing nutrients and producing high quality sludge. All other sewage plants currently are at different construction phases, and will use either MBR or SBR systems as tertiary treatment fitted with ultra-filtration membranes (Al Wahaibi, 2011).

4.3.2 Sludge composting initiative

Sludge originates mainly from the wastewater treatment process. The recycling of sludge generation depends on the process operation and efficiency of the plant, its type, cost, as well as its impact on the environment. There are many methods for utilizing sludge such as gasification converting it to fuel and using it for the manufacture of bricks. However, no legislation for these methods has been established yet in Oman. Based on the national Omani legislation of using municipal sludge in agricultural activities, composting will be the best option for reusing sewage sludge. Composting is a very useful and economic method as it contains a huge amount of organic materials, which saves power, reduces air and water pollution, improves soil in agriculture and saves landfill space.

In 2010, Haya Water (HW) implemented a project to introduce organic fertilizer compost called Kala compost which is used for agricultural activities. It is a by-product of the water reuse treatment process. The composting plant has an area of 60,000 m^2 and is located in Al-Amerat (Al-Maltaqa) town in the Governorate of Muscat. Composting is a biological process which depends mainly on the activity of naturally occurring microorganisms. The method of producing compost is to blend green waste, which consists of a mixture of grass clips, tree trimmings, dry leaves and cut shrubs with dewatered sewage sludge using windrow technology. The green waste is collected by Muscat Municipality while the biosolid (sludge) is generated by all HW reuse treatment plants.

Windrow technology which is mentioned by 1993 US-EPA guidelines is the most useful and cheapest method of treating waste materials. The temperature of the sewage sludge is maintained at 55ºC or higher for 15 days or longer, during which the windrow will be turned a minimum of 5 times.

The process in HW Company consists of three stages: First, in the mixing stage, 20% of the dewatered bio-solids (concession agreement requirement between HW and the government) are mixed with the available amount of green waste. This mixture is then placed in long piles with a specific height and width where windrows are formed and remain for 30 days. Secondly, in the turning stage, heat by bacterial activity during the aerobic state of the organic material is applied to above 55ºC for 15 days or longer to decompose plant seeds, plant pathogens and human pathogens. Finally, during the curing stage, the previous materials continue to decompose until the last decomposed raw materials are consumed by the remaining microorganisms. At this point, the compost becomes relatively stable and easy to handle after curing it for another 15 days. Samples of the final compost are tested to meet pathogen and heavy metal standards. Upon getting the laboratory results and ensuring that the Class A 1993 US-EPA guidelines have been achieved (ALSAFA, 2009); the composted materials become ready for packaging, the detailed of quantity of total dewatered sludge, bulking agent (green waste), and compost produced from 2010 up to 2025 (predicted) are presented in Appendix 1.5.

4.3.3 Cost recovery from wastewater in Oman

Developmental projects in Oman, including all water resources and other infrastructure projects, strive towards generating income through various means. Wastewater projects for instance, could contribute to the agricultural economy investments by creating job opportunities and reducing reliance on public sector funds. However, such projects have disadvantages of requiring large investments, requiring adequate maintenance and operating costs of treatment plants, along with collection and transportation of wastewater, etc. Therefore, a proper tariff is necessary to achieve the cost recovery of such projects to provide financial stability.

The report by Arab Water Council (2011) states that the HW Company in the Sultanate spent around US$ 4.3 billion to expand the collection, transmission and treatment system of wastewater projects in Muscat Government and about US$ 634 million was invested for the construction of new wastewater treatment alone.

4.3.4 Treated effluent tariffs in Oman

According to the 30-year concession agreement between HW and the government of the Sultanate to implement and operate wastewater treatment projects in Muscat, the former adjusted tariffs and charges (see Table 4.2) for the wastewater service fee, based both on metered water consumption and the places where no water meter is available. The bills, which are paid by the customers for water delivery services, also include the payment for the above service. The number of households connected to the centralized sewer system in 2010 were around 20% (Zekri et al., 2014) and was expected to rise to 93% by 2035 Al-Wahaibi (2011).

Table 4.2 Treated effluent tariffs and charges

Description	**unit**	**Unit cost (R.O.)**
Service fee for wastewater based on metered water consumption		
Domestic tariff	m^3	0.154
Governmental and institutional tariff	m^3	0.193
Commercial and industrial properties tariff	m^3	0.231
Service fee for wastewater, where no water meter is available		
Domestic tariff	month	5
Governmental, institution, commercial and industrial tariff	Tariff to reflect actual usage and based on reasonable technical evaluations	
Connection fee (monthly rental fee)		
Domestic tariff	connection/month	2
Governmental and institutional tariff	connection/month	5
Commercial and industrial properties tariff	connection/month	5
Tankered wastewater	m^3	0.193
TE water sold to Muscat Municipality	m^3	0.193
Fee for installation of connections to new properties		
Domestic tariff	connection	50
Government and institutional tariff	connection	Actual cost
Commercial and industrial properties	connection	Actual cost

Source: Al Wahaibi (2011), 1 R.O. = 2.58 USD

4.3.5 Cost of municipal sludge utilization in Oman

The income from sludge utilization is a desirable aspect for every wastewater plant owner as the price tag of converting the sludge into manufactured goods is quite high, and the marketability of its products that has human wastes in their ingredients is always considered as a challenge due to reluctance by consumers to use any product with known association with human body wastes. It is a big challenge for government-owned entities to recover full costs from consumers in most countries. Therefore, Federal irrigation projects in the U.S.A. are fortunate to cover 20% of their expenses from farmers (William and Liu, 2006).

Sludge-discarding services account for 40 to 60% of the construction charge of wastewater treatment plants (Veritas, 2000). All HW STPs and other companies are now sending their waste to the Kala compost plant in the Al-Amerat region instead of dumping it in landfill sites. As a result, 100% of all sewage sludge which is generated by the company's plants are now treated and converted to compost, which is sold commercially in the market (Times of Oman, December 11, 2012).

As reported by Times of Oman (2012), Kala was introduced to the market only in December 2010; and the price of selling the compost is Omani Rial 15 for each ton. According to ALSAFA (2009), the compost confirms Class A of 1993USEPA legislation. The project has a significant positive economic benefit for the country in terms of job creation and enhancing resource recovery.

4.4 Constraints and pre-requisites for treated wastewater reuse in irrigation

Irrespective of the many benefits, TWW use in irrigation can also create specific problems, mainly if the irrigation system and management are not properly designed and operated. The major constraints can be summarized as follows:

- Damage to the physical and chemical properties of soil, especially salinity and alkalinity;
- Decrease of crop yields, and for, some salt-sensitive crops, low quality of the produce;

- Potential environmental degradation; and
- Potential risk to public health.

4.5 The Government's role in the management of treated wastewater and sludge production in Oman

Since the establishment of the Ministry of Environment and Climate Affairs (MECA) under the royal decree No 90/2007, the responsibilities of managing wastewater in all regions for the Sultanate have been transferred to this Ministry. The Ministry of Regional Municipality and Water Resources (MRMWR) also shares the responsibility of managing wastewater in rural regions. MECA carries out several activities in its program such as monitoring, inspection, sampling, analysis and evaluation of all discharge to the environment in accordance with permits to discharge requirements (USAID, 2010).

4.5.1 Development of wastewater legislations

The government of the Sultanate realizes the importance of wastewater management, and since the 1980s, many policies have been applied to manage this source of water. The Omani legislation is issued either as laws in the form of Royal Decrees (RDs) or as regulations in the form of Ministerial Decisions (MDs). In 1984, the Royal Decree 45/84 created a Ministry for Environment and Water (MEW) making Oman the first country in the Arabian Gulf to establish such a Ministry. In 1986, special regulations for the discharge and reuse of wastewater and sludge were issued; these included prohibiting the discharge of wastewater and sludge into the environment in any form and under any condition without obtaining a permit from the Ministry (MEW). In 1991, this Ministry was merged with the Council for Conservation of the Environment and Prevention of Pollution into a new Ministry of Regional Municipalities, Environment and Water Resources (MRMEWR).

In 1993, MRMEWR amended the previous regulations for the discharge of wastewater and sludge to be more integrated, clear and in compliance with the latest technical and scientific developments. Hence, the Ministerial decision, MD 145/93 dated 13th June 1993 on "*Regulations for Wastewater Re-use and Discharge*" was issued. It defined the uses of treated wastewater that comply with the applied standards and conditions for irrigating crops, grass, ornamental plants and recharging aquifers, as well as the reuse of sludge under certain conditions.

All these regulations emphasize managing wastewater for two reasons:

- Protecting the environment and public health, and
- Recycling sewage wastewater for agricultural usage and for beautification purposes.

In 2001, two laws were issued to ensure the proper management of municipal wastewater and to protect the surroundings and public health. The first law was the law of "*the Conservation of the Environment and Prevention of Pollution*", issued under Royal Decree RD (114/2001). Article 20 of this law states that "*it is prohibited to discharge hazardous waste and substance and other environmental pollutants in wadis, watercourses, groundwater recharge areas, rainwater, flood drainage system or aflaj and their channels discharge systems*". It was also prohibited to reuse or discharge treated wastewater without obtaining a permit from MRMEWR.

In compliance with the above law, the second significant law, "*The Law on Protection of Sources of Drinking Water from Pollution*" was issued under Royal Decree No. 115/2001. This includes regulations for secure management of sewage wastewater and protection of groundwater against contamination. In addition, providing citizens with the best level of health and to protect land, soil and water resources, however, MD 145/93 became an appendix of this law. The articles of these two legislations will be mentioned latter in this chapter.

Next MD 421/98 was issued by the former Ministry of MRMEWR. It promoted the establishment of further wastewater treatment services and encouraged the residents of sparsely populated rural areas to set up sanitation units, from which wastewater could be discharged to special designed tanks according to the required technical specifications listed in the regulations of MD 421/98.

4.5.2 Standards and policies of wastewater and sludge at the national level

TWW is used as a source of irrigation water as well as a source of plant nutrients, allowing farmers to reduce or even eliminate the purchase of chemical fertilizers. Recent wastewater use practices include mostly the piped distribution of secondary treated wastewater (i.e. by mechanical and biological treatment) to farmers. Vegetable, fodder and non-food crops as well as green-belt areas and golf courses, are being irrigated.

4.5.3 Policies, standards, rules and regulations in Oman

As mentioned before, laws and regulations in Oman are promulgated under RDs (Royal Decrees) and MDs (Ministerial Decisions)**.** TWW in the Sultanate is reused by following several legal guidelines and controls. MECA has the responsibility to manage and reuse the discharge of treated effluent and sludge in the Sultanate under RD 115/2001 and MD 145/93.

Royal decree 115/2001

"*The Law on Protection of Sources of Drinking Water from Pollution*" was issued under RD 115/2001; this includes rules for protected management of sewage wastewater and protection of groundwater against pollution. In addition, providing people with the most excellent level of health and to protect land, soil and water resources. The decree of 115/2001 comprises 20 articles as follows:

- *Article 1: The provisions of the attached Law shall have effect on protection of sources of potable water from pollution.*
- *Article 2: The Minister of Regional Municipalities, Environment and Water Resources (now MECA) shall issue the regulations and decisions implementing this Law. Until then the current regulations and decisions shall remain applicable in such a manner that shall not conflict with the provisions of this law.*
- *Article 3: The Ministry shall, in coordination with concerned bodies, specify zones of protection of sources of potable water from pollution, and the activities*

prohibited to be practiced within such zones, which may pollute water and its source.

- *Article 4: Owners of wells, water tankers and distribution networks, shall abide by hygienic and environmental conditions stipulated by the ministry in coordination with the concerned bodies. Such water shall be in conformity with national standards for potable water. It is not allowed to sell potable water or to construct network pertaining thereto, unless the necessary environmental permits are obtained in accordance with the rules and principles specified by the minister.*
- *Article 5: The owner undertakes to apply the best technical and scientific methods approved by the Ministry to prevent discharge of environmental pollutants or to treat them or reduce their effect on water from all sources (surface or underground water or rain water) subject to the provisions of article (3) of this law.*
- *Article 6: The Ministry shall approve all private laboratories conducting tests of potable water and treated wastewater and shall set up the necessary rules. Test results issued by laboratories not approved by the ministry shall not be accepted.*
- *Article 7: Construction of septic tanks connected to holding tanks or soak-away shall be allowed to serve institutions and houses discharging domestic effluent with population less than (150). Large institutions shall be served by sewage treatment plants.*
- *Articles 8 to 11 deal with waste, landfills and solid non-hazardous waste.*
- *Article 12: The Minister of justice shall issue, upon request from the Minster, a decision granting judicial powers to water pollution inspectors and other persons designated by him.*
- *Article 13: States that "without prejudice to the penalties stipulated by this law, every person who pollutes water shall be bound to remove such pollution at his own expense and pay compensation for the damage. The ministry shall have the right, in the event of the failure of the violator to remove the violation within the specified period, to arrange for removal of the violation at the expense of the violator.*

- *Article 14: After coordination with the Ministry of Finance, the Minister shall issue a decision fixing the fees payable against obtaining permits pertaining to protection of sources of potable water from pollution and the services rendered by the ministry in accordance with the provisions of this law and its implementing regulations and decisions.*
- *Article15: The Minister, in case where the violation causes serious danger or harmful effect on sources of potable water or public health, shall take the necessary action to avoid the damage or mitigate its effect and to prevent the violator from practicing his activity.*
- *Article 16: No hazardous substances or waste or other water pollutants shall be discharged in aflaj and their channels, surface watercourses, wadis or places of underground water recharge*
- *Article 17: The Minister shall issue a decision specifying procedures for obtaining licenses, procedures for renewal and appeal to the concentrated bodies against decisions in this regard, in addition to determination of administrative penalties and fines payable in cases of delay of renewal of license prescribed by this law, provided that fine shall not exceed R.O 1000.*
- *Article 18: Without prejudice to any severe penalty provided for in any other law, whoever violates the provisions of articles 5 and 11 shall be punished with fine not less than 200 R.O and not more than 2000 R.O. The fine shall be increased at a rate of 10% per days as from the fourth day of the date of notifying the violator. The violator may be suspended from practicing his activity until the causes and effects of the violation are removed and the concerned bodies are notified of the same.*
- *Article 19: Without prejudice to any severe penalty provided for in any other law, whoever violates the provisions of articles (8,9,11 shall be punished with imprisonment from one month to three years and with fine not exceeding 2000 R.O. or by either of the two penalties.*
- *Article 20: Without prejudice to any severe penalty provided for in any other law, whoever prevents or causes to prevent, the water pollution inspector from exercising the powers vested in him, shall be punished with imprisonment for a*

period not exceeding two months and with fine not exceeding 1000 R.O. or by either of the two penalties. The penalty shall be doubled if the same violation is repeated."

Ministerial decision 145/93

The regulations of MD 145/93 are composed of 13 articles; and these are stated in the ministerial decision of MECA as follows:

- *Article 1: "Outlines terms and definitions*
- *Article 2: The discharge to the environment of any wastewater or sludge in whatever form or condition is prohibited without a permit to discharge issued by the Ministry. The permit to discharge may be amended by the Ministry at any time after giving reasonable notice of any change to the owner.*
- *Article 3: Details of wastewater standards shall be in accordance with Table 4.3 and 4.4. While, Table 4.14 outlines all standards for sludge re-use practice. These guidelines shall be approved by the Ministry and shall be defined under the terms of any permit to discharge which may be issued by the Ministry.*
- *Article 4: The final points of discharge of wastewater to the environment shall only be at the points marked on the drawings listed in the permit to discharge.*
- *Article 5: Wastewater quality shall at all times be within the limits that are set out in Table 4.3 as they relate to the permitted method of discharge or as may be modified and supplemented by any other limits that might be included in any specific permit to discharge.*
- *Article 6: the soil on which sludge maybe applied shall be tested by the owner for metals listed in Table 4.14, and for pH value, prior to any initial application. and the sludge quality and application constraints shall at all times be within the limits that are set out in Table 4.14 as they relate to the permitted method of sludge re-use, or as may be modified and supplemented by any other limits that might be included in any specific permit to discharge.*

- *Article 7: Any sludge having concentrations of metals greater than the limits prescribed in Table 4.14 shall be disposed of in sanitary landfills or to other facilities but only with the prior approval of the Ministry.*
- *Article 8: Facilities and equipment shall be provided and maintained by the owner to the requirements and satisfaction of the Ministry for sampling, measuring and recording the quantity and rate of discharge of the wastewater and for determining its characteristics.*
- *Article 9: Samples and readings shall be taken by the owner at intervals stated in the permit to discharge, or as required by the Ministry. All data shall be recorded and submitted at the end of each month to the Ministry in an approved format.*
- *Article 10: Wastewater or sludge should not be discharged, except in an exceptional circumstance where no form of wastewater re-use is possible.*
- *Article 11: No wastewater or sludge shall be transported from the site point of its origin without any approval of the Ministry. Approval shall be subject to conditions that will include the obligation for all transport movements to be recorded in a manner defined in the approval.*
- *Article 12: The Ministry shall have the absolute right to inspect and/or monitor any wastewater treatment plant and to take samples of any wastewater, sludge or soil at any time and place.*
- *Article 13: These regulations shall not apply to discharges from septic tanks or to discharges of wastewater to the marine environment, or discharges of wastewater or sludge which contains radioactive materials, which are subject to separate legislations"*

Omani standards for treated wastewater

MD 145/93 under RD 115/2001 mentioned two types of TWW standards reuse for agricultural purposes of various chemical pollutants, these are standards for Class A and Class B as presented in Table 4.3, these standards detail the explanation of both classes on the areas where the TWW is applied as follows:

- Reuse for unrestricted and restricted agricultural irrigation.

- Artificial recharge of groundwater, and reuse anywhere accordance with MECA's approval. The above information is described in Table 4.4.

Table 4.3 Wastewater-maximum quality limits

Parameter	Units	Standard (Class A)	Standard (Class B)
Biochemical Oxygen. (5 days @ 20 0C)	mg/l	15	20
Chemical Oxygen Demand (COD)	mg/l	150	200
Suspended Solids	mg/l	15	30
Total Dissolved Solids (TDS)	mg/l	1500	2000
Electrical Conductivity (EC)	micro S/cm	2000	2700
Sodium Absorption Ratio (SAR)		10	10
pH		6-9	6-9
Aluminum	mg/l	5	5
Arsenic	mg/l	0.100	0.100
Barium	mg/l	1	2
Beryllium (Be)	mg/l	0.100	0.100
Boron (B)	mg/l	0.500	1
Cadmium (Cd)	mg/l	0.010	0.010
Chloride (Cl)	mg/l	650	650
Chromium (Cr)	mg/l	0.050	0.050
Cobalt (Co)	mg/l	0.050	0.050
Copper (Cu)	mg/l	0.500	1
Cyanide (Cn)	mg/l	0.050	0.100
Fluoride (F)	mg/l	1	2
Iron (Fe)	mg/l	1	5
Lead (Pb)	mg/l	0.100	0.200
Lithium (Li)	mg/l	0.070	0.070
Magnesium (Mg)	mg/l	150	150
Manganese (Mn)	mg/l	0.100	0.500
Mercury (Hg)	mg/l	0.001	0.001
Molybdenum (Mo)	mg/l	0.010	0.050
Nickel (Ni)	mg/l	0.100	0.100
Nitrogen: Ammoniacal (N)	mg/l	5	10
Nitrogen: Nitrate (NO3)	mg/l	50	50
Nitrogen: Organic (Kjeldhal) (N)	mg/l	5	10
Oil & Grease	mg/l	0.500	0.500
Phenols (Total)	mg/l	0.001	0.002

Phosphorus (P)	mg/l	30	30
Selenium (Se)	mg/l	0.020	0.020
Silver (Ag)	mg/l	0.010	0.010
Sodium (Na)	mg/l	200	300
Sulphate (SO4)	mg/l	400	400
Sulphide (S)	mg/l	0.100	0.100
Vanadium (V)	mg/l	0.100	0.100
Zinc (Zn)	mg/l	5	5
Fecal Coliform bacteria (per litre)	mg/l	200	1000
Viable Nematode Ova (per litre)	mg/l	<1	<1

Source: MRMWR (1993)

Table 4.4 Wastewater reuse areas of application of standards A and B

#	Specification	Unrestricted irrigation (Class A)	Restricted irrigation (Class B)
1	**Crops**	Vegetables likely to be eaten raw Fruit likely to be eaten raw and within 2 weeks of any irrigation	Vegetables to be cooked or Processed Fruit if no irrigation within 2weeks of cropping Fodder, cereal and seed crops
2	**Grass & Ornamental areas**	Public parks, hotel lawns recreational areas Areas with public access Lakes with public contact (except place which may be used for praying and hand washing)	Pastures Areas with no public access
3	**Aquifer Recharge**	All controlled aquifer recharge	
4	**Method of irrigation**	Spray or any other method of aerial irrigation not permitted in areas with public access unless with timing control	
5	**Any other re-use applications**	Subject to the approval of the Ministry	

Source: MRMWR (1993)

4.6 Selected international standards of treated wastewater and policies

An extensive range of standards and practices associated with the reuse of TWW for agricultural production and public health risks around the world are available. The standards and concentrations of TWW and sludge vary from country to county, therefore each country should adopt their own standards, but most countries adopted the international guidelines and standards to assure the safe use of these resources. In this regard, the World Health Organization (WHO) and US Environmental Protection Act (US-EPA) guidelines and standards are the most well-known (Hussain, 2009), the other guidelines like European guidelines emphasis mainly on management of TWW instead of representing their regulations or standard values and use TWW whenever suitable (Angelakis et al.,1999). Therefore, Omani national guidelines of treated wastewater will be compared to these standards to ensure that Omani national guidelines are providing the same level of protection provided by the WHO and US-EPA guidelines.

4.6.1 WHO guidelines

The World Health Organization (WHO) criteria, which were issued in 1989, are the most useful ones (Alhumoud et al., 2003; Margane & Steinel, 2011). WHO published the guidelines in 1989 as "*Guidelines for the Safe Use of Wastewater and Excreta in Agriculture and Aquaculture"*. These guidelines focus on the following aspects:

- Ensuring the effective safe use of wastewater and excreta in agriculture and aquaculture for those involved and working in this field.
- Emphasizing microbiological contents rather than other chemical pollutants as the former are much present in domestic wastewater;
- Addressing the practice of treated effluent reuse in agriculture conditions by selecting certain irrigation methods and methods for destruction of pathogens to protect human health, and
- finally, economic considerations to achieve the guidelines' aims.

The 1989 WHO guidelines were revised in 2006 as "*Guidelines for the safe use of wastewater, excreta and grey water in Agriculture and Aquaculture*"; the revised guidelines are helpful to all those are dealing with the secure use of wastewater, excreta, greywater, community health, water resources development and wastewater management issues. In Volume 2, the Guidelines deal with health-based targets of workers, their families and consumers and describe the measures to compute the risks which are associated with wastewater to meet these guidelines. In addition, the revised strategies of the 1989 WHO guidelines are concerned with the use of sewage wastewater in food production, still the guidelines of 2006 WHO for reuse of treated wastewater for agricultural purposes are adapted in Europe and through the world successfully (US-EPA, 2012).

The major features of the 1989 WHO guidelines

As mentioned above, the 1989 WHO guidelines focus on the following aspects:

- **Safe use of wastewater**

Farmers and their families who are involved in the reuse of wastewater for agricultural purposes, consumers of crops and its handlers have higher risks of infections. Therefore, appropriate methods for irrigation are needed to be used. Farmers should wear protective clothing, gloves and footwear, and consumers should cook vegetables and apply high levels of personal hygiene.

- **Microbiological contents in treated sewage wastewater**

The 1989 WHO guidelines described only the contents of *faecal* or total coliforms and intestinal nematodes eggs; given that these are the main significant indictors to ensure the appropriateness for reusing treated sewage effluent. Health risks depend on the presence of helminthes diseases which are associated with the present of *E.coli*. Thus the helminthes eggs (*Ascaris*, *Trichuris* species and hookworms) in the safe use of wastewater in agriculture or aquaculture should contain 1 or <1 per liter, keeping in mind that the other pathogens are difficult and expensive to be monitored for many developing countries (Al Salem, 2000). Table 4.5 illustrates pathogen contents in TWW by using stabilization ponds. Three categories of contents are follows:

A: direct contact between crops, water, staff, customers and community.

B: direct contact only between employees and water or soil.

C: no contact with the above-mentioned in Category B.

Table 4.5 WHO guidelines for using treated wastewater in agriculture [a]

Category	Reuse conditions	Intestinal nematodes[b] (arithmetic mean no. of eggs per liter)[c]	Fecal coliforms (geometric mean no. per 100 ml)[c]	Wastewater treatment expected to achieve the required microbiological guideline
A	Irrigation of crops likely to be eaten uncooked, sports fields, public parks [d]	≤1	≤1000	A series of stabilization ponds designed to achieve the microbiological quality indicated, or equivalent treatment
B	Irrigation of Cereal crops, industrial crops, fodder crops, pasture and trees [e]	≤1	No standard recommended	Retention in stabilization ponds for 8–10 days or equivalent helminthes and fecal coliform removal
C	Localized irrigation of crops in category B if exposure to workers	Not applicable	Not applicable	Pretreatment as required by the irrigation technology, but not less than primary sedimentation

a In specific cases, local epidemiological, socio-cultural and environmental factors should be taken into account and the guidelines modified accordingly.
b *Ascaris* and *Trichuris* species and hookworms.
c During the irrigation period.
d A more stringent guideline limit (200 fecal coliforms/100 ml) is appropriate for public lawns, such as hotel lawns, with which the public may come into direct contact.
e In the case of fruit trees, irrigation should cease two weeks before fruit is picked, and no fruit should be picked off the ground. Sprinkler irrigation should not be used.

Source: WHO (2006) adapted from WHO (1989)

- **Physico-chemical criteria in treated sewage wastewater**

Municipal wastewater consists mostly of water with a small load of suspended organic and inorganic solids. BOD concentration of wastewater acts as an indicator of the quality of wastewater. However, neither records for BOD and other parameters of 1989 WHO guidelines nor in 2006 guidelines as shown in Tables 4.6 and 4.7 respectively were available for re-use of treated wastewater for irrigation and food crops. The waste stabilization ponds with adequate retention time can eliminate BOD and pathogens to meet the WHO guidelines for unrestricted irrigation (Mara, 2003).

Table 4.6 Quality limits for reuse of treated wastewater in irrigation and food crops

Parameters	**WHO 1989**
Biological Oxygen demand (BOD)	No regulations
Chemical Oxygen demand (COD)	No regulations
Total suspended solids (TSS)	No regulations
Oil and grease	No regulations
pH	6-9
Chlorine residual	No regulations

Source: Hussain (2009)

The guidelines of the 1989 WHO highlight mainly on microbiological contents in the municipal treated wastewater and add that, the health hazards of chemical pollution is of only minor importance in the re-use of domestic waste (MED-EUWI, 2007). The revised guidelines of 2006 WHO mentioned the chemical qualities which are needed by crop production see Table 4.7.

Table 4.8 shows the maximum concentrations of heavy metal pollutants that may be introduced to the soil and stay in crops while irrigated by treated effluent, in case if the industrial effluents enter the municipal wastewater (WHO, 2006).

Table 4.7 Water quality for irrigation

Parameters		**Units**	**Degree of restriction on use**		
			None	Slight to moderate	Severe
Salinity EC_w[1]		dS/m	<0.7	0.7-3.0	>3.0
TDS		mg/l	<450	450-2000	>2000
TSS		mg/l	<50	50-100	>100
SAR[2] = 0 - 3 and EC_w		meq/l	>0.7 E_{Cw}	0.7-0.2	<0.2
	3-6	meq/l	>1.2	1.2-0.3	<0.3
	6-12	meq/l	>1.9 E_{Cw}	1.9-0.5	<0.5
	12-20	meq/l	>2.9 E_{Cw}	2.9-1.3	<1.3
	20-40	meq/l	>5.0	5.0-2.9	2.9 E_{Cw}
Sodium (Na^+)					
	Surface	meq/l	<3	3-9	>9
	Sprinkler	meq/l	<3	>3	
Chloride (Cl^-)					
	Surface	meq/l	<4	4-10	>10
	Sprinkler		<3	>3	
Chlorine (Cl_2) Total		mg/l	<1	1-5	>5
Bicarbonate (HCO_3)		mg/l	<90	90-500	>500
Boron (B)		mg/l	<0.7	0.7-3.0	>3.0
Hydrogen Sulfide (H_2S)		mg/l	<0.5	0.5-2.0	>2.0
Iron (Fe) drip irrigation		mg/l	<0.1	0.1-1.5	>1.5
Manganese (Mn) drip		mg/l	<0.1	0.1-1.5	>1.5
Total Nitrogen (TN)		mg/l	<5	5-30	>30
pH		Normal range 6.5-8			

1 ECw means electrical conductivity in decisiemens per meter at 25°C
2 SAR means sodium adsorption ratio ([meq/l] 0.5)

Source: WHO (2006) adapted from Ayers & Westcot (1985); Pescod (1992); Asano and Levine (1998)

Table 4.8 Threshold levels of trace elements for crop production 2006 WHO

Parameter	Recommended maximum concentration (mg/l)
Aluminum (Al)	5.0
Arsenic (As)	0.10
Beryllium (Be)	0.10
Cadmium (Cd)	0.01
Chromium (Cr)	0.10
Cobalt (Co)	0.05
Copper (Cu)	0.20
Fluoride (F)	1.0
Iron (Fe)	5.0
Lithium (Li)	2.5
Manganese (Mn)	0.20
Molybdenum (Mo)	0.01
Nickel (Ni)	0.20
Lead (Pb)	5.0
Selenium (Se)	0.02
Tin (Sn)	-
Titanium (Ti)	-
Tungsten (W)	-
Vanadium (V)	0.10
Zinc (Zn)	2.0

Source: WHO (2006) adapted from Ayers & Westcot (1985); Pescod (1992)

- **Methods of irrigation and pathogen destruction**

Using appropriate irrigation methods can reduce health risks. The 1989 WHO guidelines mentioned five irrigation methods which are flooding, furrows, sprinkler, trickle or drip and subsurface irrigation methods where each of these methods has advantages and disadvantages of reducing health risk. However, the revised 2006 WHO guidelines in Volume 2 recommend the use of drip irrigation method for irrigating unrestricted crops.

The information which is provided by the 1989 WHO guidelines indicate that waste stabilization ponds are the best method to remove pathogens, especially helminthes, as this method is very effective in removing helminthes eggs compared with other conventional methods such as primary, secondary and tertiary methods followed by disinfection. Moreover, this method is very simple, has a low cost and can be used in warm climates but it needs a large area.

- **Economic aspects**

The cost of treating wastewater techniques should be reasonable and meet microbiological standards, especially in developing countries. Therefore, the 1989 WHO guidelines recommended that stabilization ponds are the most suitable wastewater treatment system to remove pathogen loads, in addition to its low cost and effective performance to eliminate microbiological contents in wastewater.

Limitations of WHO's guidelines and regulations

Although the WHO guidelines are generally accepted all over the world, some countries have developed other criteria for agricultural wastewater reuse, which are more strict (Alhumoud et al., 2003). Countries such as Israel, South Africa, Japan and Australia have selected regulations like the ones which are adopted in California and do not agree with the 1989 WHO guidelines, as the latter's criteria are too relaxed for community health protection (Angelakis et al., 1999). According to Hussain (2009), the Jordanian national standards for TWW for irrigation of food crops for human consumption are more specific for most parameters than the WHO standards. Moreover, the WHO guidelines require a series of stabilization ponds to reduce the microbiological loads in TWW, although different treatment techniques of advanced biological, chemical and physical treatment are used now for removing biological pollutants and reducing organic matter such as BOD and suspended solids (SS). However, Angelakis et al. (1999) explained that there is no best approach to guidelines that have been implemented yet, although many countries apply the WHO criteria.

Based on what was mentioned above, the sections regarding sludge activities management in the Sultanate, Europe and US-EPA guidelines will be used for comparison with the Omani national guidelines.

4.6.2 US-EPA water reclamation and reuse standards

In 1992, the US-EPA with the United States Agency for International Development (USAID) published their guidelines as "*Guidelines for Water Reuse*".

In 2004 the old 1992 guidelines were revised and provide information about re-using TWW for different purposes, such as industrial and agricultural reuse. In addition, it considers the latest technologies for treating sewage water and presents new health aspects. The new 2004 guidelines cover the practices of water reuse in different countries and outside the U.S.A.

In 2012, the guidelines of 2004 was updated and shared the information that has been adopted in 2004, thus the regulations which have been established have been confirmed for protecting public health in spite of their inflexibility.

The 2012 guidelines emphasized the following aspects: "*reuse of water resources management, energy use with associated of water reuse, agricultural reuse, wetlands and stream augmentation, industrial water reuse, groundwater augmentation and aquifer recharge, greywater reuse system, practice of direct and indirect potable water reuse''* (US-EPA, 2012).

The most important features for assessing TWW for agricultural purpose are: health criteria, heavy metals standards values including chemical and biological parameters, the requirements of wastewater treatments, irrigation methods set back distances and economic aspects etc. The U.S guidelines of TTW focus on the following aspects:

- **Microbiological criteria in US-EPA guidelines**

The standards of 1992 US-EPA guidelines for health criteria are very stringent and considered no detectable *faecal* coliform bacteria, especially for crops which are eaten uncooked (Al Salim, 2000). Table 4.9 below illustrates the microbiological quality.

Table 4.9 Microbiological quality guidelines and criteria for irrigation 1992 US-EPA

Reuse conditions	**Intestinal nematodes**	**Faecal Coliforms**	**Wastewater treatment requirements**
Irrigation of pasture for milking animals, fodder, fiber and seed crops and landscape improvement	No standards recommended	200/100 ml [a]	Secondary treatment followed by disinfection.
Surface or spray irrigation of any food crop including crops eaten raw	No standards	Not detectable [b]	Secondary treatment followed by filtration (with prior coagulant and or polymer addition and disinfection)

a The number of faecal coliform should not exceed 800/100 ml
b The number of faecal coliform should not exceed 14/100 ml

Source: Angelakis.et al. (1999)

However, the new guidelines of US-EPA (2012) for eliminating or pathogen reduction from wastewater, mentioned the analytic range of "*microbial log reduction*" via several types of wastewater treatments see Appendix 2.1.

- **Physico-chemical criteria in USEPA guidelines**

The specifications for utilizing treated wastewater, including physical, chemical, setback distances and heavy metal parameters are shown in Tables 4.10, 4.11 4.12 and 4.13 respectively, these information are adopted from guidelines of 2004 and 2012.

Table 4.10 US-EPA/USAID guidelines for agricultural reuse of wastewater. Report No EPA-625/R-92-004[1]

Types of Reuse	**Treatment**	**Reclaimed Water Quality**	**TWW Monitoring and set back distances**
Urban Reuse **All types of landscape irrigation (e.g. golf courses, parks, Cemeteries)**	• Secondary [2] • Filtration • Disinfection	pH = 6-9 BOD ≤10 mg/l NTU ≤2 No detectable FC/100 ml [3] Cl_2 residual (min.) =1 mg/l	Coliform – daily pH-weekly BOD – weekly Turbidity- continuous Cl_2 residual continuous
Agricultural Reuse -Food Crops Not Commercially Processed **Surface or spray irrigation of any food crop, including crops eaten raw**	• Secondary [2] • Filtration • Disinfection	pH = 6-9 BOD ≤10 mg/l NTU ≤2 No detectable FC/100 ml [3] Cl_2residual (min.) =1 mg/l	pH- weekly BOD – weekly Turbidity -continuous Coliform – daily Cl_2 residual continuous Setback distance=50 ft from potable water supply wells
Agricultural Reuse –Food Crops Commercially Processed	• Secondary [2] • Disinfection	pH = 6-9 BOD ≤30 mg/l SS ≤30 mg/l ≤200/100 FC ml[4] Cl_2 residual (min) = 1 mg/l	pH- weekly BOD – weekly Coliform – daily Cl_2 residual continuous Setback distance=300 ft from potable water supply wells
Agricultural Reuse –Non Food Crops Pasture for milking animals; fodder, fiber	• Secondary [2] • Disinfection	pH = 6-9 BOD ≤ 30 mg/l SS ≤ 30 mg/l ≤ 200/100 FC ml[4] Cl_2 residual (min) = 1 mg/l	pH- weekly BOD – weekly Coliform – daily Cl_2 residual continuous

1 Secondary treatment processes include activated sludge processes, trickling filters, rotating biological contractors, and many stabilization pond systems. Secondary treatment should produce effluent in which both the BOD and SS do not exceed 30 mg/l.
2 The number of fecal coliform organisms should not exceed 14/100 ml in any sample.
3 The number of fecal coliform organisms should not exceed 800/100 ml in any sample. Some stabilization pond systems may be able to meet this coliform limit without disinfection.

Source: US-EPA (1992), Report No. EPA-625/R-92-004

Table 4.11 US-EPA guidelines of appropriate setback distances

Types of Reuse	Setback Distances
Food crops commercially processed	300 ft from potable water supply wells
Orchards and Vineyards	100 ft from areas accessible to public
Pasturage	300 ft from potable water supply wells
Pasture for livestock	100 ft from areas accessible to public
Forestation	300 ft from potable water supply wells
Food crops not commercially processed	50 ft from potable water supply wells
Groundwater Recharge	Site-specific

Source: (US-EPA, 1992), Report No. EPA-625/R-92-004

Table 4.12 US-EPA guidelines for interpretation of water quality for irrigation

Parameters		Units	Degree of restriction on use		
			None	Slight to	Sever
Salinity (affects crop water availability)[2]					
Salinity EC_w[1]		dS/m	<0.7	0.7-3.0	>3.0
TDS		mg/l	<450	450-2000	>2000
Infiltration (affects infiltration rate of water into the soil, evaluating using EC_w and SAR together)[3]					
		EC_w	>0.7 E_{Cw}	0.7-0.2	<0.2
SAR	**3-6**		>1.2	1.2-0.3	<0.3
	6-12		>1.9 E_{Cw}	1.9-0.5	<0.5
	12-20		>2.9 E_{Cw}	2.9-1.3	<1.3
	20-40		>5.0	5.0-2.9	2.9 EC_w
Specific Ion Toxicity (affects sensitive crops)					
Sodium (Na)[4]	**Surface**	SAR	<3	3-9	>9
	Sprinkler	meq/l	<3	>3	
Chloride (Cl)[4]					
	Surface	meq/l	<4	4-10	>10
	Sprinkler	meq/l	<3	>3	
Boron (B)		mg/l	<0.7	0.7-3.0	>3.0
Miscellaneous Effects (affects susceptible crops)					
Nitrate (NO3-N)		mg/l	<5	5-30	>30
Bicarbonate (HCO_3)		mg/l	<01.5	1.5-8.5	>8.5
pH		Normal range 6.5-8			

1 EC_w means electrical conductivity in deci Siemens per meter at 25°C
2 SAR means sodium adsorption ration ratio, at a given SAR, infiltration rate increases as water salinity increases.
3 For surface irrigation, most tree crops and woody plants are sensitive to sodium and chloride; most annual crops are not sensitive.
4 With overhead sprinkler irrigation and low humidity (< 30%), sodium and chloride may be absorbed through the leaves of sensitive crops.

Source: US-EPA (2012) adapted from FAO (1985)

Table 4.13 US-EPA[a] heavy metals standards for wastewater reuse for irrigation

Parameters	Long term	Short- term [b]
Aluminum (Al)	5.0	20.0
Arsenic (As)	0.1	2.0
Boron (B)	0.75	2.0
Cadmium (Cd)	0.01	0.05
Cobalt (Co)	0.05	5.0
Chromium (Cr)	0.1	1.0
Copper (Cu)	0.2	5.0
Lithium (Li)	0.075	0.075
Molybdenum (Mo)	0.01	0.05
Nickel (Ni)	0.2	2.0
Lead (Pb)	5.0	0.075
Vanadium (V)	0.1	1.0
Zinc (Zn)	2.0	10.0
Selenium (Se)	0.02	0.02
Fluoride (F)	1.0	15.0
TDS	500 - 2,000 mg/l	

a Maximum permissible concentration in mg/l use on all soils
b Less than 20 years of continuous cropping are recommended for fine-textured neutral and alkaline soils

Source: US-EPA (2012) adapted from US-EPA (2004)

4.7 Sludge management in the Sultanate of Oman

Sludge derives mainly from the wastewater treatment process and its generation depends on the procedure operation of the plant and its type. In Oman, most of wastewater treatment plants use an activated sludge process to treat sewage wastewater, whereas secondary and tertiary treatments are adopted in the other Gulf countries for irrigation purposes (Al Enezi et al., 2004). However, HW uses tertiary treatment and some STPs which belong to the company use the Sequence Batch Reactor (SBR) process and Ultra Filter (UF) Membrane systems as well as Membrane Bioreactors (MBRs).

This section will focus only on sewage sludge that is generated from the treatment process of municipal wastewater and used for irrigation purposes according to MD 145/93.

4.7.1 National sludge guidelines and standards

The main national guidelines of MD 145/93 which are related to sludge management are defined in articles 3, 6, 7, 9, 11 and 12 as mentioned in Section 4.6.3.2. The national guidelines in MD 145/93 details all the heavy metal limits in municipal sludge that can be re-used for agricultural purposes according to Table 4.14.

Table 4.14 Reuse of sludge in agriculture: conditions for application to land

Metal	Maximum concentration (mg/kg dry solids)	Maximum application rate (kg/ha/year)	Maximum permitted concentration in soil (mg/kg dry
Cadmium	20	0.15	3
Chromium	1000	10.0	400
Copper	1000	10.0	150
Lead	1000	15.0	30
Mercury	10	0.1	1
Molybdenum	20	0.1	3
Nickel;	300	3.0	75
Selenium	50	0.15	5
Zinc	3000	15.0	300
After spreading of sludge, there must be a minimum period of three weeks before grazing or harvesting of forage crops.			
Sludge use is prohibited: - On soils whilst fruit or vegetables crops, other than fruit trees, are growing or being harvested. - For six months preceding the harvesting of fruit or vegetables which grow in contact with the soil and which are normally eaten raw. - on soils with a pH<7.0.			

Source: MRMWR (1993)

4.8 International guidelines for sludge application to agriculture

To ensure that Omani guidelines meet the international standards of sludge application to lands, two international guidelines were chosen for comparison purposes: the US Environmental Protection Agency (US-EPA) and the European Union guidelines.

4.8.1 US-EPA guidelines

In 1993, US-EPA established comprehensive federal standards and regulations of disposal of sewage sludge (part 503). These regulations are adopted in many countries although some limitations were found in it. Therefore each state of U.S.A suggested the option to use the federal standards or adopt its own standards (Harrison et al., 1999).

The 1993 EPA (part 503) regulations were reviewed, and there were suggestions to modify regulations of some pollutants like dioxin and dioxin like compounds when disposed by incineration or land application, furthermore the analysis of 9 heavy metals and Molybdenum was required Anon (2011). All the modifications will be completed in the late of 2015 US-EPA (2015), although no modified document is currently available in the public domain. The regulations of sewage sludge are revised every 2 years to safe human health and the environment.

The national guidelines for sludge management is compared to the federal standards of 1993 US-EPA (part 503) guidelines regarding the reuse and disposal of sewage sludge, as the sludge management in the sultanate use the method of windrow technology of 1993 US guidelines, and the sewage composted sludge meet with the criteria of Class A in the microbiological reduction aspect (AlSAFA, 2009). The federal standards of 1993 US-EPA (part 503) for the reuse and disposal of solids cover the following aspects:

- **Land application**

Table 4.15 shows the concentrations of biosolids (sludge) application to the land at agronomic rates (rates that provide the amount of nitrogen that is needed by crops) with respect to heavy metals concentrations. This includes bulk and bagged sludge, the former is not sold and can be applied to lawns and home gardens whereas the bagged one is sold or given in bags or other containers and can be applied to the lands. The bulk biosolids meet the limits of high quality biosolids pollutant concentrations, and the bagged bio-solids meet the limits of ceiling concentrations that should be applied to the land at annual pollutant loading rates (OWSC, 2005).

Table 4.15 The concentrations of biosolids (sludge) applications to the lands

Pollutant	**Ceiling concentration limits (mg/kg)**[a]	**Cumulative pollutant loading rates (kg/ha)**	**High quality biosolids pollutant concentration limits (mg/kg)**[b]	**Annual pollutant loading rates (kg/ha/annum)**
Arsenic	75	41	41	2.0
Cadmium	85	39	39	1.9
Copper	4300	1500	1500	75.0
Lead	840	300	300	15
Mercury	57	17	17	0.85
Molybdenum	75	-	-	-
Nickel	420	420	420	21.0
Selenium	100	100	36	5.0
Zinc	7500	2800	2800	140.0
Applies to:	All biosolids that are land applied	Bulk biosolids	Bulk biosolids and bagged biosoilds	Bagged biosolids

a: Absolute values   b: Monthly average

Source: Inglezakis et al. (2014) adapted from US-EPA (1993) part 503

- **Presence of pathogens**

The above regulations also issued two levels of biosolids quality with respect to pathogen numbers; these include class A and class B. Class A biosolids must undergo more extensive treatment than Class B biosolids to reduce pathogens. Biosolids in Class A should be treated in a way to eliminate pathogens, and Class B should be treated to reduce pathogens (Harrison et al., 1999). Class A can be applied in gardens, nurseries and golf courses whereas biosolids in class B cannot be sold or given away for land application at public-contact sites, because it still has potential to transmit dieses (Harrison et al., 1999; OWSC, 2005), instead, these are used for application to forests, and mine reclamation sites. Table 4.16 below gives the pathogen destruction methods.

Table 4.16 Pathogen reduction alternatives in class A and B

Class A
Class A biosolids should meet one of the following conditions at the time of use or disposal: (1) fecal coliform density of less than 1000 MPN/ 1g total dry solids **or** (2) salmonella density of less than 3 MPN per 4g of total dry solids The reduction of the above-mentioned pathogens must meet one of the alternatives: Alternative 1: thermal treatment of biosolids Alternative 2: alkaline treatment: when pH <12 when is reached in 72 hours, the biosolids should be air dried to at least 50% of the total solids and the temperature of biosolids should be greater than 52^{o} C for at least 12 hours. Alternative 3: if wastewater sludge is analyzed before pathogen reduction, and it is found that the biosolids contain viruses or helminthes <1 PFU/4 g total solids **or** >1 PFU/4 g total solids then these biosolids are considered as Class A. Alternative 4: if wastewater sludge is not analyzed for the presence of viruses or helminthes before the pathogen reduction, then the densities of viruses or helminthes must be <1 PFU per 4 g total solids, then this type of biosolids may be sold or put in a bag to be applied to the land.
Class B
Class B biosolids should meet the following pathogen requirements: at least 7 samples of biosolids should be collected at the time of use and disposal, for analyzing the presence of feacal coliforms. Theses pathogens should be <2 X 10^{6} MPN per 1g of total solids or <2 X 10^{6} colony forming units per 1 g of total solids.

Source: OWSC (2005) adapted from US-EPA (1993) part 503

- **Surface disposal**

For surface disposal, no pollutant concentration limits are required if the biosolids are used or disposed at lined sites, because pollutants will be collected in the leachate and are treated to avoid being close to the surface disposal sites. Table 4.17 below illustrates the concentrations of the three heavy metals that vary from the distance of surface disposal sites at non lined sites.

Table 4.17 Maximum allowable pollutant concentrations in wastewater sludge for disposal in active landfills without a liner and leachate collection system

Distance from the boundary of active bio-solids unit to surface disposal site property line (meters)	**Pollutant Concentration**		
	Arsenic (mg/Kg)	**Chromium (mg/Kg)**	**Nickel (mg/Kg)**
0 to less than 25	30	200	210
25 to less than 50	34	220	240
50 to less than 75	39	260	270
75 to less than 100	46	300	320
100 to less than 125	53	360	390
125 to less than 150	62	450	420
Equal to or greater than 150	73	600	420

Source: OWSC (2005) adapted from US-EPA (1993) part 503

4.8.2 European guidelines

On 12th June 1986 the European Union issued directive number EU 86/278/EEC on the use of sewage sludge in agricultural land. The EU community on waste legislation in 2009 reported that no complains or problems have been reported since the declaration of the directive, but it has proposed some new standards values for heavy metals in sludge Anon (2016). No amendments of the directive at present have been planned European Union (2002), the directive still in use (OWSC, 2005; Inglezakis et al., 2014; Zambrzycki, 2014) although some recommendations were considered to adopt more strict measures (Inglezakis et al., 2014). EU 86/278/EEC was declared to protect the environment, animals and humans when sewage sludge is subjected to be applied on the agricultural land. The guidelines aim to achieve the following goals:

- Waste prevention: by setting regulations to control the use of sewage sludge in agriculture, for preventing any adverse impacts to humans, animals and the environment.

- Controlling sludge disposal activities: this should protect soils from heavy metal contamination and prevent any risk of reaching groundwater.
- Improving the treatment of sludge before use in agriculture.
- Regulation of sludge transport, handling and storage.

The above directive guidelines specified the level of heavy metals for the application of sludge to agriculture lands and set the maximum annual rates of these metals that can be applied in soil. Heavy metals such as copper (Cu), zinc (Zn), cadmium (Cd), lead (Pb), nickel (Ni), chromium (Cr) and mercury (Hg) are major elements limiting the employment of sludge in agricultural usage (Fytili and Zabaniotou, 2008). If their levels exceed the maximum allowed levels, they produce toxic effects on human health and to the environment through their availability in the food-chain. Table 4.18 reports EU recommended heavy metals standards for the application of sludge to agriculture.

Table 4.18 1986 European standards for the application of sludge to agriculture

Element	**A**	**B**	**C**
	Limit values of amount of heavy metals which maybe added annually to agriculture land, based on 10-years average (Kg/ha/year)	**Limit values of heavy metals concentrations in sludge for use in agriculture (mg/kg of dry matter)**	**Limit values for concentrations of heavy metals in soil (mg/kg of dry matter in a representative sample of soil with a pH of 6 to 7)**
Cadmium	0.15	20 to 40	1 to3
Chromium	-	-	-
Copper	12	1000 to 1750	50 to 140
Lead	15	750 to 1200	50 to 300
Mercury	0.1	16 to 25	1 to 1.5
Nickel	3	300 to 400	30 to 75
Zinc	30	2500 to 4000	150 to 300

Source: Inglezakis et al. (2014) adapted from EU 86/278/EEC

European regulations also place final levels of heavy metals in sludge of different values of pH in soils as shown in Table 4.19.

Table 4.19 European Limits on application of sludge on soil (mg/kg)

Element	Limit according to pH of soil			
	5.0<5.5	**5.5<6.0**	**6.0<7.0**	**>7**
Cadmium	3	3	3	3
Chromium	-	-	-	-
Copper	80	100	135	200
Lead	300	300	300	300
Mercury	1	1	1	1
Molybdenum	-	-	-	-
Nickel	50	60	75	110
Selenium	-	-	-	-
Zinc	200	250	300	450

Source: Inglezakis et al. (2014) adapted from EU 86/278/EEC

According to the above standards in Table 4.19, EU legislations oblige the producers of sewage sludge to adhere to the following guidelines:

- Monitoring of sludge and reporting all the details, which include analysis of heavy metals every six months, providing concentrations of nitrogen, phosphorus, pH, organic matter and percentage of dry matter to the users, and informing clients about any changes that occur in the characteristics of treated wastewater techniques.
- EU guidelines of sludge usage should be banned for use in:

  - Harvesting of forage crops and grazes grassland for a period not less than 27 days.

  - Land in which fruit are growing as well as vegetable crops, excluding fruit trees.

  - Land for farming fruits and vegetables which are eaten raw for a period of 10 months during crop harvesting.

4.9 Comparisons between national and international guidelines

Based on the previous sections, this section comprises the summary comparison between national and international guidelines concerning TWW reuse and sludge applications for agricultural purposes.

4.9.1 Comparison between national and international guidelines in terms of TWW reuse for agricultural purposes

Comparison between the national and WHO guidelines

- Microbiological quality in national standards in Table 4.3 meet WHO standards in terms of the number of nematode ovae eggs which is shown in Table 4.5 whereas faecal coliforms bacteria in WHO standards is specified for crops likely to be eaten uncooked or in sports fields at ≤1000 FC/100 ml and for public parks at <200 FC/100 ml compared to 200 FC/100 ml for unrestricted irrigation applications for class A in national standards as shown in Table 4.4.
- No standards are recommended for faecal coliforms in the WHO guidelines for cereal crops, industrial crops, fodder crops, pasture and trees compared to the national standards that sets them at 1000 FC/100 ml for the same irrigation applications. It reveals that the national standards in terms of faecal coliforms are specified in the Table 4.4 for both categories A and B than WHO standards.
- National Omani limits for BOD, COD and organic contents (oil, grease, and phenols) are more precise than WHO guidelines, as no records are mentioned for the latter.
- Most heavy metals in national standards are close to WHO standards. However, these three elements are not mentioned in the national standards: Tin (Sn), Titanium (Ti) and Tungsten (W).

Generally as shown in Table 4.20 below, the Omani national standards are consistent with WHO standards in terms of health aspects. However, these are defined for the majority of the other organic and inorganic elements and are more precise than WHO standards; these remarks are in line with Hussain (2009) who states that the Jordanian standard values are more accurate for the majority of parameters, whereas concentrations of BOD, COD and TSS are not mentioned in the WHO guidelines.

Comparison between the national and US-EPA guidelines

- The value number of nematode ova eggs in the national guidelines for both categories A and B in Table 4.3 are specified at <1 nematode ova / l L compared to US-EPA guidelines as shown in Table 4.9 where no standards have been recommended for this type of pathogen for any type of re-use irrigation conditions.
- There is no mention of faecal coliforms in US-EPA guidelines for irrigation of crops likely to be eaten uncooked compared to the national standards of 200 FC/100 ml and 1000 FC/100 ml for unrestricted and restricted irrigation applications in class A and B respectively as shown in Table 4.3.
- The limit of BOD for standards A in Omani guidelines as shown in Table 4.3 is higher than the US-EPA guidelines for all types of landscape areas (e.g. golf courses, parks and for crops that are eaten raw) but lower than the values of US-EPA guidelines in the all types of restricted irrigation as shown in Table 4.10.
- The concentrations of SS in class B in the national guidelines in the Table 4.3 are consistent with the international standards in Table 4.10 in terms of agricultural re-use for commercial food crops and nonfood crops e.g. pasture, fodder, etc.
- There are no records mentioned for turbidity and chlorine residual limits in national guidelines.
- Most heavy metals in the national standards are close to the US-EPA standards.
- The national guidelines in terms of aquifer recharge use either classes A or B as shown in Table 4.3 whereas the US-EPA guidelines meet drinking water standards for direct injection into potable aquifers (US-EPA, 2012).

To sum up, the national standards in Table 4.20 below on the theme of microbiological contents and BOD concentrations are relaxed compared with the US-EPA guidelines. Furthermore, most heavy metals meet the US-EPA guidelines. Thus, this reveals that the

US-EPA guidelines are stricter than the national guidelines in terms of microbiological quality.

Table 4.20 Comparison summary between national and international guidelines in terms of TWW reuse for agricultural purposes

Health risk criteria			
Details	**Omani guidelines**	**WHO guidelines**	**USEPA guidelines**
Nematode Ovae eggs	<1 / liter (un-restricted and restricted irrigation applications)	≤1/liter (un-restricted and restricted irrigation applications)	No standards (un-restricted and restricted irrigation applications)
Faecal Coliforms	200/100 ml for un-restricted irrigation applications 1000/100 ml for restricted irrigation applications	≤1000/100 ml for un-restricted irrigation applications No standards for restricted irrigation applications	No detectable for un-restricted irrigation applications 200/100 ml for restricted irrigation applications
Agriculture criteria in terms of chemical and trace elements and irrigation methods			
Details	**Omani guidelines**	**WHO guidelines**	**US-EPA guidelines**
BOD mg/l	15-20 (un-restricted and restricted irrigation applications respectively)	No standards	10 mg/l (un-restricted irrigation) 30 mg/l (restricted irrigation applications)
COD mg/l	150-200 (un-restricted and restricted irrigation applications respectively	No standards	No standards
TSS mg/l	15-30 (un-restricted and restricted irrigation applications respectively	No standards	No standards for unrestricted irrigation 30 for restricted irrigation applications

Agriculture criteria in terms of chemical and trace elements and irrigation methods			
Details	**Omani guidelines 145/93**	**WHO guidelines**	**US-EPA guidelines**
Oil and Grease	0.500 (un-restricted and restricted applications irrigation)	No standards	No standards
Trace elements	No Tin, Titanium, Tungsten are mentioned compared to WHO guidelines	Close to national guidelines	Close national guidelines
Treatment processes	Not mentioned	Series of stabilization ponds (of restricted and unrestricted reuse conditions) and pretreatment as required but not less than sedimentation.	Secondary, filtration and disinfection for urban reuse and for not commercially food crops. Secondary and disinfection for commercially food crops and nonfood crops.
Method of irrigation	Spray or aerial irrigation	Flooding, furrows, sprinkler, drip (1989 WHO) drip irrigation only (2006 WHO)	Surface or sprinkler irrigation
Aquifer Criteria			
	Omani guidelines	**WHO guidelines**	**US-EPA guidelines**
Aquifer criteria	Uses either class A or B standards	Not mentioned	Meet drinking water standards

4.9.2 Comparison between the national and international guidelines in terms of sludge applications for agricultural purposes

Comparison between the national and US-EPA guidelines

- Omani standards for heavy metals of sludge application in Table 4.14 are stricter than the US-EPA standards which are mentioned in Table 4.15.
- Table 4.14 of MD 145/93 deals only with heavy metal concentrations in sludge re-use and does not account for any presence of pathogen contents. However, according to the concession agreement between HW and the government, the former reported that if sludge is recycled, then pathogen contents have to be consistent with class A of 1993 US-EPA requirements (AlSAFA,2009).
- No surface disposal site distances of sludge application (unlined sites) are determined in the national guidelines.
- No handling precautions of sludge transportation are mentioned in the Omani guidelines, although these issues are found to be a very essential part of sludge management.

Comparison between the national and European guidelines

- Omani regulations of heavy metals for reusing sludge in agricultural conditions are close to European standards.
- According to Table 4.19 of European standards, MD 145/93 does not state how the rates of sludge can be applied on soils with different values of pH. However, it prohibits the applications only when soils have a pH <7.0.
- National control applications for prohibiting sludge on lands are equivalent to EU controls.

To sum up the regulations of sludge reuses and its applications on the lands for agricultural purposes, Table 4.21 illustrates the comparison summary of national and international guidelines.

Table 4.21 Comparison summary between national and international guidelines in terms of sludge reuses and its applications for agricultural purposes

Land application with respect to heavy metal concentrations			
Details	**Omani guidelines**	**USEPA guidelines**	**European guidelines**
Heavy metals concentrations	Stricter than USEPA and close to European guidelines	National guidelines are more strict	Close to Omani guidelines
Levels of Heavy metals at different values of soil pH	Only if pH of soil is <7.0	Not mentioned	Mentioned for pH of soil is 5.0 <5.5, 5.5 <6.0, 6.0 <7.0 and >0.7
Health risk in terms of Pathogen destruction			
Details	**Omani guidelines**	**USEPA guidelines**	**European guidelines**
Pathogen concentrations	Not mentioned	Class A: Fecal Coliform <1000 (MPN)/ 1g of total dry solids or Salmonell <3 (MPN)/ 4g of total dry solids Class B: Fecal Coliform $<2 \times 10^6$ MPN per 1g of total solids or $<2 \times 10^6$ colony forming units per 1 g of total solids.	Not mentioned
Surface disposal			
Details	**Omani guidelines**	**USEPA guidelines**	**European guidelines**
Concentrations of heavy metals and the distance of sludge application to the sites	Not mentioned	Mentioned only for Arsenic, Chromium and Nickel.	Not mentioned

The RD 115/2001 and MD 145/93 framework of national guidelines are aimed to provide elevated safety standards for health and social benefits; these protect the land and water resources against any sort of contamination from treated wastewater and sludge reuse. On the other hand, these are too short and do not cover all possibilities (Mott Macdonald, 1999). In addition, these are general and lack legislation when compared to international guidelines. Furthermore, the overlapping of duties and tasks between many governmental organizations does not satisfy the assessment and components of such strategies.

4.10 Limitations in the national guidelines

Based on the comparison between international and existing national guidelines which were mentioned in Section 4.10, this section will outline the limitations and gaps of the national policy in terms of health risk, agricultural usage, aquifers recharge and their distances in terms of reuse of wastewater, as well as handling, techniques and transporting in terms of sludge management, and suggest solutions from the mentioned international guidelines of WHO,US-EPA and European and other international regulations such as FAO.

4.10.1 In terms of treated wastewater management

Health risks

- MD 145/93 addresses the quality limits of only two types of pathogens: *faecal* coliforms and intestinal nematodes. HMR (2006) reported that the risk of disease transmission from consumption of low growing crops, such as green vegetables, could be increased within six weeks. However, the WHO (1989) guidelines mentioned the number of biological pathogens and their survival times as mentioned in Appendix 2.2.
- MD 145/93 does not comprise any detailed text about the elimination of pathogens for treating sewage wastewater; it provides only treated wastewater standards. However, private sector companies such as HW use tertiary treatment with MBR or SBR systems to eliminate the load of pathogens. On the contrary, the secondary treatment method which is recommended by the 2004 US-EPA

guidelines is not very efficient to eliminate pathogens (Marganea nd Steinel, 2011), nor the stabilization ponds which are recommended by WHO are useful, as the latter guidelines are used at different places without knowing the ill effects (Mott Macdonald,1999), but may guidelines of pathogens removal by several methods which is mentioned by US-EPA (2012) should be under considerations for national guidelines as shown in Appendix 2.1.

- Only numerical values of all physico-chemical parameters and heavy metal concentrations of treated effluent are given in the national guidelines without mentioning the procedures of sampling and analyzing these parameters.

Agriculture usage

- No examples are mentioned in the national legislation for vegetables and fruit that are eaten cooked or raw and their suitable irrigation techniques. However, FAO (1992) publications mentioned some selected crops which can be irrigated by TWW and the irrigation methods for these crops as mentioned in Appendix 2.3 and Appendix 2.4 respectively.
- There is no variation for parameters in national standards for those which can be reused for agricultural purposes for both standards A and B, or which can be recharged to groundwater aquifers or could be reused for any purposes like wetlands and standards for grey or black water, this is in line with Prathapar et al. (2004) that national standards of TWW reuse do not differentiate between black and greywater.
- There are no details in the national guidelines regarding the possible usage of treated wastewater for industrial, oil production water purposes or other applications. This conclusion is consistent with Al Muselhi (2011), that the national regulatory structure of the Sultanate presents strategies and standards for all kinds of irrigation applications without addressing other usage.
- MD 145/93 does not involve standards or textures for soils to cultivate certain types of crops which can be grown by reclaimed wastewater. In fact, the physical characteristics of soil quality and its classifications are important aspects when

assessing and utilizing treated wastewater for irrigation purposes (Saskatchewan Environment, 2004).

Aquifer recharge

- The current national legislation for TWW has no such standards for recharging freshwater aquifers (Al Wahaibi, 2011). In fact, MD 145/93 allows using either class A or B of treated wastewater for recharging all controlled aquifers. However, Mott Macdonald (1999) recommended US-EPA guidelines to use drinking water standards for injecting potable water into the aquifers.
- There are no detailed explanations of the performance and tecniques to recharge aquifers by TWW.

4.10.2 In terms of sludge application reuse management

Health risk

- The type of pathogens such as bacteria, viruses, protozoa, helminthes and their destruction methods are not presented in MD 145/93 guidelines, although these pathogens can cause risk to human health and to flora and fauna see (http://www.fao.org/documents).

Agricultural usage

- MD 145/93 framework does not specify nutrient concentrations in sewage sludge such as N, P and K in which sludge can play an important role for providing nutrient characteristics when spreading on land, as these elements are important to the agricultural market and are main concerns in the economy of farmers (Al Salim, 2000). Furthermore, the excess amount of these nutrients can also be a source of groundwater pollution.
- MD 145/93 does not state how the rates of sludge can be applied on soils with different values of pH. However, it prohibits the applications only when soils have pH <7.0, as the pH of most soils in the Sultanate goes beyond 7.0 (HMR, 2006).

Distance to freshwater aquifers

- MD 145/93 guidelines do not determine the geological and hydro-geological aspects and the distances from sludge lands to active residential areas and groundwater resources, as the spreading of sludge to the land for larger communities may sometimes pollute groundwater if the place is close to wadis or other groundwater sources. As a result, there may be health problems posed to people and animals which feed on the vegetation from the applied sludge. This can be supported by Margane and Steinel (2011) that the activities which can negatively affect groundwater, such as reuse of treated wastewater or sludge application should not be permitted in neighborhoods near fresh water aquifers.

Sewage sludge techniques

- No handling of sewage sludge is mentioned in the national guidelines of MD 145/93. This can be the most important aspect in wastewater management (Fytili and Zabaniotou, 2008).
- Storage techniques of sewage sludge are not mentioned in both MD 145/93 and RD 115/2001, to ensure minimizing odor concentrations. Therefore, Burea Veritas (2000) mentioned that holding tanks with sludge storage neighboring sensitive surroundings such as groundwater aquifers should be situated on hard surfaces to avoid leakages and outflows.
- MD 145/93 does not cover appropriate methods of sludge treatment and control which is suitable to Omani conditions, FAO describes the effective sludge treatment process to reduce health risk, these include: "*Sludge Pasteurization, Mesophilic Anaerobic Design, Thermophilic Aerobic Design, Composting (Windrows or Aerated Piles), Lime Stabilization of liquid Sludge, Liquid Storage and dewatering and storage*" which are illustrated in Appendix 2.5. As discussed previously in section 4.3.2 HW uses composting windrows according to the criteria of 1993 US-EPA guidelines, as this process is much more suitable to Omani environments, and is much cheaper and more effective in dry and relatively hot climates.

4.11 Suggestions for developing national guidelines

To improve the acceptability and adequacy of the national framework, reflection from skilled employees is needed to supply decision-makers with sufficient information about the complexities of implementing the national strategies, as well as suggestions regarding all possibilities to overcome difficulties. This section will bring up a number of suggestions which should be considered in case if the MD 145/93 policy should be amended:

- As MD 145/93 belongs to RD 115/2001 and the latter focuses only on the "*legislative frameworks for safe management of sewage wastewater rather than wastewater as a whole"*, the title of MD 145/93 should be changed to regulations for sewage wastewater reuse and discharge, to distinguish between the regulations for treated sewage wastewater and other fields such as, industrial wastewater, greywater and oil field wastewater.
- Penalties and offences should be covered in MD 145/93.
- Public health risk assessment should be specified and regulated. This can be in coordination with the Ministry of Health (MOH).
- All techniques and technologies for treating wastewater and sludge should be reported.

4.11.1 Suggestions to be included in MD 145/93 associated to reuse of treated sewage water management

- The guidelines of reuse of treated greywater, industrial water, re-use for wetland practices and other activities should be integrated and exist individually within the national frame-work of treated wastewater and sludge re-use, rather than outlining regulations of non-household use as mentioned in RD 115/2001, which can be considered as guidelines for all applications (except for irrigation purposes).

- It is necessary to detail all pathogenic vectors present in wastewater, and their quality standards, with destruction methods given to realize the microbiological risk hazards for crops which are irrigated by reclaimed wastewater.
- All types of vegetables and fruits which are irrigated by treated sewage effluent and their suitable irrigation technique should be mentioned.
- As Article 6 in MD 145/93 standards agrees to "exclude the general law of RD115/2001", it is therefore suggested to follow international US-EPA guidelines to inject potable aquifers with drinking water standards to ensure there is no significant effect on people's health. This is consistent with Mott Macdonald (1999) and Zekri et al. (2014) that the standard values for recharging the Sultanate managed aquifers should meet drinking water values.
- There should be a distinction between acceptable concentration, in national standards of chemical parameters for reusing treated wastewater for agricultural application and those for re-use in other applications like aquifers recharging, wetlands etc.
- Additional regulations are required to specify the allowable depth of the water level for injecting TSE into freshwater aquifers for recharging purposes.
- MD 145/93 should state the suitable treatment of sewage wastewater to recharge potable aquifers with drinking water standards. RO is an optional technique for recharging purposes as it can act as a quaternary treatment (Zekri et al., 2014).
- Antibiotic resistance bacteria should be considered guidelined in MD 154/93

4.11.2 Suggestions to be included in MD 145/93 associated to sewage sludge management

- It is suggested that the following measures should be taken and reported in the article no 11 of MD145/93:

- Supply all workers with protective clothing (overalls, gloves, boots, hats and eye protection, as required for individual tasks).
- Provide first aid kits in vehicles and on site.

- Number and type of pathogens and their destruction methods should be addressed in MD 145/93 regulations in a separate table.
- An article about the appropriate method of sludge treatment and control should be added to the ministerial decision which is suitable to Omani conditions.
- Loads of N, P and K in sludge contents nutrients should be specified in Table 4.14 of MD 145/93, as sludge provided good nutrient properties for crops when applied on land.
- Buffer zones or setback distances should be specified in national guidelines when sludge is applied on the lands which are close to people's houses and water sources, as specified by US-EPA guidelines that appeared in Table 4.11. Moreover, geological, hydrogeological and topographical characteristics should be determined to avoid any potential contamination of groundwater.

4.12 Recommendations

- Rules should be specified in such a way that the relationships between the sewage, soil, and aquifers and the topography of the area are highlighted.
- The government sector is responsible for formulating new plans, modifying strategies and policies that go in parallel with the change of population and infrastructure in Oman. These include wetlands management, details of aquifer recharge water treatment systems, utilization of various types of wastewater such as industrial wastewater, production water and greywater.
- The Omani guidelines should also be compared to Australian guidelines.
- Different ministries and authorities need to publish combined national joint guidelines. This will help avoid any duplication of effort, eliminate any confusion, and give definitive measures. These responsibilities should be then transferred to only one governmental institution to avoid any duties overlapping.
- The modifications to laws and policies for environmental protection should be made in agreement with various fields of environmental and public work.
- Public awareness needs to be raised regarding environmental protection topics through a range of forums, lectures, conferences, environmental

trips, activities like marathons, environment festivals and social media programs.

- Studies on water policies are needed with the application of a range of methodologies and analysis.
- It is necessary to update the national regulations of pathogens and chemical standards within the present technical values to protect community health.
- Inspection and monitoring programs should be very comprehensive in order to maintain the best levels of environmental protection. This can be achieved through data collection, sampling, testing increasing the number of employees, supporting staff with training courses, etc.
- Evaluation of risk assessment is essential for managing TWW before maintaining and preserving water quality regulations.

4.13 Conclusions of the study

The present national framework is related to the regulations for wastewater reuse and discharge does not cover any comprehensive research on treated wastewater and sludge reuse. It only illustrates environmental standards with very brief descriptions of a few articles. In this sense, the national polices and guidelines should be reformed and revised by all concerned stakeholders to achieve an integrated sustainable developmental plan and strategies to implement them.

CHAPTER FIVE

RESULTS AND DISCUSSIONS

The chapter highlights the results and discussion of the practical parts (laboratory and field) of the present study: the degradation of diesel through phytoremediation method in combination with compost, the removal of diesel fuel from the contaminated soil by biroremediation method in combination with compost and finally, the quality of soil and the two crops (Radish and Beans) and the capacity yield of these crops when applying Kala compost and NPK fertilizer using groundwater and treated municipal wastewater.

5.1 Effects of Kala compost on plant species in enhancing phytoremediation technique

Some of the basic characteristics of the background soil (BG) or the clean soil as well as the contaminated soil used in this study are presented in Table 5.1.

Table 5.1 Basic physical and chemical characteristics of the contaminated soil and BG soil

Soil	pH	EC (μS/cm)	TN %	Texture	TOC %	TPH %	
Contaminated soil	7.90	1750	0.025	Loamy sand	10.50	1.15	
Background soil (BG soil)	8.00	955	0.013	Sandy loam	1.81	-	
Heavy metals (mg/kg)							
	Cr	**Fe**	**Cu**	**Zn**	**Cd**	**Ni**	**Ag**
Contaminated soil	<0.40	0.37	<0.3	0.95	<0.010	0.64	<0.010
Background soil (BG soil)	<0.30	0.36	<0.3	<0.30	<0.010	<0.50	<0.010

The compost (Kala compost) which was obtained from Haya Water Company (HW) has the chemical composition as follows: Pb 63.8 mg/kg, Cu 225.4 mg/kg, Zn 519.2 mg/kg, Cd 1.03 mg/kg, Cr 119.5 mg/kg, Ni 87.07 mg/kg, Hg 1.524 mg/kg, Mo 6.78

mg/kg and Se 0.5096 mg/kg and biological analysis (*E.coli*, Total coliforms, Salmonella) were not detected (Haya, 2013).

Additional testes were done at SQU laboratories and the results were as follows: pH 7.6, EC 31 mS/cm, Total Organic Carbon (TOC) 28.04, Total Nitrogen (TN) 2.6-2.8, moisture content 25%.

5.1.1 Growth of plants

No undesirable effects were observed during the growth period of the two plants (Bermuda grass and Ryegrass) in the diesel-contaminated soil without addition of any amendments. This revealed the tolerance of these plants to the diesel fuel in the soil which is in line with the findings by Ghanem et al. (2013) that there was no effect of pyrene on leaf development of Ryegrass, alfalfa and oil seed rape plant species. When Kala compost was added at the level of 10% (mass basis), most positive growth of both plants was observed especially for the Bermuda grass which showed a better growth than Ryegrass. However, the addition of compost at the level of 20% showed stunted growth with yellowish leaves for Ryegrass compared to Bermuda grass. This could be because of the high salinity level of Kala compost (31 mS/cm) which may explain the stunted growth of the plants.

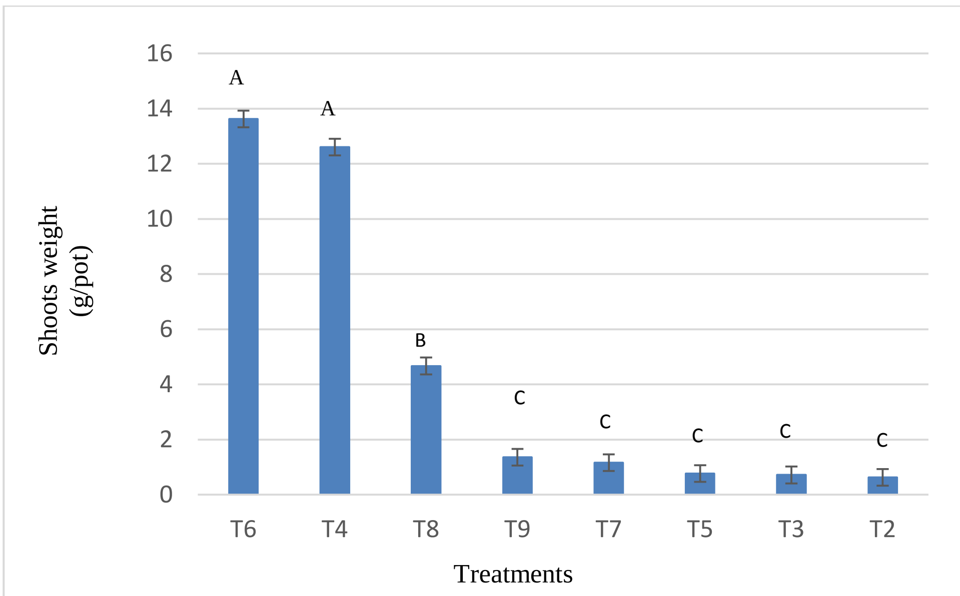
5.1.2 Biomass of shoots and roots

- **Shoot biomass**

The mean shoot growth (g/pot) for both plants is shown in Figure 5.1. This shows that, in the pots of contaminated soil only (without compost), the mean shoot weight for Bermuda grass was recorded at 0.63 g/pot, while mean shoot weight for Ryegrass was recorded at 0.71 g/pot.

Bermuda grass was grown in the contaminated soil with 10% compost addition recorded a mean shoot biomass of 12.60 g/pot, whereas Ryegrass grown in similar conditions recorded a mean shoot biomass of 0.77 g/pot.

The addition of 20% compost to the contaminated soil increased the mean shoot biomass of Bermuda grass to 13.63 g/pot and for Ryegrass to 1.16 g/pot. This is in line with reports by Ghanem et al. (2013) that the addition of compost could increase the dry weight of shoots of plants grown in the soil contaminated with pyrene, compared to the growth of plants in the soil without any amendments or lower levels. The positive effect of compost on plant growth may be due to the triggering of helpful soil microbes by providing good conditions for their growth (Duong, 2013).



Means followed by similar letters are not significantly different at $p<0.05$

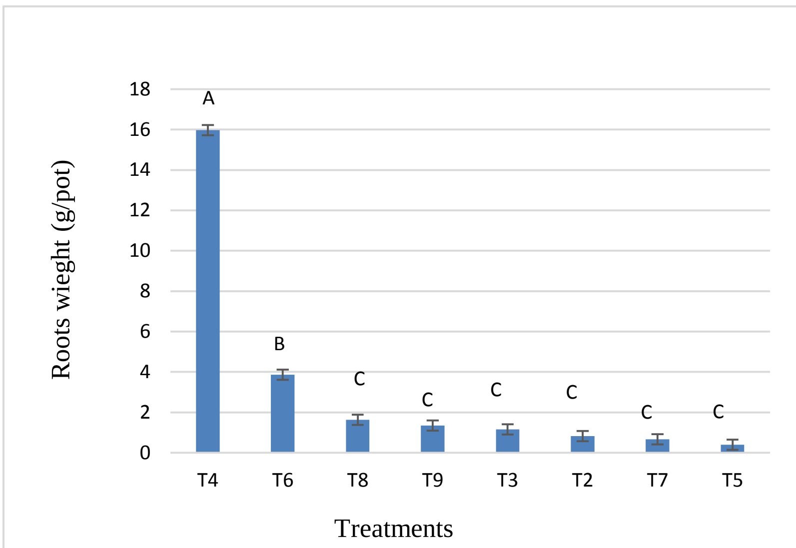
Figure 5.1 Mean values of shoot weight

T1: contaminated soil, T2: contaminated soil cultivated with Bermuda grass, T3: contaminated soil cultivated with Ryegrass. T4: contaminated soil applied with 10% compost and cultivated with Bermuda grass. T5: contaminated soil applied with 10% compost and cultivated with Ryegrass. T6: contaminated soil applied with 20% compost and cultivated with Bermuda grass. T7: contaminated soil applied with 20% compost and cultivated with Ryegrass, T8: Clean soil a Bermuda grass .T9: Clean soil and Ryegrass.

As shown in Figure 5.1, the highest biomass of shoots was found in T4 and T6 when Bermuda grass was grown in the contaminated soil applied with 10 and 20% of compost respectively. These two treatments showed significant differences ($p<0.05$) compared to other treatments (T2, T3, T5, T7 and T9) which show no significant differences among them (p>0.05) except for T8 which was significantly different from them.

- **Root biomass**

The mean root biomass in non-contaminated soil which is shown in Figure 5.2 was recorded at 0.82 g/pot for Bermuda grass and 1.35 g/pot for Ryegrass. When 10% compost was added; Bermuda grass recorded the highest root biomass of 15.97 g/pot, whereas Ryegrass gave a mean root biomass of 0.4 g/pot. Nevertheless, the contaminated soil amended with 20% Kala compost gave low root biomass at 3.87 and 0.66 g/pot for Bermuda grass and for Ryegrass respectively.



Means followed by similar letters are not significantly different at p<0.05

Figure 5.2 Mean values of root weight

T1: contaminated soil, T2: contaminated soil cultivated with Bermuda grass, T3: contaminated soil cultivated with Ryegrass. T4: contaminated soil applied with 10% compost and cultivated with Bermuda grass. T5: contaminated soil applied with 10% compost and cultivated with Ryegrass. T6: contaminated soil applied with 20% compost and cultivated with Bermuda grass. T7: contaminated soil applied with 20% compost and cultivated with Ryegrass, T8: Clean soil a Bermuda grass .T9: Clean soil and Ryegrass.

The root biomass for both plants as shown in Figure 5.2, shows that the treatments (T2, T3, T5, T7 and T8) showed no significant differences ($p>0.05$) between each other, which indicates that these have similar effect on the biomass of roots for Ryegrass. However, T4 where Bermuda grass was grown in the pots of contaminated soil applied with 10% of compost showed significant different ($p<0.05$) compared to the other treatments, which indicates that this treatment has the highest effect on the biomass of Bermuda grass roots. But generally, the statistical analysis of shoot and root biomasses was highly significant among the treatments (see Appendix 3.1).

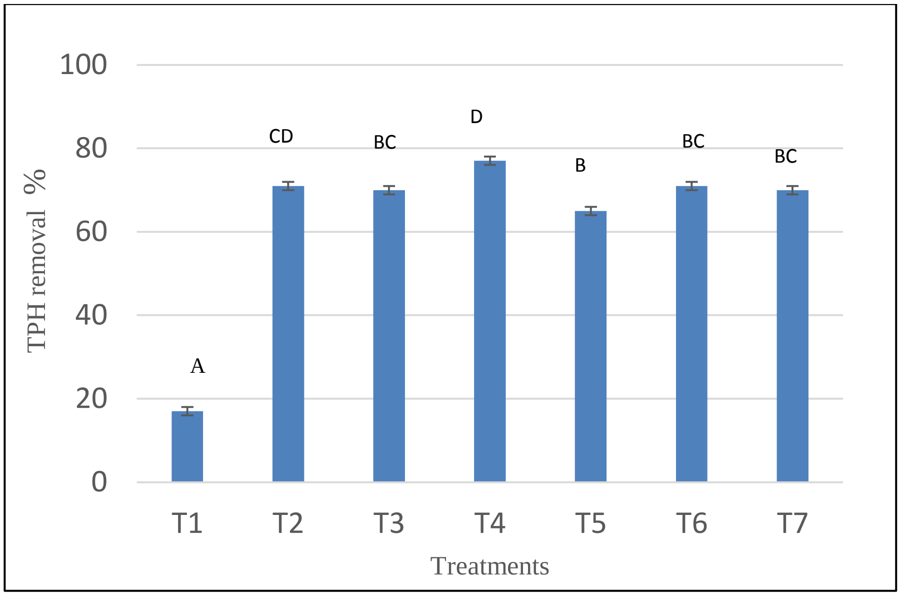
The observation of slow growth of roots of Ryegrasses in our study is consistent with the remarks of Kechavarzi et al. (2007) when they noted that Ryegrass prefers to grow in uncontaminated zones in their experiment before moving to the diesel-contaminated zones where the acceleration of growth is reduced there. Moreover, Ghanem et al. (2013) noted that roots and shoots biomass of *Lolium perenne* was adversely influenced by the presence of pyrene in soil, and this may explain that the decreased root biomass of Ryegrass in our study may be due to the presence of diesel fuel.

The improved growth of Bermuda grass in our study in terms of plant biomass is consistent with the findings of Razmjoo and Adavi (2012), when they noted that the root weights of this plant species increased in oil-contaminated soil.

5.1.3 Results of soil and plant samples after harvesting

- **TPH removal in soil and plant samples**

The removal of hydrocarbons was calculated using gravimetrical analysis as: $\%\text{Degradation} = \frac{\text{TPH of original sample} - \text{TPH of treated sample}}{\text{TPH of original sample}} \times 100$. (The amount of TPH in each treatment is given in Appendix 3.2), thus the mean values of TPH removal with different treatments conditions in the contaminated soils are shown in Figure 5.3 as follows:



Means follows similar letters are not significantly different at $p<0.05$

Figure 5.3 TPH% removals in contaminated soil

T1: contaminated soil, T2: contaminated soil cultivated with Bermuda grass, T3: contaminated soil cultivated with Ryegrass. T4: contaminated soil applied with 10% compost and cultivated with Bermuda grass. T5: contaminated soil applied with 10% compost and cultivated with Ryegrass. T6: contaminated soil applied with 20% compost and cultivated with Bermuda grass. T7: contaminated soil applied with 20% compost and cultivated with Ryegrass, T8: Clean soil a Bermuda grass .T9: Clean soil and Ryegrass.

In the Figure above, the removal rates of hydrocarbons, showed that in the non-vegetated pots with contaminated soil only (T1), the mean removal of hydrocarbon was 17%, indicating that diesel degradation was slow, because of no microorganisms to enhance the degradation of diesel and thus hydrocarbons could stick to the soil particles (Ling and Isa, 2006) and the removal of oil was due to the evaporation process through natural attenuation route (Dadrasnia and Agamuthu, 2013a).

The mean removal of TPH in contaminated soil when cultivated with Bermuda grass (T2) was at 71%, wherease with Ryegrass was almost 70% (T3), this indicates the high ability of these plants to degrade hydrocarbons in the diesel-contaminated soil.

Application of 10% compost in the contaminated soil further enhanced the phytoremediation efficiency in Bermuda grass. The average removal of hydrocarbon was approximately at 77% and 65% when grown with Bermuda grass and Ryegrass respectively (T4and T5).

In the pots of contaminated soil with the application of 20% compost, the mean removal of contamination was recorded at 71% and 70% when cultivated with Bermuda grass and Ryegrass respectively (T6 and T7).

As shown in Figure 5.3, the highest removal of TPH by the growth of plants was in T4 (where contaminated soil was applied with 10% of compost cultivated with Bermuda grass) which showed significant difference ($p<0.05$) compared to the other treatments, this may be due to the root excretions in this treatment, as the highest root biomass of 15.97 g/pot of Bermuda grass might be the attributing factor for high TPH degradation, because the organic compounds which are released by the roots may trigger the microbial growth in the rhizosphere zone which can stimulate the root contaminant interactions and favor more degradation of TPH in soils (Palmroth et al., 2002; Padmavathiamma et al., 2014). In addition, the activities of microorganisms to degrade diesel in the rhizosphere zone through the rhizo-degradation mechanisms is very fast especially when supplying with organic manure such as sewage sludge or compost ,as these can act as nutrients for these microorganisms (Namkoong et al., 2002).

The statistical analysis as detailed in Appendix 3.3 showed, the removal of hydrocarbons was highly significant ($P<0.05$) among all treatments.

The plant samples (both root and shoot) were analyzed for TPH content. However, the values obtained were negligible showing that the uptake of TPH by plants was not occurring. This reveals the fact that whatever TPH reduction in soil obtained was mainly by the degradation of hydrocarbons by the influence of plants as well as the microorganisms associated with them. This is consistent with the observation of Dadrasnia and Agamuthu (2013a) when they reported no fuel remains in the plants in their study using different species of plants under different climatic conditions.

- **Heavy metals analysis in soil and plant samples**

The mean values of total and DTPA extractable metals in soil are shown in Tables 5.2 and 5.3 below. Whereas, the mean values of heavy metals in shoots and roots of both plants are illustrated in Tables 5.4 and 5.5.

Table 5.2 Mean values of total metals extractable in soil in mg/kg

Treatments/ Metals	Mn	Fe	Zn	Pb	Ni	Cu
T1	173.01[a]	499.40 [a]	17.00 [a]	217.00 [a]	90.67 [a]	0.059[c]
T2	116.67 [a]	399.30 [a]	18.33 [a]	230.33 [a]	88. 70 [a]	0.31[bc]
T3	162.67 [a]	360.91 [a]	29.67 [a]	200.00 [a]	86. 32 [a]	0.54[bc]
T4	99.13 [a]	343.92 [a]	19.67 [a]	212.67 [a]	82.00 [a]	0.84[ab]
T5	116.67 [a]	349.70 [a]	18.67 [a]	217.67 [a]	99.24 [a]	0.96[ab]
T6	102.33 [a]	369.60 [a]	20.67 [a]	215.33 [a]	86.66 [a]	1.29[a]
T7	162.67 [a]	450.80 [a]	26.33 [a]	260.33 [a]	89.66 [a]	1.20[a]
T8	139.67 [a]	411.10 [a]	23.33 [a]	254.33 [a]	80.56 [a]	0.33[bc]
T9	149.67 [a]	476.21 [a]	30.33 [a]	284.00 [a]	89.00 [a]	0.14[c]

Means followed by similar letters are not significantly different at $p<0.05$.

Table 5.3 Mean values of DTPA extractable metals in soil in mg/kg

Treatments/ Metals	Mn	Fe	Zn	Pb	Ni	Cu
T1	0.59[ab]	1.04[c]	0.83[bc]	0.43[c]	0.094[bc]	N.d
T2	0.93[a]	2.28[ab]	0.41[c]	0.41[c]	0.20[abc]	N.d
T3	0.92[a]	1.39[b]	0.20[d]	0.40[c]	0.22[abc]	N.d
T4	0.97[a]	2.88[a]	1.95[a]	0.46[b]	0.30[a]	N.d
T5	0.93[a]	2.99[a]	1.32[ab]	0.47[b]	0.25[ab]	N.d
T6	0.93[a]	2.79[a]	1.11[b]	0.64[ab]	0.30[b]	N.d
T7	0.96[a]	2.99[a]	1.38[ab]	0.74[a]	0.23[ab]	N.d
T8	0.58[ab]	1.16[bc]	0.29[d]	0.46[b]	0.15[abc]	N.d
T9	0.23[b]	1.05[c]	0.74[bc]	0.48[b]	0.053[c]	N.d

N.d: Not detecte

Means followed by similar letters are not significantly different at $p<0.05$.

Table 5.4 Mean values of heavy metals in shoots in mg/kg for both plants

Treatments/ Metals	Mn	Zn	Cu	pb	Ni	Cr
T2	21.13^{c}	4.30^{ab}	10.45^{a}	N.d	N.d	N.d
T3	25.04^{b}	5.26^{a}	11.29^{a}	N.d	N.d	N.d
T4	25.26^{b}	1.26^{c}	6.78^{ab}	N.d	N.d	N.d
T5	32.77^{a}	2.54^{ab}	5.34^{b}	N.d	N.d	N.d
T6	31.53^{ab}	3.03^{b}	6.34^{ab}	N.d	N.d	N.d
T7	25.92^{b}	1.02^{d}	10.85^{a}	N.d	N.d	N.d
T8	28.38^{ab}	1.63^{ac}	6.74^{ab}	N.d	N.d	N.d
T9	30.42^{ab}	3.42^{b}	5.050^{b}	N.d	N.d	N.d

N.d: Not detected

Means followed by similar letters are not significantly different at $p<0.05$

Table 5.5 Mean values of heavy metals in roots in mg/kg for both plants

Treatments/ Metals	Mn	Zn	Ni	Cu	Pb	Cr
T2	30.61^{a}	5.88^{ab}	3.00^{b}	15.29^{a}	N.d	N.d
T3	28.16^{ab}	4.060^{b}	3.53^{ab}	18.66^{a}	N.d	N.d
T4	22.63^{b}	13.79^{a}	0.13^{c}	19.24^{a}	N.d	N.d
T5	11.30^{c}	11.05^{a}	5.10^{a}	17.10^{a}	N.d	N.d
T6	16.93^{bc}	11.54^{a}	0.13^{c}	15.25^{a}	N.d	N.d
T7	19.042^{b}	12.13^{a}	3.025^{b}	18.45^{a}	N.d	N.d
T8	28.38^{ab}	5.62^{ab}	0.13^{c}	16.74^{a}	N.d	N.d
T9	22.62^{b}	5.92^{ab}	0.13^{c}	14.50^{a}	N.d	N.d

N.d: Not detected

Means followed by similar letters are not significantly different at $p<0.05$

The concentration levels of studied total and dissolved extractable heavy metals for soil and for plants (shoots and roots) in the all above tables are lower compared to the standard values for safe limits as shown in Tables 5.6 and 5.7.

As shown in Table 5.2 and Appendix 3.4, the statistical results for total extractable metals in soil samples were not significantly different ($p>0.05$) among all treatments except for Cu. However, all DTPA extractable metals showed significant differences ($p<0.05$) between treatments for the various metals and were not even detected in the soil samples (Table 5.3 and Appendix 3.5). This indicates that the total extractable metals such as Cd, Mn, Zn and Fe are dominant elements in soil samples especially after application of composted sludge (Al-Dughaishi, 2009).

Moreover as shown in Tables 5.4 and Appendixes 3.6, there were significant differences($p<0.05$) for the various heavy metals for shoot samples among all treatments in both plants compared to the root samples (Table 5.5 and Appendix 3.7) which showed no significant differences ($p>0.05$) for all heavy metals except for Mn and Ni. This may be due to the uptake of these metals by these types of plants, and the fact that metal accumulation in plant tissue is more in the leafy portions than in the roots (Gupta et al., 2010; El- Nahhal et al., 2013).

Table 5.6 Concentration of heavy metals in soils and plants

Element	**Normal Range in soils†**	**Critical soil total conc†(mg/kg) †**	**Normal Range in plants***	**Critical concentrations in plant‡(mg/kg)**	
				a	b
Ag	0.01-8	2	0.1-0.8		1.0-4
As	0.1-40	20-50	0.2-7	5.0-20	1.0-20
Au	0.001-0.02	-	0.0017		<1
Cd	0.01-2.0	3.0-8.0	0.1-2.4	5.0-30	4-200
Co	0.5-65	25-50	0.02-1	15-50	4.0-40
Cr	5-1500	75-100	0.03-14	5.0-30	2.0-18
Cu	2-250	60-125	5.0-20	20-100	5.0-64
Hg	0.01-0.5	0.3-5	0.005-017	1.0-3	1.0-8
Mn	20-10000	1500-3000	20-1000	300-500	100-7000
No	0.1-40	2.0-10	0.03-5	10.0-50	
Ni	2-750	100	0.02-5	10-100	8-220
Pb	2-300	100-400	0.2-20	30-300	
Sb	0.2-10	5-10	0.0001-0.2		1.0-2
Se	0.1-5	5.0-10	0.001-2	5.0-30	3.0-40
Sn	1-200	50	0.2-6.8	60	63
Ti	0.1-0.8	1	0.03-3	20	
U	0.7-9		0.005-0.06		
V	3-500	50-100	0.001-1.5	5.0-10	1.0-13
W	0.5-83		0.005-0.015		
Zn	1-900	70-400	1-400	100-400	100-900

* Data mainly from Bowen, H.J.M., Environmental Chemistry of the elements. Academic Press, London (1979)

† The critical soil total concentration is the range of values above which toxicity is considered to be possible. Data from Kabata-Pendias, A. and Pendias, H., Trace Elements in Soils and Plants , 2nd edn. CRC Press, Boca Raton, Fla (1992),

‡ The critical concentration in plants is the level above which toxicity effects are likely. a, data from Kabata-Pendias and Pendias (1992); b, values likely to cause 10% depression in yield; data from Macnicol, R.D. and Beckett, P.H.T., Plant and Soil 85 (1985), 107-129.

Source: Alloway (1995).

Table 5.7 Standard threshold limit values of heavy metals in soils and fruits

Samples	Standards	Fe	Zn	Cu	Pb	Cd	Mn	Cr	Ni	AS
Soil (mg/kg)	Indian Standard (Awashthi, 2000)	NA	300 - 600	135 - 270	250 - 500	3-6	NA	NA	75 - 150	-
	WHO/FAO (2007)	-	-	-	-	-	-	-	-	-
	European Union Standard (EU 2002)	NA	300	140	300	3	NA	150	75	-
	USEPA (2010)	NA	200	50	300	3	80	NA	-	-
	Kabata-Pendias (2010)	1000	NA	NA	NA	NA	NA	NA	-	-
Plant (mg/kg)	Indian Standard (Awashthi, 2000	NA	50	30	2.5	1.5	NA	20	5	1.1
	WHO/FAO (2007)	450	60	40	5.0	0.2	500	5	10	-
	European Union Standard (EU 2002)	NA	60	40	0.3	0.2	NA	NA	-	-
	USEPA (2010)	-	-	-	-	-	-	-	-	-

Source CPCB (2002)

5.1.4 Conclusions of the experimental study

1. The promising effect of Bermuda grass (*Cynodon dactylon*) in the remediation of petroleum hydrocarbons (TPH) in the diesel contaminated soils was more than of Ryegrass (*Lolium perenne).*
2. Applying 10% of Kala compost could enhance the reduction of TPH in soils grown with Bermuda grass.

5.2 The effects of isolated diesel-degrading bacteria, Kala compost and urea on the bioremediation of diesel-contaminated soil

The basic characteristics of the first type of sandy loam soil which was used for isolating bacteria and the second type of sandy soil (artificially contaminated soil with diesel) are presented in Table 5.8 below:

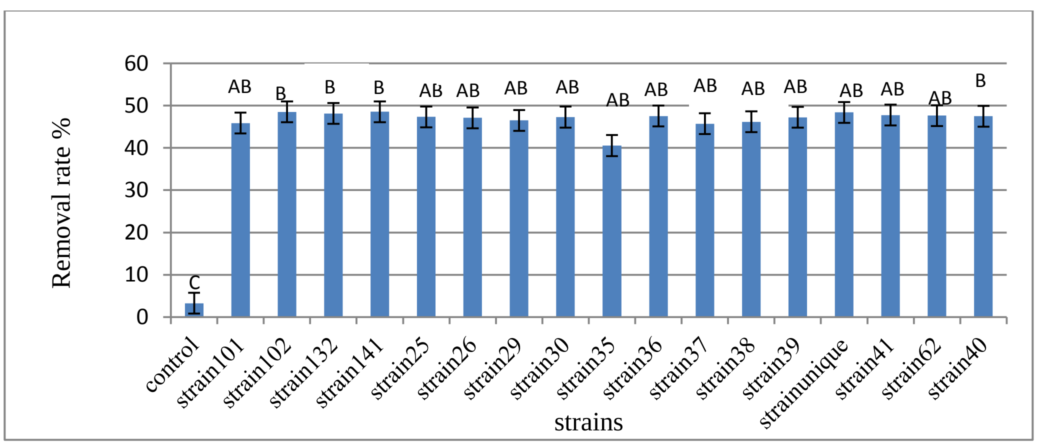
Table 5.8 Basic physical and chemical characteristics of soil samples

Soil Texture	pH	EC	%Moisture Content	%TOC	%TN	P mg/l	K mg/l
1. sandy loam	8.0	955 µS/cm	25	1.81	0.013	15	100
2. sandy	8.4	825 µS /cm	21.6	2	0.046	39.1	30

5.2.1 TPH removal through bioaugmentation method

The initial amount of TPH content in the contaminated soil and the amount of TPH content in the same contaminated soil inoculated with 17 strains (3 replicates) are shown in Appendix 4.1:

Using gravimetric analysis, the mean removal rates of hydrocarbons with different strains is shown in Figure 5.4 below:



Means followed by similar letters are not significantly different at $p<0.05$

Figure 5.4 TPH removals through bioaugmentation method

As shown in the Figure 5.4, the mean removal rate of hydrocarbons in the control soil samples (without addition of bacteria) was 3%, and this could be explained, that the little degradation of hydrocarbons may occur from non-biological factors like evaporation or photodegradation if there is no addition of microorganisms in the polluted soil (Dadrsina and Agamuthu, 2013a). However, the mean removal rates of diesel fuel by 17 strains was between 40% and 48.6% compared to the control sample, this indicates that all the mentioned strains were able to degrade hydrocarbons successfully and therefore the statistical analysis was significantly different $p<0.05$ in all treatments (See Appendix 4.2).

Out of 17 isolates, three strains namely numbers 102, 141 and unique (because it has different shape and size compared to the other strains) showed slightly higher removal of hydrocarbons compared to the other strains, therefore these strains showed no significant differences ($p>0.05$) between each other as shown in Figure 5.4.

The strains 102, 141 and unique were selected for further study in the bioremediation of diesel-contaminated soil through the biostimulation method.

5.2.2 TPH removal through biostimulation method

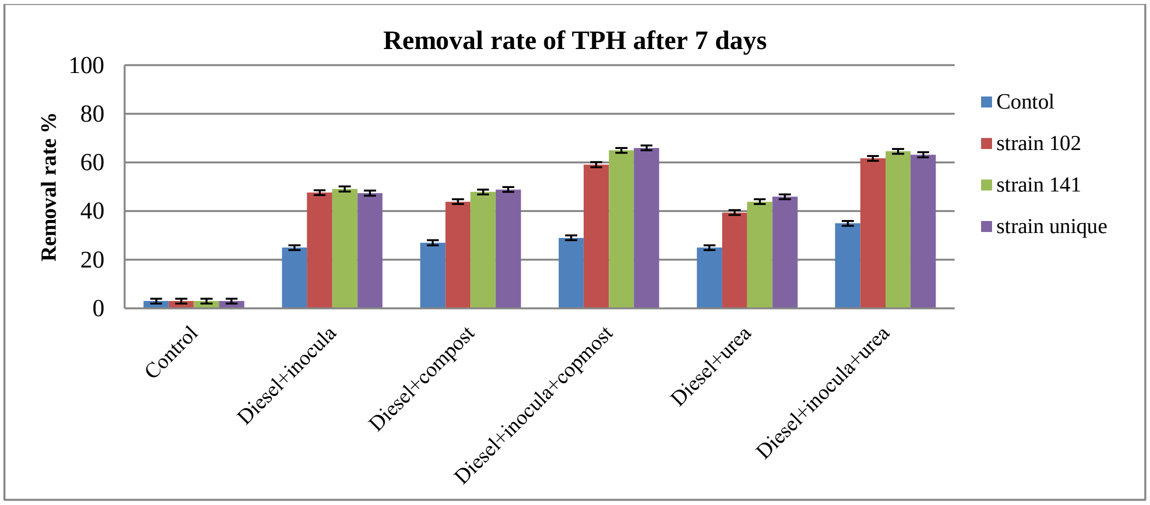
The three strains which were selected from the bioaugmentation method (102,141 and unique) were examined through the biostimulation method to check their efficacy for degrading hydrocarbons after incubation periods of one week and then after two weeks through 6 treatments as mentioned in Section 3.3.1.

The removal of TPH through biostimulation method was measured using Gas Chromatogram-Mass Spectrophotometer (GC-MS) analysis, fitted with Turbo Mass Software which was adjusted and calibrated to integrate n-alkane areas of diesel fuel from C10-C30. The bioremediation in each treatment was recognized from the area and retention time of each carbon atom compared to the area and retention time of the control sample. It was calculated by the addition of the total areas of all alkanes above the retention time-line by using the equation:

$$\%Biodegradation = \frac{\text{Total area of original sample} - \text{Total area of treated sample}}{\text{Total area of original sample}} \times 100$$

- **Rate of hydrocarbons removal in biostimulation experiment**

The results of removal rates of hydrocarbons for all treatments after one and two weeks incubation periods are shown in Figure 5.5 below: .



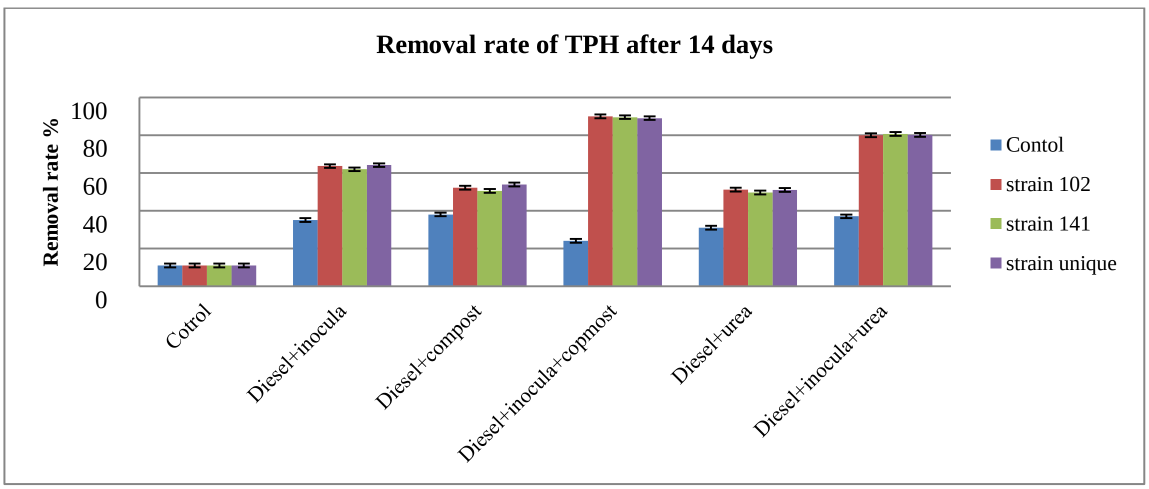


Figure 5.5 Comparison rates of diesel fuel removal through biostimulation method after incubationg for 7 and 14 days

The results of the biostimulation method showed that the control sample (no addition of bacteria) in treatment A has the lowest TPH removal of 3% and 10% after one and two weeks incubation period, this could be explained that if there is no addition or occurrence of microorganisms in the soil, oil particles could not be degraded and may stick in the pores of the soil (Ling and Isa, 2006).

The removal rate of diesel in contaminated soil in treatment B, when inoculated with micoorganisms number 102, 141 and unique after incubating for 7 days was calculated at 47.61, 49.12 and 47.40% respectively. However, after 14 days, the rate increased to 63.61, 61.92 and 64.10% respectively which indicates that bacteria were active in consuming the contaminants.

Samples which were amended with compost only in treatment C, showed the removal rates of 46.91% after 7 days, and after 14 days of 52.10%. The result of removing diesel fuel after 14 days incubation in this trial is consistent with the findings of Dadrsina and Agamuthu (2013a) in which the biodegradation in contaminated soil with diesel fuel amended with organic waste reached 55% at the end of 14 days incubation. However, Saviozzi et al. (2009) reported that there was a little effect of 1% compost in their experiment when this amount was added to the contaminated and un sterilized soil stimulating the indigenous bacteria to degrade TPH. Morover, as reported by Namkoong et al. (2002); Taccari et al. (2012), and Ghanem et al. (2013) the high percentage of organic matter and dissolved organic carbon in compost may degrade hydrocarbon in the contaminated soil, therefore the removal of TPH in this trial of our expriment was an excellent amount since the organic matter in Kala compost was 51%. In addition, the adequate values of N and P in compost acts as a nutrient for some microorganisms which could be present there, thus enhancing the speed of fuel removal (Dadrsina and Agamuthu, 2013a ; Taccari et al., 2012).

Nevertheless, when contaminated soil (c.s) was inoculated with bacterial solution amended with compost in tratment D, the removal of fuel increased from 59.11,65.10 and 66.0% in the first week, and to 90.0, 89.51 and 89.0% in the second week for the same strains 102, 141 and unique respectively. The high rate of fuel removal in this trial could be explained from the combination of two approaches, that biostimulation and bioaugmentation together can improve the biodegradation (Abed et al., 2014; Tacari et al., 2012). Also, compost provides extra nutrition for the microorganisms (Taccari et al., 2012) and it unlocks soil pores, smoothes the progress of air flow and provides a source of carbon for microbes (Ghanem et al., 2013).

The pots which were supplemented with urea only in treatment E, showed the lowest removal of hydrocarbons. The removal was calculated at 43.07% in the first week to 50.57% in the second week for three replicates. The removal of hydrocarbons was low because the addition of nitrogen may not produce an important result on bacterial growth (Wong et al., 2002; Speight and Arjoon, 2012), thus little degradation occurred.

However, the removal of TPH increased when the contaminated soils (c.s) were treated with bacteria and urea (Treatment F), the removal was at 61.71 , 64.60 and 63.22% in the first week and increased to 80.0,80.61 and 80.13% for 102, 141 and unique strains respectively. This means that the microorganisms used urea as a nutrient, thus enhancing the rate of hydrocarbon removal.

Figures 5.6 and 5.7 illustrate all GC-MS chromatograms of all treatments for the same mentioned incubation periods.

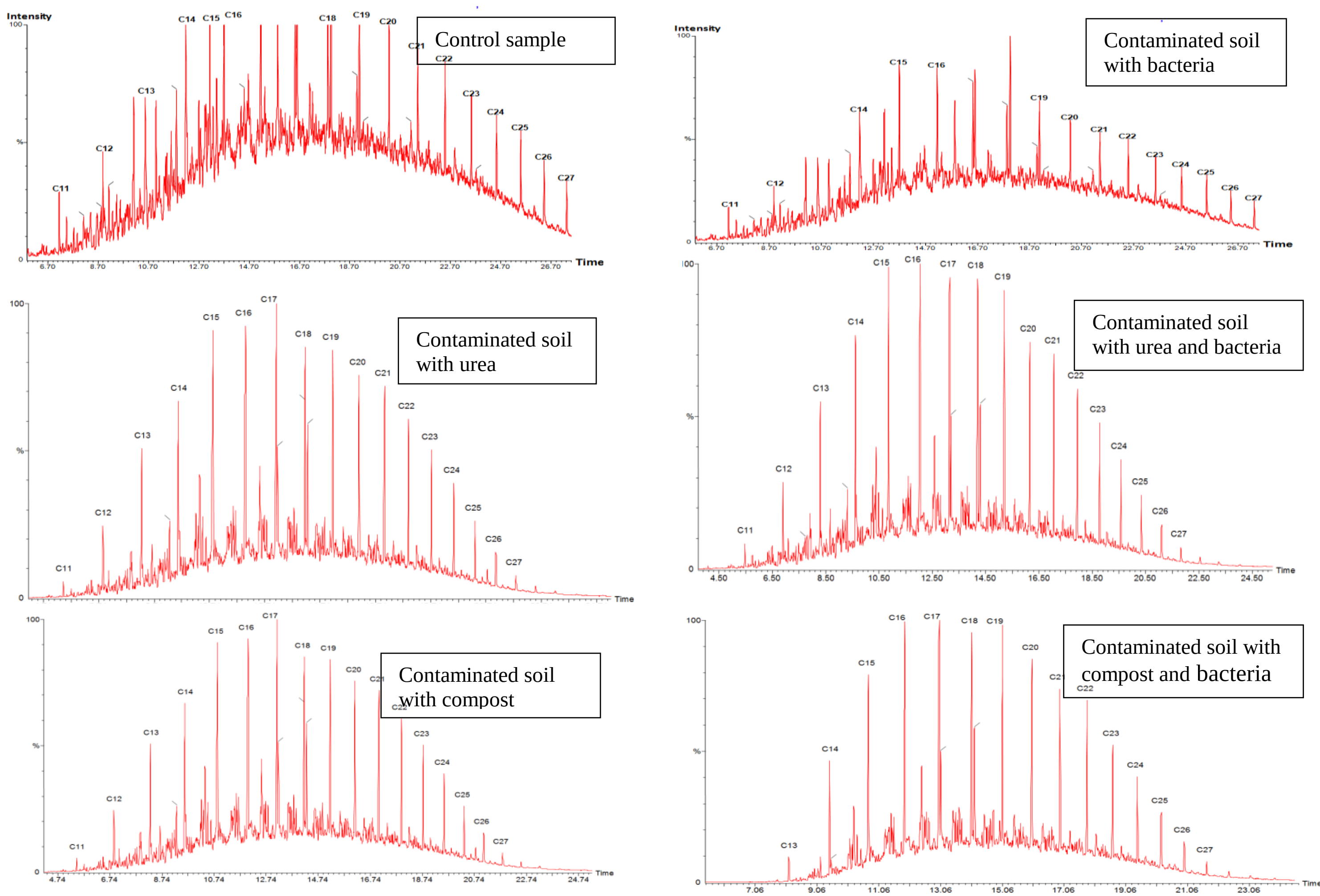


Figure 5.6 Chromatograms of GC-MS through biostimulation method after incubating for 7 days

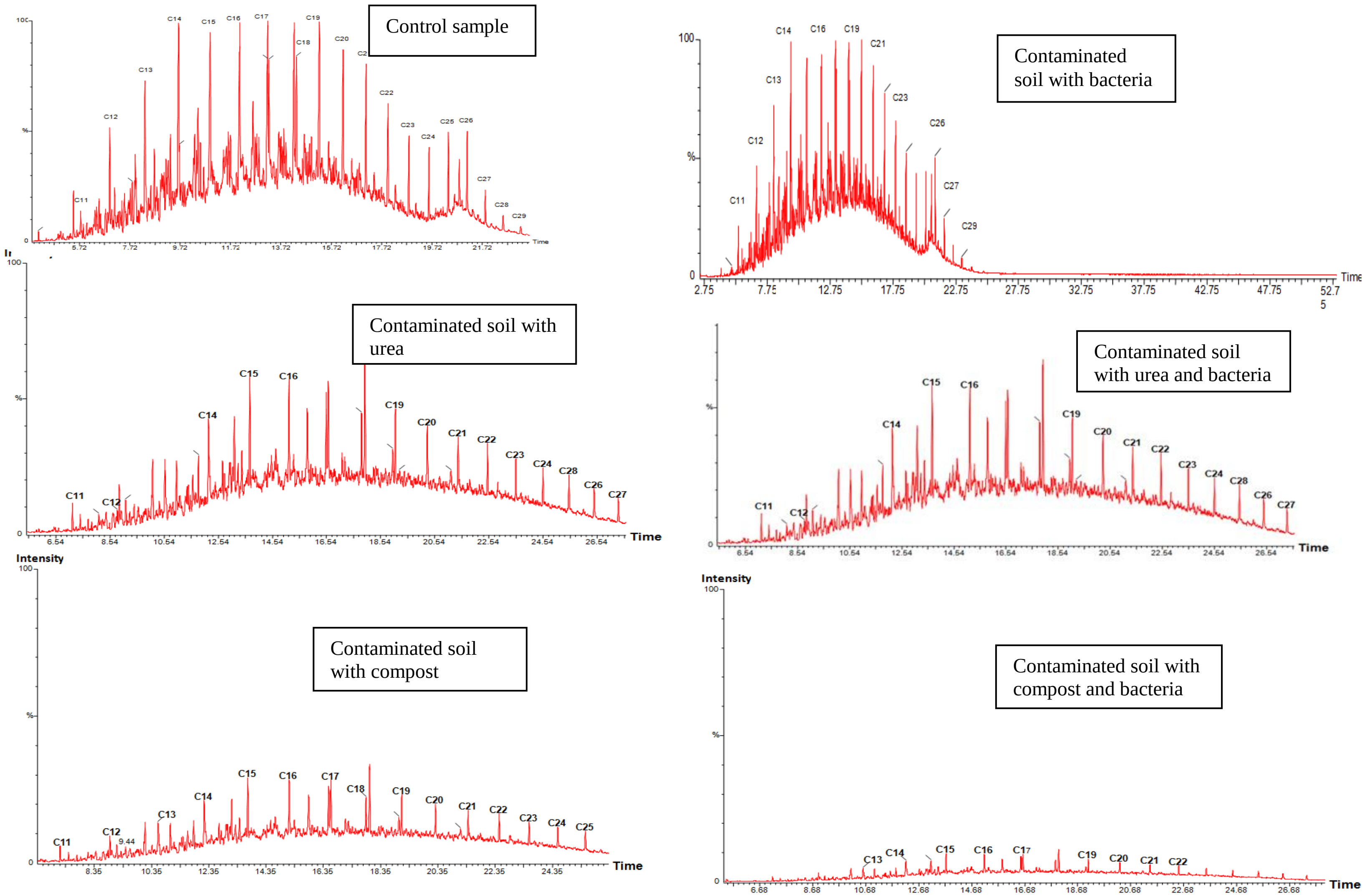
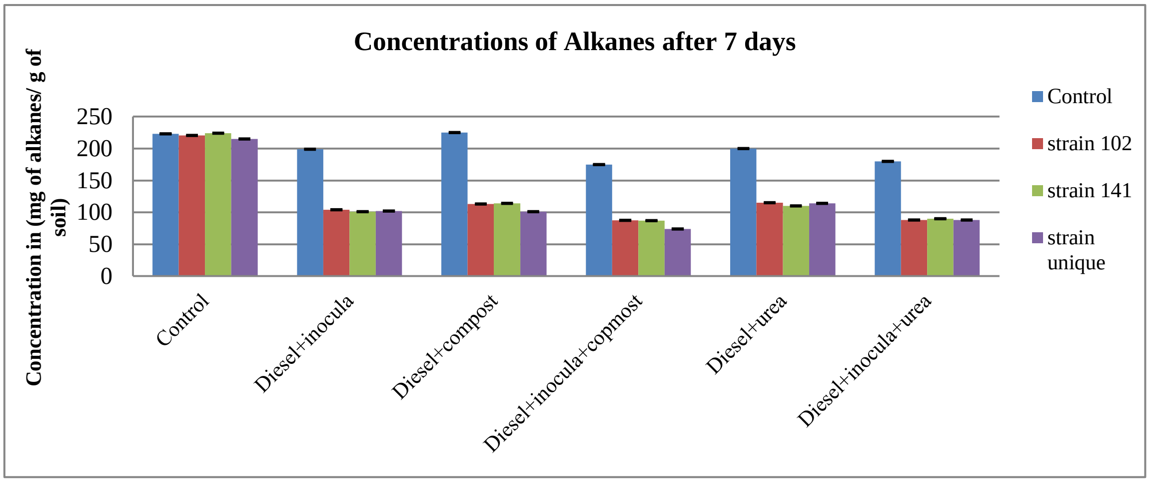


Figure 5.7 Chromatograms of GC-MS through biostimulation method after incubating for 14 days

- **Estimating concentrations of alkanes through biostimulation method**

The concentrations of alkanes (C10-C30) in this experiment were calculated in mg of alkanes/g of soil using GCMS instrument, and were based on the calibration curves of the standard mix solution of the concentrations 20, 40, 60, 80, and 100 ppm. The results of alkanes concentrations after 7 days and 14 days incubating periods are shown in Figure 5.8 below:



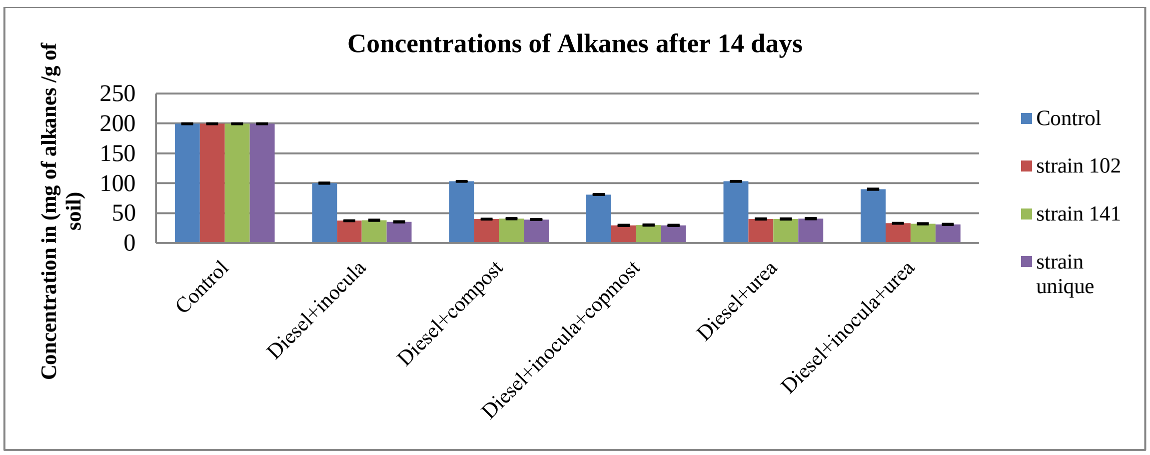


Figure 5.8 Alkanes concentrations through biostimultion method

The concentration of the control sample after incubating for one week was at 223.30 mg of alkanes g^{-1} of soil and dropped to 190.30 mg of alkanes g^{-1} of soil after an incubation period of 2 weeks.

For the pots with diesel and bacteria solution (102,141 and unique) in treatment B, the concentrations of alkanes were at 104.10, 102.15 and 102.9 mg of alkanes/g of soil when incubated for one week. However, the concentrations dropped to 37.21, 37.80 and 35.50 mg of alkanes/g of soil for all treatments when they were incubated for 2 weeks.

When compost was added only to the contaminated soil (c.s) in treatment C, the concentration was at 109.36 mg of alkanes/g of soil when incubated for one week, but decreased to 40.02 mg of alkanes/g of soil for all treatments after 2 weeks.

The concentrations of alkanes for contaminated soil with bacteria solution (102,141 and unique) and compost in treatment D were at 87.00, 87.19 and 87.01 mg of alkanes/g of soil after one week and were 29.00, 29.90 and 29.61 mg of alkanes/g of soil after 2 weeks.

In the treatment E when urea was added only to the contaminated soil for one week incubation, the concentrations of alkanes was calculated 113.41 mg of alkanes/g of soil and calculated at 40.32 mg of alkanes/g of soil for the same treatments after 14 days incubation.

The pots of diesel contaminated soil with strains (102,141 and unique) and urea (treatment E) the concentrations of alkanes were at 88.00, 90.01 and 88.02 mg of alkanes/g of soil which then decreased to 32.91, 31.90 and 31.10 mg of alkanes/g of soil when were incubated for 14 days.

The results of all treatments revealed that the isolated strains were very effective in reducing hydrocarbons and their concentrations to low values, especially when Kala compost was added to the treatments.

The Analysis of Variance (ANOVA) for removing hydrocarbons through the biostimultaion method and for estimating the concentration of alkanes showed that, the interaction between the factors (contaminated soil, compost and urea) when treating with and without the isolated bacteria was significantly different ($p<0.05$) for both incubation periods among all treatments (see Appendix 4.3, 4.4, 4.5, and 4.6), noted that the removal of diesel fuel was accelerated with the combination of bacteria and compost when incubated for two weeks.

The results of two experiments for both methods (bioaugmentation and biostimulation) revealed that, the removal rates of diesel fuel increased more through the biostimulation method compared to the bioaugmentation method, which was in agreement with Abed et al. (2014) who found that the biostimulation method increases the removal rates of hydrocarbons in contaminated soils more than the bioaugmentation approach.

5.2.3 Growth of strains in various salty media and temperatures

All the selected strains showed excellent growth at salinity of concentration from 5% to 10% of NaCl concentration, but there was no growth at 15% salinity for all of these strains (Figure 5.9).

Figure 5.9 The growth of strains in 5% and 10% salty media

For the growth of the isolates at different temperatures, Table 5.9 and Figure 5.10 below show the following results.

Table 5.9 The growth of isolates at different temperatures

Strains	**35°C**	**40°C**	**45°C**	**50°C**	**55°C**
102	yes	yes	yes	yes	No
141	yes	yes	yes	yes	No
Unique	yes	yes	yes	yes	yes

Figure 5.10 The growth of strains at different temperatures

Strains 102 and 141 grew at temperatures up to 45°C and 50°C respectively; whereas the unique strain showed it can tolerate temperatures up to 55°C. This meets with Abed at al. (2014) conclusion that the isolated strains have the features of halotolerance and are mesophilic, and can grow in the coastal sediments where the salinity could reach up to 10%, and can survive in the Omani hot summer months. As a result, they can contribute to the bioremediation of hydrocarbon-polluted soil of the same environmental conditions. The same results were obtained by Das and Tiwary (2012) that the strain which was isolated in their research was described as thermo-tolerant in nature, and can grow in temperatures ranging from 30^{o}C to 40^{o}C. Also, it can act as a good degrader for cleaning oil spills in the marine environment as it can survive at 3% salinity assuming that salinity of marine bodies is 3-3.5% of NaCl concentrations.

Based on the above studies our strains could be suggested as halo-tolerant and mesophilic and may have abilities to degrade hydrocarbons in the saline environments.

5.2.4 Identification of selected microorganisms

The identification by the Genetic Analyzer using method at S.Q.U showed that all 3 strains (102,141 and unique) which were selected during the experiment were *Bacillus* genera which belong to diesel degrading bacteria species.

5.2.5 Conclusions of the experimental study

1. The 17 strains which were isolated from the artificially-contaminated soil with diesel fuel had shown their capabilities to remove diesel fuel in the bioaugmentation approach between 40 and 48.6%.
2. Moreover, the rate of hydrocarbons removal increased from 66 and to 90% and the concentration of alkanes dropped from 87 to 29 mg/g of soil through the biostimulation approach when Kala compost was added to the contaminated soil and inoculated with the isolated strains after incubating for 7 days and then for 14 days respectively.
3. The isolated strains have demonstrated survival in the different ranges of salinities and temperatures, as they can exist in the saline environments which can reach up to 10% of NaCl concentration, and could survive at the temperatures of 55^{o}C.

5.3 The effect of municipal sewage sludge on the quality of soil and crops

5.3.1 Soil analysis before harvesting

Chemical analysis of groundwater (GW) and treated wastewater (TWW) are given in Appendix 5.1, soil texture was determined as sandy loam of 70.21% sand, 18.82% silt and 11.01% clay. EC and pH before adding fertilizers were at 955 µS/cm and 8.0 respectively. The initial mean results before adding any fertilizers of heavy metals in soil samples are given in Table 5.10, and the statistical analysis results are given in Appendix 5.2.

Table 5.10 Initial mean values of heavy metals concentration in soil samples in mg/kg

Element (mg/kg)	Treatment			
	T1	**T2**	**T3**	**T4**
Fe	0.25[a]	0.39[a]	0.20[a]	0.29[a]
Zn	0.50[a]	0.55[a]	0.52[a]	0.51[a]
Cu	1.080[a]	0.86[a]	0.57[a]	0.53[a]
B	0.023[a]	0.014[a]	0.030[a]	0.030[a]
Cr	N.d	N.d	N.d	N.d
Ni	0.64	N.d	N.d	N.d
Ag	N.d	N.d	N.d	N.d
Cd	N.d	N.d	N.d	N.d

N.d: Not deteacted

Means followed by similar letters are not significantly different at $p<0.05$.

5.3.2 Soil analysis after harvesting

- **EC and pH analysis**

Mean EC in (mS/cm) and pH values for two sites of the experimental plots (the third site was ignored due to operational problems) are shown in Table 5.11 below:

Table 5.11 Mean EC values in (mS/cm) and pH values of soil samples (saturation extract)

Parameters	Treatments			
	T1 Kala+GW	**T2 NPK+GW**	**T3 Kala+TWW**	**T4 NPK+ TWW**
EC (mS/cm)	4.51^{b}	6.90^{a}	4.72^{b}	5.40^{ab}
pH	7.83^{a}	7.51^{b}	7.70^{ab}	7.71^{ab}

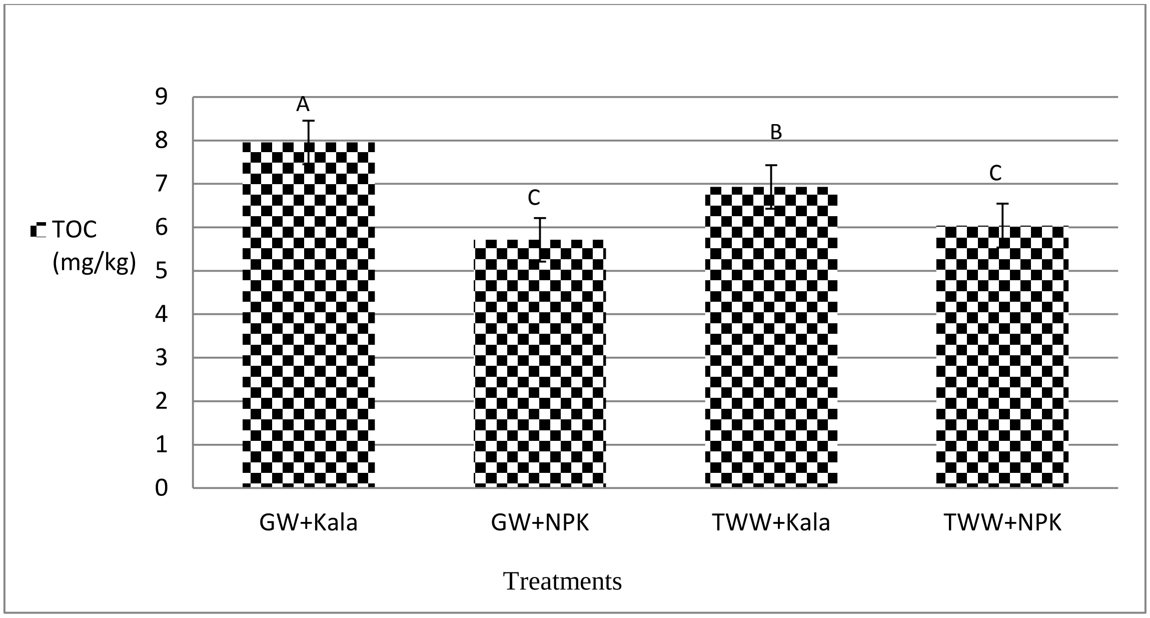
Means followed by similar letters are not significantly different at $p<0.05$.

EC values for the sites which were amended with NPK fertilizer and irrigated with GW and TWW as shown above were higher than the sites which were amended by Kala compost. This revealed that, although Kala compost has a high EC value of 31 mS/cm its EC when amended in the soil gave lower value than NPK fertilizer; this maybe a leachate from salts was occurred.

The statistical results of ANOVA for both EC and pH showed that the treatments and the sites were significantly different at $p<0.05$ but the interaction between them was not (see Appendix 5.3).

- **TOC analysis**

Figure 5.11 below shows the analysis of Total Organic Carbon (TOC) in soil samples



Means followed by similar letters are not significantly different at p<0.05.

Figure 5.11 TOC in soil samples in mg/kg

Soil samples with Kala compost media in the Figure 5.11 shows higher TOC values than in the media of NPK fertilizer. This observation is consistent with Singh and Agrawat (2011) and Mi et al. (2016) that the application of organic fertilizers leads to increase in the values of organic carbon compared to NPK fertilizer. In addition, adding municipal sewage sludge compost results in increasing of TOC values (Peña, 2015).

Moreover, the TOC in Kala compost amended soil is 39% (irrigated with GW) higher than TOC in soil samples under the application of NPK as shown in the above figure. The corresponding increase was 14% when soils were irrigated with TWW. The TOC level in the treatments when Kala compost was used showed a significant difference ($p<0.05$) compared to the treatments when NPK was used.

The Analysis of Variance indicated that TOC levels were strongly significant different ($p<0.05$) among all treatments, but both the sites and the interaction (sites with treatments) were not significantly different (see Appendix 5.3).

- **Heavy metal analysis**

The results of heavy metals of soils for the two sites of the experimental plots are given in Table 5.12 below:

Table 5.12 Mean values of heavy metals in soil in mg/kg after harvesting

Element (mg/kg)	**Treatments**			
	T1 Kala+GW	**T2 NPK+GW**	**T3 Kala+TWW**	**T4 NPK+ TWW**
Fe	49.80[a]	64.74 [a]	19.67 [a]	29.61 [a]
Zn	1.25 [a]	3.98 [a]	2.52 [a]	2.24 [a]
Mn	0.10	N.d	N.d	N.d
Cu	1.63 [a]	1.013 [a]	1.36 [a]	0.95 [a]
B	0.093 [a]	0.036 [a]	0.11 [a]	0.071 [a]
Cr	8.60 [a]	5.041 [a]	7.21 [a]	4.20 [a]
Ni	1.041 [a]	0.57 [a]	0.74 [a]	0.66 [a]
Ag	0.13 [a]	0.093 [a]	0.096 [a]	0.092 [a]
Cd	0.11 [a]	0.072 [a]	0.071 [a]	0.068 [a]

N.d: not detected.

Means followed by similar letters are not significantly different at $p<0.05$.

In our experiment, all metals in soil samples after harvesting showed higher concentrations compared to their initial concentrations (Table 5.10). When heavy metals enter soil through application of sludge or compost, a variety of mechanisms especially ion exchange with soil components occur, thus converting into insoluble forms, which make heavy metals bound to various soil phases (Navas and Lindhorfer, 2003), but all metals concentrations were within the standard range of the limits as given in Tables 5.6 and Table 5.7 in Section 5.1.3. However, Fe had the highest concentration in our results which agrees with the findings of Nogueirol et al. (2013) who reported that Fe concentration was the highest in their research especially at a depth ranging from 0 to 10 and from 10 to 20 cm, showing that Fe was mostly stuck to the mineral fraction found in the soil.

The Analysis of Variance of heavy metals in soil was not significantly different ($P>0.05$) within all treatments as shown in Table 5.12 and Appendix 5.4. This may be due to the reason of decomposition of organic compounds at the top layer as mentioned previously by Al-Busaidi et al., (2015b), and also for the high retention of heavy metals in the soil with different amendments (Singh and Agrawal, 2011).

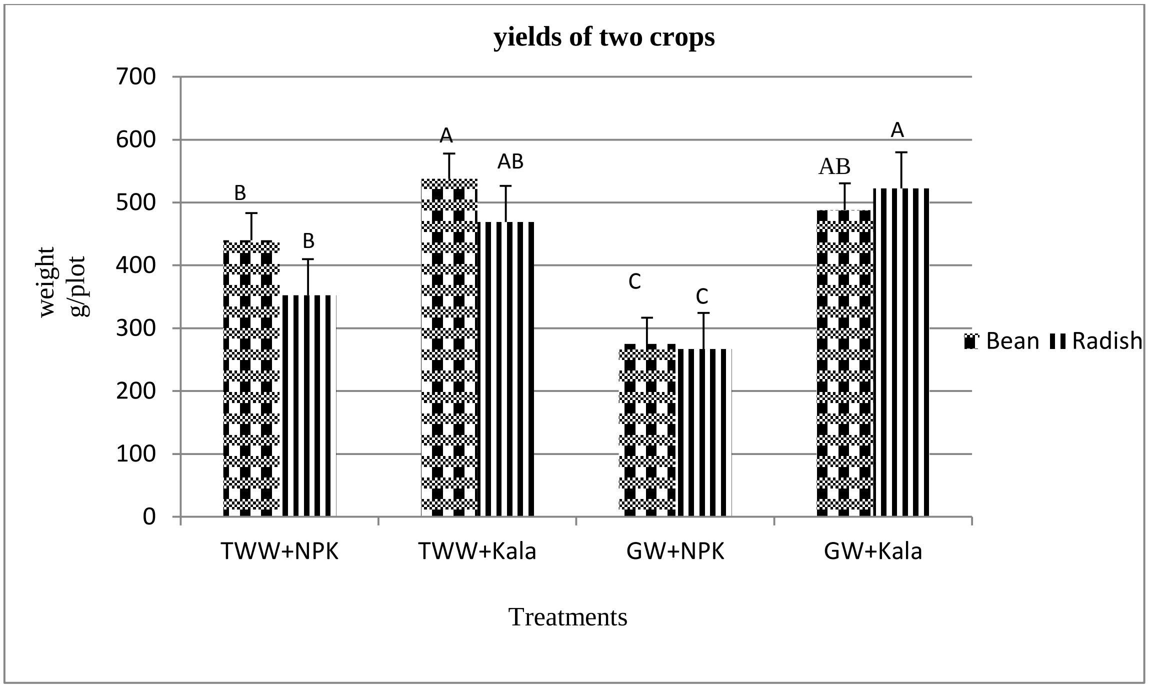
5.3.3 Plant analysis after harvesting

- **Biomass of crops and yield**

No phytotoxicity signs were detected in this experiment for both crops. As shown in Figure 5.12, the biomass of both plants was the highest under the application of Kala compost compared to NPK fertilizer. This result is in accord with Al-Tobi (2015) who found that the yield of cucumbers was grown higher in Kala media compared to Diwan compost and SQU agricultural compost. In addition, the same finding was found by Al-Saadi (2016) that the yield and biomass of tomato were increased by applying Kala compost. Furthermore, Singh and Agrawal (2011) found that, Spinach plant *Spinacia oleracea* had the highest yield when it was amended with organic fertilizer. Therefore, applying sewage sludge improves soil physicochemical properties, thus increasing the yield of the plants (Antonkiewicz and Pelka, 2014).

As shown in Figure 5.12, the yield of Beans increased under the application of Kala compost compared to NPK fertilizer. The increases were 22% (TWW) and 77% (GW) for Beans and 33% (TWW) and 96% (GW) for Radish. There was a significant different ($p<0.05$) in the treatments when Kala was used compared to the treatments where NPK was used.

The statistical analysis as summarized in Appendix 5.5 points that the yield of both crops were significantly different ($p<0.05$) among all treatments. The site factor for Radish was significant but not for Beans. Also the interaction (sites with treatments) for both plants was not significant.



Means followed by similar letters are not significantly different at $p<0.05$

Figure 5.12 Mean yields of two crops per plot in grams

- **Total Nitrogen in soil and plant samples**

As shown in Figure 5.13, soil samples showed the lowest levels of TN compared to the plant samples, this can be explained that Nitrogen had been taken from soil and accumulated in the plants, therefore the statistical analysis of TN in soil samples was not significant ($p>0.05$) among all treatments in both sites as well as their interaction (Appendix 5.6).

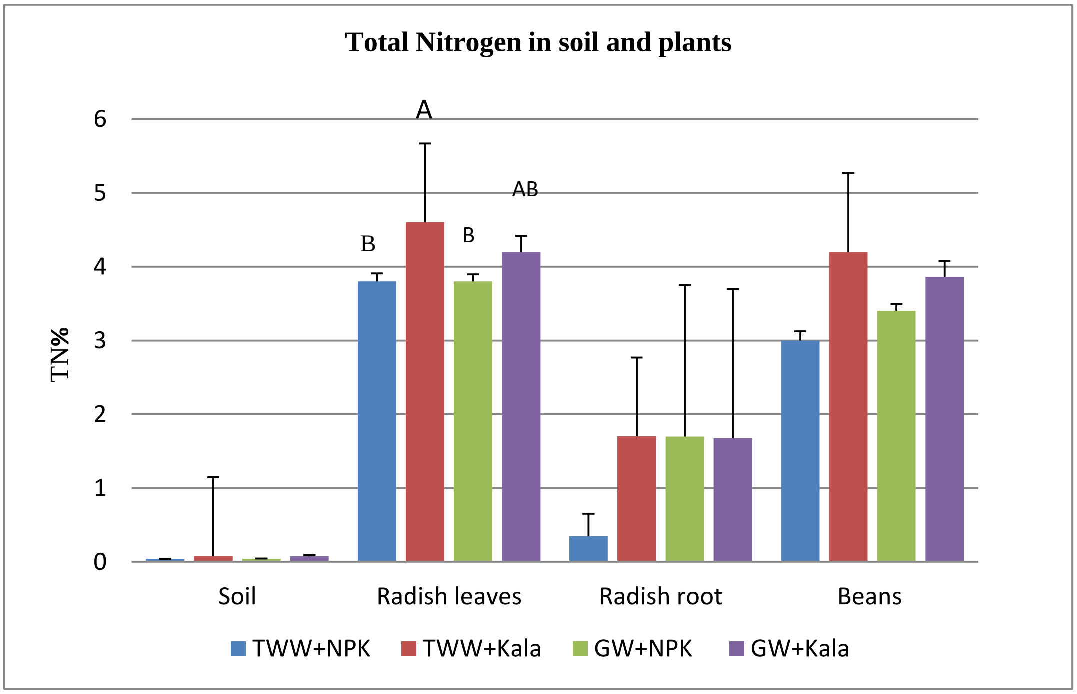
In addition, TN in plants which were amended with Kala compost and irrigated with GW and TWW showed higher values than the plants which were amended by NPK, this observation agrees with Al-Tobi (2015) that TN was higher with Kala compost than with the other two types of Diwan and AES compost.

As shown in Figure 5.13, TN showed an increase between 13% and 40% for Beans and an increase between 10.3% and 21% in leaves for Radish under the application of Kala compost when both plants were irrigated with GW and TWW, respectively.

However, TN showed no increase in the roots of Radish when the plant was irrigated with GW using either NPK or Kala fertilizers, but showed an increase of 3.86% when it was irrigated with TWW under the application of Kala compost, but showed

The Analyses of Variance (see Appendix 5.6) for roots in Radish indicated that TN levels did not show significant affected by treatments ($p>0.5$) as well as for the sites and their interaction (sites with treatments). However, there was a strong significant difference ($p<0.05$) identified for the leaves of Radish among all treatments, this maybe because of the nitrogen level which relates to the high Chlorophyll content is found in the leaf part of plants (Daughtry et al., 2000).

In Beans there was no significant difference ($p>0.05$) of TN among all treatments as well as for the site and their interaction (see Appendix 5.6).

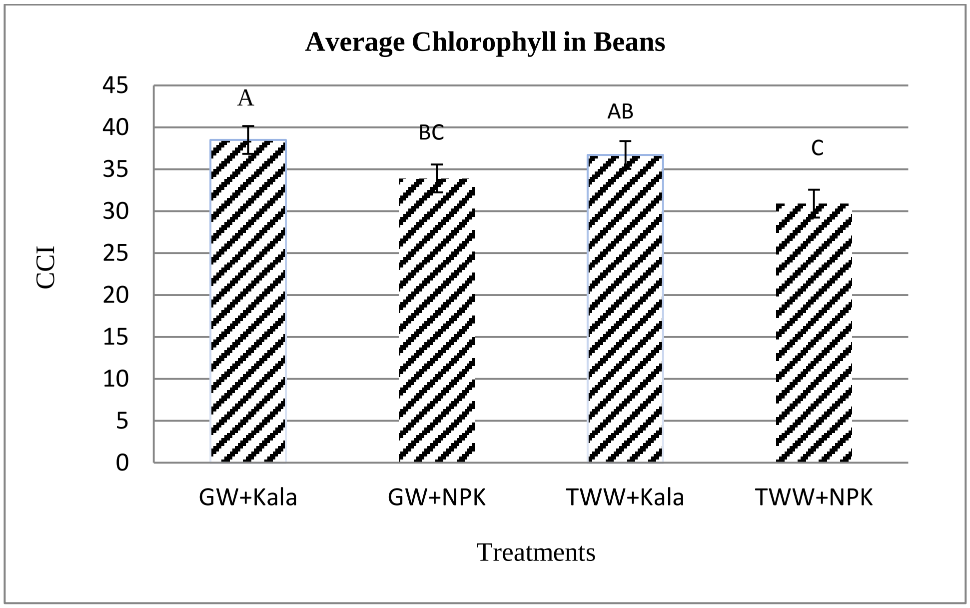


Means followed by similar letters are not significantly different at $p<0.5$.

Figure 5.13 Total Nitrogen in soil and plants

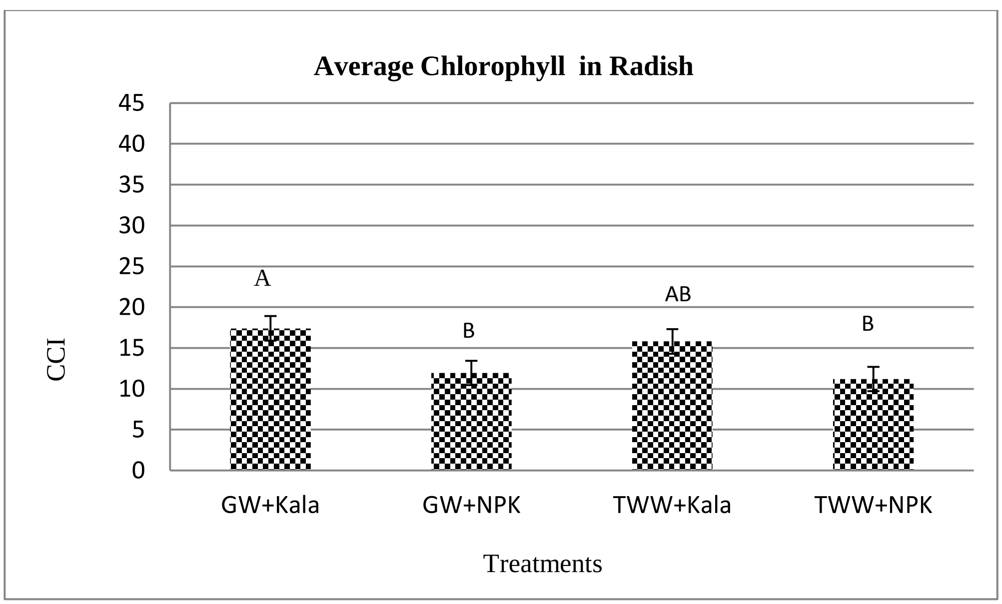
- **Measurements of Chlorophyll**

Chlorophyll Contents Index (CCI) of the two crops was measured 2 times weekly by a Chlorophyll meter, the mean values are shown in Figures 5.14 and 5.15.



Means followed by similar letters are not significantly different at $p<0.05$

Figure 5.14 Average Chlorophyll in Beans



Means followed by similar letters are not significantly different at $p<0.05$

Figure 5.15 Average Chlorophyll in Radish

As shown from the above two figures (5.14 and 5.15), the average Chlorophyll contents in the treatment using Kala compost was higher than in the treatment using NPK fertilizer. A similar result was found by Al-Tobi (2015) that Chlorophyll contents in Kala media had the highest values over the other two fertilizers of Diwan and AES compost. Also, Singh and Agrawal (2011) reported that, Chlorophyll measurement was the highest in Spinach plant *Spinacia oleracea* with organic fertilizer using farm-yard manure.

The Chlorophyll contents of Beans increased by 19% and 13.6% and increased for Radish by 41% and 45.6% when both plants were irrigated with TWW and GW respectively using Kala compost compared to NPK. Therefore, there was a significant different ($p<0.05$) among all treatments when Kala was applied.

As detailed in Appendix 5.7 , the Analysis of Variance (ANOVA) for the Chlorophyll contents in both plants showed that there was a high significant difference ($p<0.05$) in all treatments but neither the sites nor their interaction (sites with treatments) were significantly different.

- **Heavy metal analysis**

The results of heavy metals in plant samples are given in the Tables 5.13, 5.14, and 5.15 below:

Table 5.13 Mean values of heavy metals in roots of Radish plant in mg/kg

Elements (mg/kg)	Treatments			
	T1 GW+ Kala	T2 GW+NPK	T3 TWW+Kala	T4 TWW+NPK
Fe	125.28^{c}	299.50^{a}	175.07^{ab}	145.59^{b}
Zn	32.13^{a}	23.10^{b}	29.67^{ab}	23.55^{b}
Mn	0.42^{b}	7.33^{a}	7.37^{a}	N.d
Cu	7.96^{a}	5.39^{ab}	3.089^{c}	4.074^{b}
B	21.53^{a}	23.1^{a}	26.083^{a}	22.26^{a}
Cr	1.37^{b}	2.11^{ab}	1.27^{b}	7.28^{a}
Ni	2.40^{c}	5.40^{b}	1.68^{c}	7.97^{a}
Ag	0.14^{b}	0.19^{ab}	0.28^{a}	0.22^{ab}
Cd	0.13^{a}	0.10^{a}	0.20^{a}	0.20^{a}

Means followed by similar letters are not significantly different at $p<0.05$

Table 5.14 Mean values of heavy metals in leaves of Radish plant in mg/kg

Elements (mg/kg)	Treatments			
	T1 GW+Kala	T2 GW+NPK	T3 TWW+Kala	T4 TWW+NPK
Fe	349.82^{ab}	426.74^{a}	264.19^{b}	226.14^{c}
Zn	56.76^{a}	44.37^{b}	17.20^{c}	46.23^{ab}
Mn	0.41^{b}	N.d	1.70^{a}	N.d
Cu	5.32^{a}	7.24^{a}	3.092^{a}	7.095^{a}
B	7.087^{c}	14.95^{a}	9.51^{b}	11.50^{ab}
Cr	N.d	1.24^{a}	3.41^{a}	2.60^{a}
Ni	2.059^{c}	4.11^{ab}	3.96^{b}	4.80^{a}
Ag	0.20^{ab}	0.23^{ab}	0.19^{b}	0.26^{a}
Cd	0.79^{a}	0.22^{b}	0.14^{c}	0.65^{a}

N.d: Not detected

Means followed by similar letters are not significantly different at $p<0.05$.

Table 5.15 Mean values of heavy metals in Beans in mg/kg

Elements (mg/kg)	Treatments			
	T1 GW+Kala	T2 GW+NPK	T3 TWW+Kala	T4 TWW+NPK
Fe	40.51^{a}	43.90^{a}	47.23^{a}	50.10^{a}
Zn	60.69^{a}	48.44^{ab}	36.68^{b}	38.35^{b}
Cu	6.63^{a}	5.98^{a}	5.70^{a}	4.84^{a}
B	27.078^{a}	25.32^{a}	29.61^{a}	22.36^{a}
Cr	5.32^{a}	4.53^{a}	2.46^{a}	2.76^{a}
Ni	6.90^{a}	3.73^{c}	5.71^{b}	6.45^{ab}
Ag	1.32^{a}	0.153^{b}	0.26^{b}	0.22^{b}
Cd	1.34^{a}	0.12^{b}	0.23^{b}	0.14^{b}

Means followed by similar letters are not significantly different at $p<0.05$

As appears in Tables 5.13, 5.14 and 5.15, Fe has the highest values in our results found in plant samples, which agreed with the findings of Nogueirol et al. (2013) who reported that Fe concentration was the highest when using the method of EPA 3052 among the other methods. Therefore, maybe the EPA 3050 method which was used to extract Fe in our study has the similar effect. In addition, Zn concentration also showed high values in all results, which is in line with Ji et al. (2012) and Safarri and Saffarri (2013) who found that the high value of Zn in plants may be related to the addition of organic manure as fertilizers and that Zn is mobile element in compost Garrido et al., (2005).

The statistical analysis for most heavy metals in roots and leaves parts of Radish plant were significantly different ($P<0.05$) as shown in Tables 5.13 and 5.14 and summarized in Appendixes 5.8 and 5.9. This is in agreement with Al-Busaidi et al. (2015a; 2015b) who found that the high concentrations of some metals such as Fe, Zn, and Ni in date plant leaves, which were irrigated either with TWW or GW were significant in all locations due to the plant growth especially when Kala compost or organic manure was applied. Also, Gupta et al. (2010) found that metal accumulation per gram dry weight of plant tissue is more in the leafy portions than in the roots; in addition El- Nahhal et al. (2013) found a high concentration of heavy metals in the leaves of Chinese cabbage and corn when irrigated either with treated wastewater or with freshwater in their experiment.

On the other hand, the statistical results showed that the differences in some trace elements in Beans were not significantly different ($p>0.05$) which maybe because of using different fertilizers and water quality (Al-Busaidi, 2015b). However, Zn, Ni, Ag and Cd were significantly different ($p<0.05$) in all treatments for this plant as shown in Table 5.15 and detailed in Appendix 5.10.

Generally, all metals concentrations in plant samples were within the standard range of the limits as given in Table 5.6 and Table 5.7 (see Section 5.1.3), which indicates no risk of heavy metals accumulation, was found in the plant samples.

5.3.4 Conclusions of the experimental study

1. Kala compost creates good media for producing higher crop yield as compared to NPK.
2. Chemical analyses of heavy metals in soil and in the two crops were within the standard limits.

3. Both groundwater and treated wastewater irrigation develop the growth of plants especially when Kala compost was used for growing the two crops.
4. Plants yield, chlorophyll contents and TOC in soil increased when GW irrigation was used with the application of Kala compost.
5. Irrigation with treated wastewater did not show any toxicity or contamination in the soil and the two plants used.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

The following conclusions and recommendations have been drawn on the basis of this study which encourages the reuse of treated wastewater and sewage sludge.

6.1 Conclusions

6.1.1 Treated wastewater and sludge reuse management

- The present national framework does not cover any inclusive documents and texts on treated wastewater and sludge reuse but only demonstrates the environmental standards with very short descriptions of a few articles.
- The national policies and guidelines should be revised under different technical, economic, environmental and social contexts by all sectors of decision makers, achieving an integrated sustainable developmental plan and strategies.
- Sludge and wastewater utilization can add up positively in the economic aspects of the country in terms of creating jobs and improving annual economy.
- The following flow charts maybe follow in managing sludge and treated wastewater by decision makers:

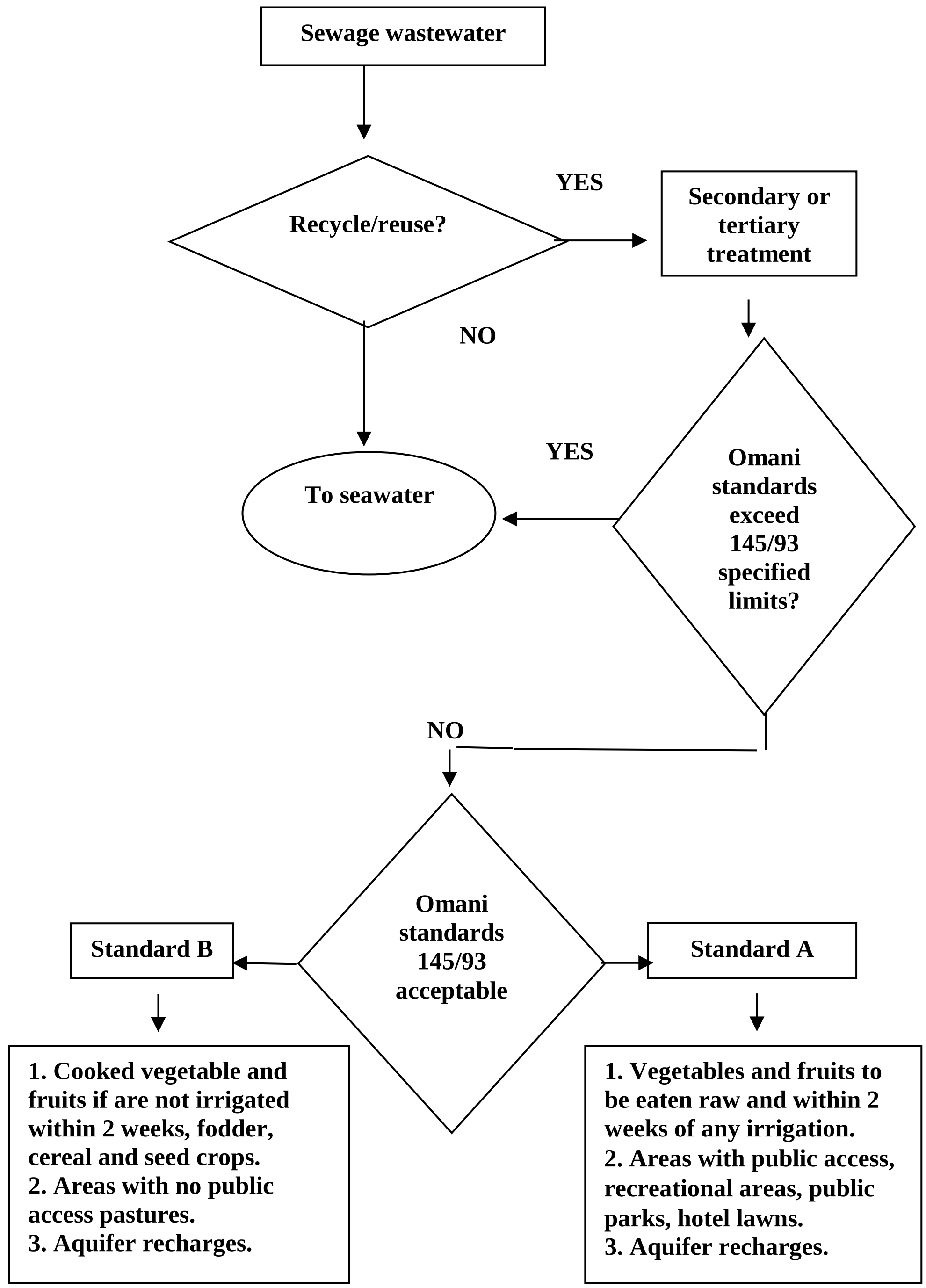


Figure 6.1 Flow chart of recycling of sewage treated wastewater

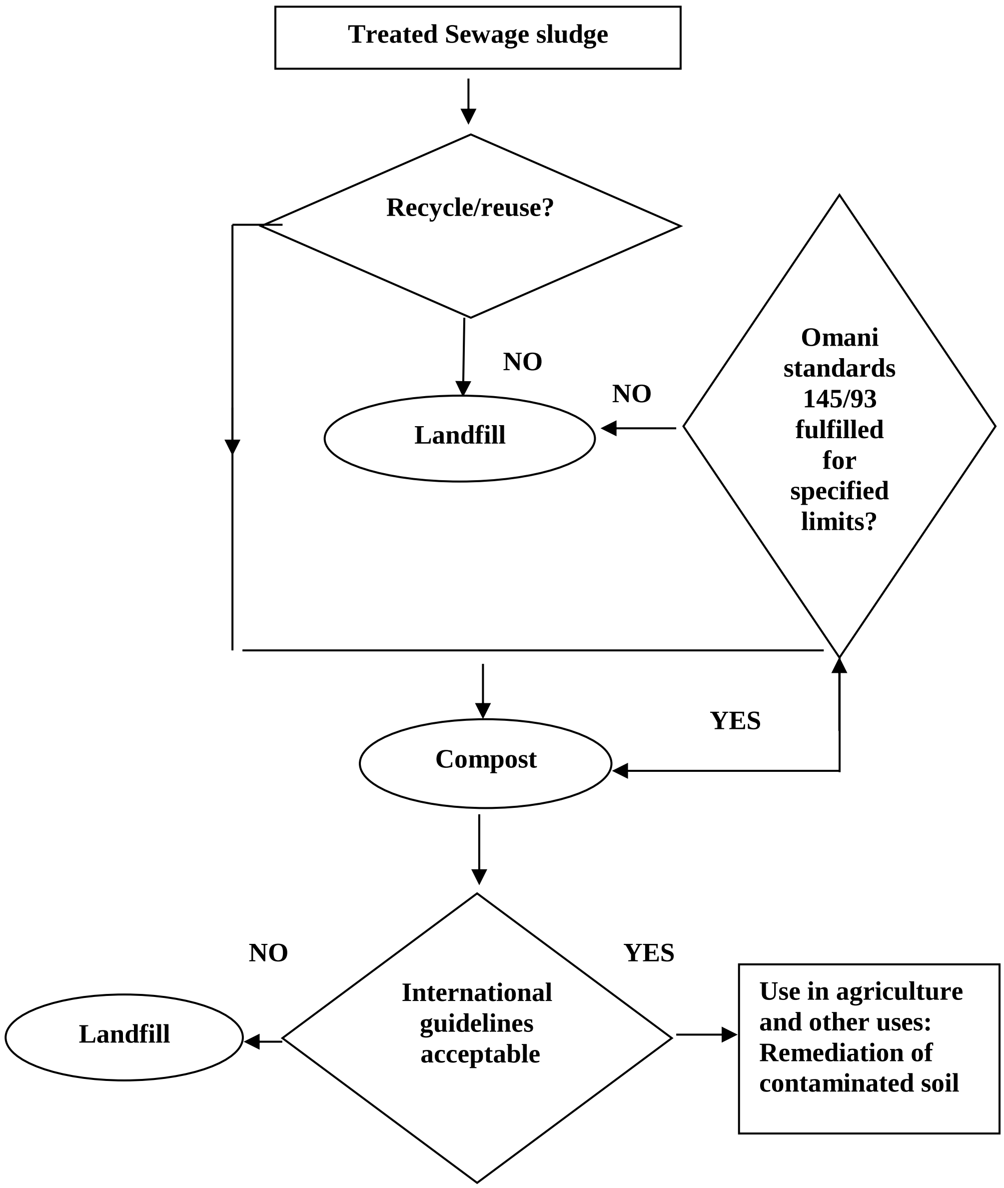


Figure 6.2 Flow chart of recycling of sewage sludge

6.1.2 Phytoremediation technique

- The study revealed the promising effect of Bermuda grass in the remediation of petroleum hydrocarbons in the diesel-contaminated soils compared to Ryegrass.
- Application of Kala compost at 10% complemented the phytoremediation process, and enhanced the reduction of hydrocarbon loads up to 77%.

6.1.3 Bioremediation technique

- The identified strains in this study were *Bacillus* genera which belong to diesel degrading bacteria species, showed the capability to reduce petroleum hydrocarbons up to 90% in the diesel-contaminated soil with addition of 1% Kala compost after two weeks incubation period.
- The concentration of alkanes dropped from 87 to 29 mg of alkane/g of soil after incubating for one week and then after two weeks respectively, through biostimulation approach when 1% Kala compost was added to the diesel-contaminated soil and inoculated with the isolated strains.
- The removal rates of hydrocarbons in the diesel-contaminated soil increased more through biostimulation method compared to bioaugmentation method.
- The strains improved their survival in the different ranges of salinities up to 10% NaCl concentration and could survive at temperatures up to 55^{o}C.

6.1.4 The effect of Kala compost on agricultural activities

- Kala compost application resulted in higher crops yield than NPK fertilizer.
- There was no heavy metal accumulation in the two crops after chemical analysis.
- Plants yield, Chlorophyll contents and TOC in soil increased when groundwater irrigation was used with the application of Kala compost.
- Heavy metal analyses in soil and in two crops when irrigated with treated waste water were within the standard limits.

6.2 Recommendations

- A framework for the analysis of treated wastewater and sludge reuse management systems in the Sultanate is urgently needed to evaluate and improve the current guidelines.
- Municipal sewage sludge (Kala compost) is recommended to be a good medium for enhancing the remediation of diesel-hydrocarbon contaminated soil and for land application in the agricultural purposes in terms of high-yield crop production and in improving soil physico-chemical properties.
- Sludge utilization has been a challenge due to its association with human waste. Therefore, composting of sewage sludge is the best option especially in the agricultural activities.
- The research projects related to land application of sewage sludge should be encouraged for its beneficial use in Oman in terms of economic and environmental aspects.
- Considering that the agricultural experiment was a short duration one, there is a need for more continuous long-term experiments that will improve the understanding on the effects of composted sewage sludge (Kala) on soil fertility and crop yield to contribute to the development of sustainable agricultural practices in an arid environment of Oman.

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APPENDICES

Appendix 1 National policies of wastewater and sludge management

1.1 Capacity of treated wastewater plants in different regions in the Sultanate and their production rates (m^3/day) in 2016

N.O	Willayate	Design Capacity	Flow Received	TE Produced	TE Utilized
1	Suwaiq	120	130	125	39
2	Khabora	500	480	433	13
3	Saham	5000	3352	3213	360
4	Liwa	3000	2846	2703	358
5	Shinas	600	328	311	297
6	Rustaq	7200	2922	2037	823
8	Blad Seet	200	30	28	0
9	Barkha	2000	2091	2049	1313
10	Musana	950	765	715	288
11	Khasab	1800	2089	2089	114
12	Daba	120	76	69	61
13	Madha	180	39	21	20
14	Bukha	180	103	102	47
15	Birami	3000	3073	3039	2936
16	Mahda	2500	1647	1492	71
17	Abri	1800+500	2192	2734	2003
18	Dank	250	280	276	54
19	Yankl	250	192	181	49
20	Hamer Al Drooa	180	116	132	10
21	Nizwa	5600	7113	6690	2584
22	Suq Nizwa	250	71	68	56
23	Wadi Kalbo	450	307	299	283
24	Nizwa Al Rahbha	100	33	30	5
25	Manah	180	108	103	29
26	Sah Al Qatna	120	139	134	112
27	Seeq	150	47	43	30
28	alsheraga	60	0	0	0
29	Izki	180	133	126	125
30	Samail	2700	2245	2022	1598
31	Alhamra	120	65	51	10
32	Adam	250	180	176	150
33	Bahla	600	265	259	10

No#	Willayate	Design Capacity	Flow Received	TE Produced	TE Utilized
34	Bedbid	250	112	104	33
35	Mudhabi	600	146	144	116
36	Algzee	180	13	12	0
37	Ibra	4500	1475	1271	118
38	Bidiya	300	259	235	160
39	Qabil	250	171	166	104
40	Snaw	120	81	77	55
41	Maqal	150	5	4	5
42	Badha	500	167	162	150
43	Sur	1400	1165	1149	286
44	Jalan Bni Bu Ali	600	581	525	109
45	Jaalan Bni Bu Hassn	600	507	455	162
46	Kamil Wafi	250	298	263	82
47	Sur- Bond	2000	2262	2093	161
48	Maserah	1800	940	916	97
49	Haima	120	62	60	0
50	Nagdha	120	159	153	0

Source: Haya (2016)

All these plants use tertiary treatment (mostly activated sludge).

1.2 Wastewater flow projections for years 2013, 2015, 2020 and 2025 (m^3/day)

2013	2015	2020	2025
90,865	165,750	324,134	364,282

Source: Al Muselhi (2014)

1.3 Raw wastewater characteristics at Al Ansab STP, Muscat Governorate, Oman

Parameter	Units	Minimum	Maximum
Biological Oxygen Demand (BOD5)	mg/l	350	400
Chemical Oxygen Demand (COD)	mg/l	600	900
Total Suspended Solids (TSS)	mg/l	350	500
Volatile Suspended soli(VSS)	mg/l	280	400
VSS/TSS ratio	%	75	85
Total Kjeldahl Nitrogen (TKN)	mg/l	50	70
Ammonia Nitrogen (NH3-N)	mg/l	35	45
Total Phosphorus (TP)	mg/l	9	15
Total Alkalinity (as CaCO3)	mg/l	100	200
Oil and Grease (O&G)	mg/l	-	200
pH	mg/l	6.0	8.0
Temp	℃	20	35

Source: Al Waheibi (2015)

1.4 Treated wastewater quality from the Al Ansab plant parameters

Treated wastewater quality parameters	Units	Effluent from Al Ansab Plant	Class A (Agricultural irrigation permissible limits)
BOD	mg/l	< 3	15
TSS	mg/l	1	15
NH3 as N	mg/l	< 0.16	5
Organic N as N	mg/l	-	5
NO3 as N	mg/l	16	50
Total P as P	mg/l	3	30
pH	-	7	6-9
Fats, Oil & Greases	mg/l	-	0.5
Total Dissolved Solids (TDS)	mg/l	-	1500
Total alkalinity (as CaCO3)	mg/l	-	-
Faecal Coliform Bacteria	MPN/100 mg/l	10	200
Visible Helminth ova	Number mg/l	0	< 1
Turbidity	NTU	-	-

Source: Haya (2016)

1.5 The quantity of total dewatered sludge, bulking agent (green waste), and compost produced in Muscat for years 2010, 2015, 2020 and 2025 (kg/day)

year	**2010**	**2015**	**2020**	**2025**
Total dewatered sludge (kg/day)	97,690	200,886	252,615	281,790
Quantity of bulking agent to be added (kg/day)	97,690	200,886	252,615	281,790
Quantity of produced compost (tons/day)	36,233	75,332	94,731	105,671

Source: OWSC (2005)

Appendix 2 International standards and policies of treated wastewater and sludge management

2.1 Indicative log removal of indicator microorganisms and enteric pathogens during various stages of wastewater treatment

	Indicator microorganisms			**Pathogenic microorganisms**				
Type of Microorganisms	*E.Coli*	*Clostridium perfringens*	*Phag*	*Enteric bacteria*	*Enteric viruses*	*Giardia lamblia*	*Crypotosporidium parvum*	*Helminths*
Bacteria	X	X		X				
Protoza and Helminths						X	X	X
Viruses			X		X			
Indicative log reduction in various stages of wastewater treatment								
Secondary treatment	1-3	0.5-1	0.5-2.5	1-3	0.5-2	0.5-1.5	0.5-1	0-2
Dual media filtration	0-1	0-1	1-4	0-1	0.5-3	1-3	1.5-2.5	2-3
Membrane filtration(UF, NF, RO)	4-> 6	>6	2-> 6	> 6	2-> 6	> 6	4-> 6	> 6
Reservoir storage	1-5	NA	1-4	1-5	1-4	3-4	1-3.5	1.5-> 3
Ozonation	2-6	0-.5	2-6	2-6	3-6	2-4	1-2	NA

UV disinfection	2-> 6	NA	3-> 6	2-> 6	1-> 6	3-> 6	3-> 6	NA
Advanced Oxidation	> 6	NA	> 6	> 6	> 6	> 6	> 6	NA
Chlorination	2-> 6	1-2	0-2.5	2-6	1-3	0.5-1.5	0-0.5	0-1

Source: US-EPA (2012)

2.2 Survival of excreted pathogens (at 20-30 °C)

Type of pathogen	**In the soil**	**On crops**
Enteroviruses	< 100 but usually < 20 days	< 60 but usually < 15days
Bacteria	70 but usually < 20 days	< 30 but usually <15 days
***Salmonella* spp.**	70 but usually < 20 days	< 30 but usually < 15 days
Vibrio cholerae	20 but usually <10 days	< 5 but usually < 2 days
Protozoa		
Entamoeba histolytica cysts	<20 but usually <10 days	<10 but usually < 2 days
Helminths		
***Ascaris lunbricoides* eggs**	Many months	< 60 but usually < 30 days
Hookworm Larvae	90 but usually < 30 days	< 30 but usually < 10 days
***Taenia saginata* eggs**	Many months	< 60 but usually < 30 days
***Trichuris trichiura* eggs**	Many months	< 60 but usually < 30 days

Source: WHO (2006) adapted from WHO (1989)

2.3 Water requirements, sensitivity to water supply of some selected crops

Crop	Water requirements (mm/growing period)	Sensitivity to water supply (ky)
Alfalfa	800-1600	low to medium-high (0.7-1.1)
Banana	1200-2200	High (1.2-1.35)
Bean	300-500	Medium-high (1.15)
Cabbage	380-500	Medium-low (0.95)
Citrus	900-1200	Low to medium-high (0.8-
Cotton	700-1300	Medium-low (0.85)
Groundnut	500-700	Low (0.7)
Maize	500-800	High (1.25)
Potato	500-700	Medium-high (1.1)
Rice	350-700	High
Safflower	600-1200	Low (0.8)
Sorghum	450-650	Medium-low (0.9)
Wheat	450-650	Medium high (spring: 1.15; winter: 1.0)

Source: FAO (1992)

2.4 Irrigation methods for some selected crops

Irrigation method	**Topography**	**Crops**
Widely spaced borders	Land slopes capable of being graded to less than 1% slope and preferably 0.2%	Alfalfa and other deep rooted close-growing crops and orchards
Graded contour furrows	Variable land slopes of 2-25 % but preferable less	Row crops and fruit
Rectangular checks (levees)	Land slopes capable of being graded so single or multiple tree basins will be levelled within 6 cm	Orchard
Sub-irrigation	Smooth-flat	Shallow rooted crops such as potatoes or grass
Sprinkler	Undulating 1- > 35% slope	All crops
Localized (drip, trickle, etc.)	Any topographic condition suitable for row crop farming	Row crops or fruit

Source: FAO (1992)

2.5 Examples of effective sludge treatment processes

Process	Descriptions
Sludge Pasteurization	Minimum of 30 minutes at 70°C or minimum of 4 hours at 55°C (or appropriate intermediate conditions), followed in all cases by primary mesophilic anaerobic digestion
Mesophilic Anaerobic Digestion	Mean retention period of at least 12 days of primary digestion in temperature range 35 °C +/- 3°C or of at least 20 days primary digestion in temperature range 25°C + /- 3°C followed in each case by a secondary stage which provides a mean retention period of at least 14 days
Thermophilic Aerobic Digestion	Mean retention period of at least 7 days digestion. All sludge to be subject to a minimum of 55°C for a period of at least 4 hours
Composting (Windrows or Aerated Piles)	The compost must be maintained at 40 °C for at least 5 days and for 4 hours during this period at a minimum of 55 °C within the body of the pile followed by a period of maturation adequate to ensure that the compost reaction is substantially complete.
Lime Stabilization of Liquid Sludge	Addition of lime to raise pH to greater than 12.0 and sufficient to ensure that the pH is not less than 12 for a minimum period of 2 hours. The sludge can then be used directly
Liquid Storage	Storage of untreated liquid sludge for a minimum period of 3 months
Dewatering and Storage	Conditioning of untreated sludge with lime or other coagulants followed by dewatering and storage of the cake for a minimum period of 3 months if sludge has been subject to primary mesophilic anaerobic digestion, storage to be for a minimum period of 14 days

Source: (FAO,1992)

Appendix 3 Statistical analyses in phytoremediation method (plant growth)

3.1 Statistical analysis of shoots and roots biomass in (g/pot) for both plants

Source	DF	Shoots biomass			Roots biomass		
		Sum of Squares	F Ratio	Prob>F	Sum of Squares	F Ratio	Prob>F
Treatment	7	639.7904	331.9998	< .0001	580.6992	429.6179	< .0001
Rep	2	1.17671	2.1372	0.1549	0.73257	1.8969	0.1866
Error	14	3.85416			2.70333		
C. Total	23	644.8213			584.1351		

3.2 TPH% content in all treatments of Phytoremediation method

Treatment	**T1**	**T1**	**T1**
TPH%	0.97	0.95	0.89
Treatment	**T2**	**T2**	**T2**
TPH%	0.30	0.33	0.34
Treatment	**T3**	**T3**	**T3**
TPH%	0.34	0.35	0.32
Treatment	**T4**	**T4**	**T4**
TPH%	0.28	0.24	0.28
Treatment	**T5**	**T5**	**T5**
TPH%	0.42	0.38	0.39
Treatment	**T6**	**T6**	**T6**
TPH%	0.33	0.31	0.33
Treatment	**T7**	**T7**	**T7**
TPH%	0.33	0.38	0.33

The initial amount of TPH in the contaminated soil was 1.15%

T1: contaminated soil, T2: contaminated soil with Bermuda grass, T3: contaminated soil with Rye grass. T4: contaminated soil with 10% compost with Bermuda grass. T5: contaminated soil with 10% compost and Rye grass. T6: contaminated soil with 20% compost and Bermuda grass. T7: contaminated soil with 20% compost and Rye grass, T8: Clean soil and Bermuda grass .T9: Clean soil and Rye grass.

3.3 Statistical analysis of TPH% removal in contaminated soil

Source	DF	Sum of Squares	F Ratio	Prob>F
Treatment	6	1.647676	423.9828	< .0001
Rep	2	0.000512	0.5273	0.6001
Error	12	0.007772		
C. Total	20	1.655961		

````**1** **3.4** Statistical analysis of total extractable metals in (mg/kg) in soil

| Source | DF | Mn | | | Fe | | |
|---|---|---|---|---|---|---|---|
| | | Sum of Squares | F Ratio | Prob>F | Sum of Squares | F Ratio | Prob>F |
| **Treatment** | 8 | 24457.290 | 0.590 | 0.772 | 7827659 | 0.447 | 0.874 |
| **Rep** | 2 | 3743.170 | 0.361 | 0.702 | 24528790 | 5.610 | 0.0142 |
| **Error** | 16 | 82864.810 | | | 34976438 | | |
| **C. Total** | 26 | 111065.270 | | | 67332887 | | |
| **Source** | **DF** | **Zn** | | | **Pb** | | |
| | | **Sum of Squares** | **F Ratio** | **Prob>F** | **Sum of Squares** | **F Ratio** | **Prob>F** |
| **Treatment** | 8 | 7513.333 | 0.954 | 0.502 | 24866.3 | 0.621 | 0.748 |
| **Rep** | 2 | 530.667 | 0.269 | 0.767 | 54558.74 | 5.449 | 0.0157 |
| **Error** | 16 | 15738.667 | | | 80087.26 | | |
| **C. Total** | 26 | 23782.667 | | | 159512.3 | | |
| **Source** | **DF** | **Ni** | | | **Cu** | | |
| | | **Sum of Squares** | **F Ratio** | **Prob>F** | **Sum of Squares** | **F Ratio** | **Prob>F** |
| **Treatment** | 8 | 63146 | 1.905 | 0.1296 | 5.0328 | 12.26 | 0.001 |
| **Rep** | 2 | 1338.89 | 0.161 | 0.8521 | 0.364 | 3.55 | 0.053 |
| **Error** | 16 | 66263.78 | | | 0.820 | | |
| **C. Total** | 26 | 130748.67 | | | 6.2168 | | |
````

3.5 Statistical analysis of DTPA extractable metals in (mg/kg) in soil

Source	DF	Mn			Fe		
		Sum of Squares	F Ratio	Prob>F	Sum of Squares	F Ratio	Prob>F
Treatment	8	1.577929	6.1513	0.001	60.29159	8.7473	0.0001
Rep	2	0.047523	0.741	0.4923	3.577325	2.076	0.1579
Error	16	0.513041			13.78522		
C. Total	26	2.138492			77.65414		
Source	**DF**	**Zn**			**Pb**		
		Sum of Squares	**F Ratio**	**Prob>F**	**Sum of Squares**	**F Ratio**	**Prob>F**
Treatment	8	57.99431	24.5735	< .0001	0.377609	7.4873	0.0003
Rep	2	0.803419	1.3617	0.2844	0.011595	0.9196	0.4187
Error	16	4.720077			0.100867		
C. Total	26	63.51781			0.490071		

Source DF		Ni		
		Sum of Squares	F Ratio	Prob>F
Treatment	8	0.18391	6.1121	0.0011
Replicates	2	0.043804	5.8231	0.0126
Error	16	0.060179		
C. Total	26	0.287893		

3.6 Statistical analysis of heavy metals in (mg/kg) in shoots for both plants

Source	DF	Mn			Zn		
		Sum of Squares	F Ratio	Prob>F	Sum of Squares	F Ratio	Prob>F
Treatment	7	119165.6	353.2023	< .0001	721.2016	5.4312	0.0035
Rep	2	44.07	0.4572	0.6422	4.13411	0.109	0.8975
Error	14	674.77			265.578		
C. Total	23	119884.5			990.9137		

Source	DF	Cu		
		Sum of Squares	F Ratio	Prob>F
Treatment	7	624.250	7.9609	0.0005
Replicates	2	6.15396	0.2747	0.7638
Error	14	156.829		
C. Total	23	787.234		

3.7 Statistical analysis of heavy metals in (mg/kg) in roots for both plants

Source	DF	Mn			Zn		
		Sum of Squares	F Ratio	Prob>F	Sum of Squares	F Ratio	Prob>F
Treatment	7	19033.33	18.045	< .0001	8917.173	1.2783	0.3285
Rep	2	556.073	1.8452	0.1944	1985.894	0.9964	0.394
Error	14	2109.546			13952.12		
C. Total	23	21698.94			24855.19		
Source	**DF**	**Ni**			**Cu**		
		Sum of Squares	**F Ratio**	**Prob>F**	**Sum of Squares**	**F Ratio**	**Prob>F**
Treatment	7	1383.437	5.2729	0.0041	336.2506	1.1637	0.3816
Rep	2	0.1579	0.0021	0.9979	64.56299	0.782	0.4765
Error	14	524.7388			577.9216		
C. Total	23	1908.334			978.7352		

Appendix 4 Statistical analyses in the bioremediation method

4.1 TPH% content in all treatments of bioaugmentation method

Initial amount of TPH in the contaminated soil was 0.90%

Strains	**Control**	**Control**	**Control**
TPH%	0.87	0.86	0.87
Strains	**Strain 25**	**Strain 25**	**Strain 25**
TPH%	0.470	0.470	0.48
Strains	**Strain 26**	**Strain 26**	**Strain 26**
TPH%	0.48	0.48	0.47
Strains	**Strain 29**	**Strain 29**	**Strain 29**
TPH%	0.50	0.50	0.44
Strains	**Strain 30**	**Strain 30**	**Strain 30**
TPH%	0.48	0.47	0.47
Strains	**Strain 35**	**Strain 35**	**Strain 35**
TPH%	0.53	0.52	0.56
Strains	**Strain 36**	**Strain 36**	**Strain 36**
TPH%	0.47	0.48	0.46
Strains	**Strain 37**	**Strain 37**	**Strain 37**
TPH%	0.44	0.51	0.52
Strains	**Strain 38**	**Strain 38**	**Strain 38**
TPH%	0.52	0.47	0.47
Strains	**Strain 39**	**Strain 39**	**Strain 39**
TPH%	0.48	0.49	0.46
Strains	**Strain 40**	**Strain 40**	**Strain 40**
TPH%	0.46	0.47	0.49
Strains	**Strain 41**	**Strain 41**	**Strain 41**
TPH%	0.45	0.44	0.52
Strains	**Strain 62**	**Strain 62**	**Strain 62**
TPH%	0.48	0.45	0.48
Strains	**Strain 101**	**Strain 101**	**Strain 101**
TPH%	0.49	0.49	0.48
Strains	**Strain 102**	**Strain 102**	**Strain 102**
TPH%	0.47	0.50	0.42
Strains	**Strain 132**	**Strain 132**	**Strain 132**
TPH%	0.46	0.47	0.47
Strains	**Strain 141**	**Strain 141**	**Strain 141**
TPH%	0.47	0.48	0.44
Strains	**Strain unique**	**Strain unique**	**Strain unique**
TPH%	0.44	0.52	0.43

4.2 Probabilities of hydrocarbon degradation through bioaugmentation method

Source	**Df**	**Sum of Squares**	**F Ratio**	**Prob>F**
Treatments	17	0.02680231	2.3	0.0174
Rep	2	0.0006747	0.49	0.6114
Error	34	0.0229793		
C. total	53	0.05045631		

4.3 Probabilities through biostimulation method after incubating for 7 days

Source	**DF**	**Sum of Squares**	**F Ratio**	**Prob>F**
Bacteria	3	0.13767356	15.345	0.0002
Factors	2	0.07766706	12.985	0.001
Replicates	2	0.00001838	0.0061	0.938
Bacteria *factors	6	0.12658961	7.055	0.0021
Bacteria*Replicates	6	0.00931346	1.038	0.410
Factors* Replicates	4	0.00556225	0.931	0.421
Bacteria*Factors* Replicates	12	0.00966042	0.538	0.769

Bacteria (no strains, strain 102, strain 141 and strain unique)

Factors (contaminated soil, compost and urea)

4.4 Probabilities through biostimulation method after incubating for 14 days

Source	**DF**	**Sum of Squares**	**F Ratio**	**Prob>F**
Bacteria	3	0.22113653	48.070	< .0001
Factors	2	0.113442	36.989	< .0001
Replicates	2	0.00026004	0.169	0.687
Bacteria*factors	6	0.04145956	4.506	0.012
Bacteria*Replicates	6	0.00048113	0.104	0.955
Factor* Replicates	4	0.00518108	1.689	0.225
Bacteria*Factors* Replicates	12	0.00244725	0.266	0.942

4.5 Probabilities of alkanes concentration after incubating for 7 days

Source	**DF**	**Sum of Squares**	**F Ratio**	**Prob>F**
Bacteria	3	2475891	196.969	< .0001
Factors	2	1358790	152.650	< .0001
Replicates	2	5269873	2.66	0.1289
Bacteria*factors	6	0.0687921	5.080	0.0082
Bacteria*Replicates	6	25263	2.427	0.116
Factor* Replicates	4	5732	1.832	0.2021
Bacteria*Factors*	12	59782	3.010	0.0493

4.6 Probabilities of alkanes concentration after incubating for 14 days

Source	**DF**	**Sum of Squares**	**F Ratio**	**Prob>F**
Bacteria	3	2354949	327.315	< .0001
Factors	2	1278379	266.524	< .0001
Replicates	2	5233.1	2.182	0.165
Bacteria*factors	6	1309465	91.001	< .0001
Bacteria*Replicates	6	23000.4	3.196	0.0624
Factor* Replicates	4	5532.6	1.153	0.348
Bacteria*Factors* Replicates	12	53681.2	3.730	0.0249

Appendix 5 Statistical analysis in agricultural experiment

5.1 Chemical analysis of GW and TWW (mg/l) for growing Radish and Beans at AES In agricultural experiment

Water	Mn	Fe	Zn	Cu	Cr	Cd	Pb	Ni	B
GW	0.002	0.013	0.013	0.008	< 0.002	< 0.001	<0.001	< 0.001	0.29
TWW	0.002	0.016	0.064	0.024	< 0.002	< 0.001	0.066	< 0.001	0.50
EPA Standard	0.200	5.000	5.000	0.500	0.100	0.010	0.100	0.100	0.75
FAO Standard	0.200	5.000	2.000	0.200	0.100	0.010	0.500	0.200	0.75
Omani Standard	0.500	5.000	5.000	1.000	0.050	0.010	0.200	0.100	0.75

5.2 Statistical analysis of heavy metals concentration in (mg/kg) for initial soil samples

Source	DF	Fe			Zn		
		Sum of Squares	**F Ratio**	**Prob>F**	**Sum of Squares**	**F Ratio**	**Prob>F**
Treatment	3	0.00314	2.08654	2.158	1.708209	30.857	0.1104
Site	1	0.000212	0. 511	0.40171	0.000210	0.01301	0.9522
Rep	3	0.000809	0.8314	0.4350	0.01358	0.6254	0.40547
Treatment *site	3	0.0002841	0.11721	0.93126	0.009300	1.90258	0.1087

Source	DF	Cu			B		
		Sum of Squares	**F Ratio**	**Prob> F**	**Sum of Squares**	**F Ratio**	**Prob>F**
Treatment	3	50.2145	245.021	0.101	20.650540	0.6110	0.5821
Site	1	0.11151	3.51306	0.0729	0.03241	0.3478	0.5814
Rep	3	0.530061	8. 9490	0.0614	11.280083	0.9451	0.4047
Treatment *site	3	0. 4312	4.03510	0.0785	0.063214	1.2308	0.2107

5.3 Statistical analysis in soil samples after harvesting in agricultural experiment

Source	**DF**	**pH**			**EC (mS/cm)**		
		Sum of Squares	**F Ratio**	**Prob>F**	**Sum of Squares**	**F Ratio**	**Prob>F**
Treatment	3	4.043	5.51	0.005	26.387	5.24	0.006
Site	1	4.06	16.59	<0.001	12.363	7.37	0.012
Replicates	3	0.180625	0.5736	0.4736	2.052056	0.6782	0.4374
Treatment*site	3	6.301	8.58	<0.001	4.971	0.99	0.415

Source	**DF**	**TOC (mg/kg)**		
		Sum of Squares	**F Ratio**	**Prob > F**
Treatment	3	11.043925	86.8599	< .0001
site	1	0.009025	0.2129	0.6585
Replicates	3	0.099225	2.3412	0.1698
Treatment*site	3	0.215925	1.6982	0.2536

5.4 Statistical analysis of heavy metals in (mg/kg) in soil samples after harvesting

Source	DF	Fe			Zn		
		Sum of Squares	F Ratio	Prob>F	Sum of Squares	F Ratio	Prob>F
Treatment	3	2451.3964	4.0856	0.139	7.0982093	3.8577	0.1484
Site	1	0.012012	0.01561	0.90160	0.23375	0.8998	0.3522
Rep	3	824.8094	4.124	0.1352	0.4227401	0.6892	0.4673
Treatment *site	3	2.723363	1.17972	0.33826	0.93755	1.20297	0.30087
Source	**DF**	**Cu**			**B**		
		Sum of Squares	**F Ratio**	**Prob>F**	**Sum of Squares**	**F Ratio**	**Prob>F**
Treatment	3	0.600249	5.0224	0.109	29.550546	0.7513	0.5901
Site	1	0.06151	1.52306	0.2291	0.03087	0.3133	0.5808
Rep	3	0.2308601	5.7949	0.0953	12.381085	0.9444	0.4028
Treatment *site	3	0.04308	0.35561	0.7855	0.06025	1.0508	0.2008
Source	**DF**	**Ni**			**Ag**		
		Sum of Squares	**F Ratio**	**Prob>F**	**Sum of Squares**	**F Ratio**	**Prob>F**
Treatment	3	0.2521671	1.8337	0.3154	0.00255838	1.7022	0.3365
Site	1	0.15345	1.5694	1.5694	0.008418	25.929	<0.0001
Rep	3	0.0918061	2.0028	0.252	0.000578	1.1537	0.3615
Treatment *site	3	0.178935	0.6100	0.61504	0.015178	15.585	0.7745
Source	**DF**	**Cd**			**Cr**		
		Sum of Squares	**F Ratio**	**Prob>F**	29.7014	25.2117	0.87496
Treatment	3	0.002109	2.1391	0.2742	0.02547	0.02972	0.8671
Site	1	0.000274	0.52062	0.4775	1.545872	6.25424	0.09547
Replicates	3	0.000270	0.8223	0.4314	0. 5478	0.35547	0.83765
Treatment*site	3	0.000228	0.14458	0.93214	25.7140	28.24	0.1147

5.5 Statistical analysis of yield in plant samples

Source	DF	Beans plants			Radish plants		
		Sum of Squares	F Ratio	Prob>F	Sum of Squares	F Ratio	Prob>F
Treatment	3	326.6624	10.4848	0.00013	24960057	4.3988	0.01332
Site	1	0.08	0.00770	0.93078	23538375	12.4493	0.00172
Replicates	3	2889.06	0.0285	0.8706	5670352	2.1708	0.1841
Treatment*site	3	31.15322	0.99992	0.40987	9460006.5	1.66719	0.20058

5.6 Statistical analysis of TN% in soil and plant samples

Source	DF	Soil			Beans plant		
		Sum of Squares	F Ratio	Prob>F	Sum of Squares	F Ratio	Prob>F
Treatment	3	0.001434	1.5216	0.2497	0.20166	3.3611	0.5123
Site	1	0.0009	2.8653	0.1112	0.01333	0.33333	0.7230
Replicates	3	0.00015	0.4776	0.5001	0.24000	0.02000	0.3254
Treatment*site	3	0.001019	1.0809	0.3871	0.14333	1.1944	0.3722
Source	**DF**	**Radish leaves**			**Radish roots**		
		Sum of Squares	**F Ratio**	**Prob>F**	**Sum of Squares**	**F Ratio**	**Prob>F**
Treatment	3	1.84833	19.982	≤ 0.0001	0.070545	1.8322	0.1950
Site	1	0.120000	1.9459	0.1854	0.262252	10.217	0. 2600
Replicates	3	0.370000	0.90025	0.1225	0.154013	0.9587	0.3547
Treatment*site	3	0.186667	1.0090	0.4633	0.050590	0.65695	0.6854

5.7 Statistical analysis of Chlorophyll content in (CCI)

Source	DF	Beans plants			Radish plants		
		Sum of Squares	F Ratio	Prob>F	Sum of Squares	F Ratio	Prob>F
Treatment	3	119.5225	39.8408	0.00024	326.662	10.4848	0.00013
Site	1	4.35125	1.04618	0.316588	0.08	0.00770	0.93078
Replicates	3	28.89062	2.3755	0.1672	78.10141	2.8886	0.133
Treatment*site	3	8.62125	0.69094	0.56649	31.15322	0.99992	0.40987

5.8 Statistical analysis of heavy metals in (mg/kg) in root of Radish plant

Source	DF	Fe			Zn		
		Sum of Squares	F Ratio	Prob>F	Sum of Squares	F Ratio	Prob>F
Treatment	3	100995.7	9628.60	< .0001	120.7479	14.0585	0.0285
Site	1	74.36816	0.84158	0.36807	6.940607	6.63373	0.0165
Rep	3	1.18	0.3363	0.6027	0.36551	0.1277	0.7445
Treatment *site	3	273.6576	1.03228	0.396	2.02938	0.64655	0.59273

Source	DF	Cu			B		
		Sum of Squares	F Ratio	Prob> F	Sum of Squares	F Ratio	Prob>F
Treatment	3	26.69038	27.43	0.0111	49.71175	4.1705	0.1357
Site	1	0.015225	0.02513	0.8753	7.09702	5.0845	0.0335
Rep	3	2.536878	7.8215	0.068	4.43201	0.1115	0.7604
Treatment *site	3	0.606089	0.33356	0.8011	2.245234	0.5361	0.6619

Source	DF	Cr			Ni		
		Sum of Squares	F Ratio	Prob>F	Sum of Squares	F Ratio	Prob>F
Treatment	3	49.41523	243.78	0.0004	50.3369	46.692	0.0051
Site	1	0.118037	3.2617	0.0834	0.163878	1.1387	0.2965
Rep	3	0.597325	8.8404	0.0589	0.876157	2.4382	0.2163
Treatment *site	3	0.434987	4.0066	0.01910	0.795084	1.8415	

Source	DF	Ag			Cd		
		Sum of Squares	F Ratio	Prob>F	Sum of Squares	F Ratio	Prob>F
Treatment	3	0.021366	14.459	0.0274	0.028552	5.0204	0.109
Site	1	0.001219	2.4651	0.1294	0.001365	1.3297	0.26020
Rep	3	0.002211	4.4892	0.1243	0.004851	2.559	0.208
Treatment *site	3	0.001268	0.8545	0.47798	0.006524	2.1184	0.12436

		Mn		
Source	**DF**	**Sum of Squares**	**F Ratio**	**Prob>F**
Treatment	3	64.127002	22.92636	0.0316
Site	1	1.612359	0.406795	0.52964
Replicates	3	0.0282031	4.2635	0.1309
Treatment*site	3	5.834936	0.490714	0.692028

5.9 Statistical analysis of Leaves in (mg/kg) of Radish plant

Source	DF	Fe			Zn		
		Sum of Squares	F Ratio	Prob>F	Sum of Squares	F Ratio	Prob>F
Treatment	3	150353.74	98.1241	0.0017	1707.5221	103.123	0.0016
Site	1	57.65232	0.25765	0.61637	0.38658	0.08837	0.7688
Rep	3	428.45	0.8388	0.4273	5.2237	0.9464	0.4024
Treatment* site	3	340.4163	0.50711	0.68109	6.713518	0.51159	0.67811

Source	DF	Cu			B		
		Sum of Squares	F Ratio	Prob>F	Sum of Squares	F Ratio	Prob>F
Treatment	3	8.560941	4.8669	0.1132	19.534873	11.334	0.0382
Site	1	0.010332	0.03373	0.8558	2.608956	13.753	0.0010
Rep	3	0.11496	0.1961	0.6879	0.95082	0.1655	0.7114
Treatment* site	3	0.21517	0.23421	0.8716	1.398073	2.4568	0.08747

Source	DF	Ni			Ag		
		Sum of Squares	F Ratio	Prob>F	Sum of Squares	F Ratio	Prob>F
Treatment	3	71.005501	11.170	0.029	0.30464534	11.174	0.039
Site	1	0.393833	0.8655	0.3614	0.000857	0.9760	0.33030
Rep	3	0.9786	0.4619	0.5455	0.00935028	1.0289	0.3851
Treatment* site	3	1.253595	0.9183	0.4468	0.004686	1.7780	0.17822

Source	DF	Mn			CD		
		Sum of Squares	F Ratio	Prob>F	Sum of Squares	F Ratio	Prob>F
Treatment	3	10.866672	16.742	0.0443	0.60740563	30.607	0.0095
Site	1	0.010804	0.0499	0.82505	0.005382	1.1813	0.28787
Rep	3	0.12569	0.8654	0.7547	0.02820313	4.2635	0.1309
Treatment* site	3	0.0246342	0.0379	0.98986	0.01593	1.1655	0.34345

Source	DF	Cr		
		Sum of Squares	F Ratio	Prob>F
Treatment	3	40.13214	20.58742	0.0812
Site	1	1.212012	0.302148	0.5325
Replicates	3	0.035471	3.12547	0.10214
Treatment*site	3	4.21540	0.3254	0.78745

5.10 Statistical analysis of heavy metals in (mg/kg) of Bean plant

Source	DF	Fe			Zn		
		Sum of Squares	F Ratio	Prob>F	Sum of Squares	F Ratio	Prob>F
Treatment	3	2000.3062	4.1359	0.209	734.5753	15.2586	0.0254
Site	1	1.233039	0.18613	0.67000	0.1682	0.05039	0.82427
Rep	3	810.800	4.294	0.1550	0.24851	0.0155	0.9088
Treatment *site	3	3.901877	0.19634	0.89785	3.209911	0.32059	0.81038

Source	DF	Cd			Cu		
		Sum of Squares	F Ratio	Prob>F	Sum of Squares	F Ratio	Prob>F
Treatment	3	2.11059	34.643	0.0079	3.274065	0.2982	0.8266
Site	1	0.04132	4.8652	0.03722	0.076392	0.0708	0.79230
Rep	3	0.01390	0.6846	0.4687	5.1216	1.3993	0.322
Treatment *site	3	0.15081	5.9178	0.00358 8	3.247658	1.0047	0.40779

Source	DF	B			Cr		
		Sum of Squares	F Ratio	Prob>F	Sum of Squares	F Ratio	Prob>F
Treatment	3	22.88429	6.6719	0.0767	11.44817	3.0334	0.1932
Site	1	1.881315	0.8869	0.35568	0.42389	2.4560	0.13016
Rep	3	1.07494	0.094	0.7792	0.41907	0.3331	0.6043
Treatment *site	3	3.809025	0.5985	0.62213	0.843594	1.6293	0.20887

Source	DF	Ni			Ag		
		Sum of Squares	F Ratio	Prob>F	Sum of Squares	F Ratio	Prob>F
Treatment	3	12.07093	13.015	0.0317	1.869015	30.511	0.0095
Site	1	0.083999	0.4031	0.53161	9.3 E-06	0.0100	0.9211
Rep	3	0.010224	0.0331	0.8673	0.012641	0.6191	0.4888
Treatment *site	3	0.195905	0.3134	0.81549	0.005526	1.9722	0.14504