INFLUENCE OF TREATED SEWAGE EFFLUENTS ON SAUDI COAST OF THE ARABIAN GULF FROM WATER QUALITY AND MICROBIAL ASPECTS

BY

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A Thesis Presented to the
DEANSHIP OF GRADUATE STUDIES

KING FAHD UNIVERSITY OF PETROLEUM & MINERALS

DHAHRAN, SAUDI ARABIA

In Partial Fulfillment of the
Requirements for the Degree of

MASTER OF SCIENCE

In

ENVIRONMENTAL SCIENCE

MAY, 2015

KING FAHD UNIVERSITY OF PETROLEUM & MINERALS

DHAHRAN- 31261, SAUDI ARABIA

DEANSHIP OF GRADUATE STUDIES

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This thesis is dedicated to my parents, may Allah be merciful on them in this world and the hereafter.

ACKNOWLEDGMENTS

All thanks and adorations are due to Allah, the Gracious Lord for seeing me through the completion of this thesis work. My gratitude goes to the government of Saudi Arabia, and King Fahd University of Petroleum and Minerals for the award of a postgraduate scholarship.

I sincerely appreciate my thesis advisor and co-advisor, Dr. Amjad Khalil and Dr. Mohammed Qurban respectively for their unwavering support, guidance, patience and understanding. Likewise, my profound acknowledgement goes to members of the thesis committee, Dr. Manikandan K. P, Dr. Ruben Roa-Ureta and Dr. Alexis Nzila for their invaluable ideas and constructive criticisms.

I am grateful to the Marine Studies Section of the Centre for Environment and Water, Research Institute, King Fahd University of Petroleum and Minerals for affording me the facilities and logistical support required for field and lab work. I register my appreciation to the following staff of the centre for the assistance they provided during field sampling and laboratory analysis: Mr. Anwar M. Al-Mosawi, Mr. Sultan, Mr. Premlal Panickan, Mr. Zahid Umar, Mr. Jesusito Vicente, Mr. Arvin Balala, Mr. Zain and Mr. Hassan Al-Muhanna. Thanks to Dr. Wafa for his technical advice on nutrient analysis. My acknowledgement also goes to Mr. Sudibyo Solikan for helping in the creation of the field map.

Thanks to the Department of Life Science for providing me the laboratory space and resources for the microbiological studies. I express my gratitude to the research technicians in the Biology Lab - Mr. Deepak Anand and Mr. Saravanan Sankaran. I

acknowledge the respectable faculties, staff and student colleagues of the Department of Earth Sciences. Thanks to all my friends and members of the Nigerian Community in KFUPM for making my stay in the university socially worthwhile.

Special thanks to my parents, my wife and baby, and my siblings for their prayers and moral supports.

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LIST OF ABBREVIATIONS

STP - Sewage Treatment Plants

TCC - Total Coliform count

HPC - Heterotrophic plate count

CFU - Colony forming units

BOD - Biochemical Oxygen Demand

COD - Chemical Oxygen Demand

DO - Dissolved oxygen

Chl a - Chlorophyll a

TSS - Total suspended solids

PME - Presidency of Meteorology and Environment

ABSTRACT

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Thesis Title : Influence of Treated Sewage Effluents on Saudi Coast of the Arabian Gulf from Water Quality and Microbial Aspects

Major Field : Environmental Science

Date of Degree : May, 2015

This study investigated the influence of sewage effluents on water quality and microbial aspects of Saudi Arabian coastal waters of the Arabian Gulf. Water samples were analysed for nutrients, chlorophyll *a*, heterotrophic plate counts, total Coliforms and environmental variables in three outfall locations and a control location (Half Moon Bay). All three outfalls were found to serve as important sources of inorganic nutrient into the coastal waters. Significantly higher concentrations of nitrate and nitrite in Dammam and Rahima; ammonia in Al-Khobar and Dammam; and phosphate in all three outfall locations were recorded relative to the control. The mean nitrate, nitrite, ammonia, and phosphate concentrations in sampling stations ranged from 0.30 to 29.12 µmol/L, 0.17 to 10.66 µmol/L, 0.51 to 1.15 µmol/L, 0.35 to 25.00 µmol/L and 0.62 to 64.76 µg/L respectively. Chlorophyll *a* concentrations were significantly high only in Dammam at levels indicative of poor water quality. Total Coliform counts in sampling stations ranged from 4000 to 310000 CFU/100ml while heterotrophic plate counts ranged from 70 to 210000 CFU/ml. The lowest bacteria counts in effluent samples were recorded in Rahima. Microbiological quality of stations close to the outfalls is generally not safe for activities such as recreation and shell-fish harvesting based on international standards.

Keywords: Sewage pollution, Nutrients, Chlorophyll *a*, Bacteria, Saudi Arabian Gulf coast

ملخص الرسالة

الاسم الكامل:عبد الرحمن أكينكونمى يوسف

عنوان الرسالة:تأثير مخلفات الصرف الصحي المعالجة على جودة المياه ومحتوى الأحياء الدقيقة لمياه الساحل السعودى في الخليج العربى

التخصص:علوم البيئة

تاريخ الدرجة العلمية: مايو 2015

بحثت هذه الدراسة فى تأثير مخلفات مياه الصرف الصحي على جودة المياهومحتوى الأحياء الدقيقةلمياه الساحل السعودى في الخليج العربى وقد تم تحليل المحتوي الغذائي , كلوروفيل أ, الطبقات الغذائية المختلفة المحسوبة, محتوى بكتيريا القولون والمتغيرات البيئية لبعض عينات المياه فى ثلاث مصبات ونقطة التحكم (فى شاطئ نصف القمر).وقد وجد أن الثلاث مصبات تساهم كمصدرمهم للمواد الغذائيةغير العضوية فى المياه الساحلية حيث وجدت تراكيزعالية للنترات والنيتريت فى الدمام والرحيمة ; بينما وجدت الأمونيا فى الخبر والدمام ; أما الفوسفات فقد وجد فى الثلاث أماكن مقارنة بنقطة التحكم. وقد كان متوسط التراكيز للنترات, النيتريت, الأمونيا والفوسفات فى مواقع الدراسة يتراوح من 0.3 إلى 29.12 ميكرومول لكل لتر , 0.17 إلى 10.66 ميكرومول لكل لتر , 0.51 إلى 1.15 ميكرومول لكل لتر , 0.35 إلى 25 ميكرومول لكل لتر و 0.62 إلى 64.64 ميكرومول لكل لتر على التوالي . وقد كانت تراكيز كلوروفيل أ عالية جدا فى الدمام فقط وهذا أشار الي أن المياه قليلة الجودة (ملوثة نسبيا). وقد كانت إجمالي بكتيريا القولون في أماكن الدراسة تتراوح من 4000 إلى 310000 وحدة كولوني لكل 100مليلتر بينما كانت الطبقات الغذائية المختلفة المحسوبة تتراوح من 70 إلى 210000 وحدة كولوني لكل مليلتر. أقل نسبة بكتيريا تم حسابها في مياه المجاري السائلة تم تسجيلها في الرحيمة.المحطات القريبة من المصبات الرئيسية في الغالب غير آمنة للنشاطات مثل الترفيه و إصطياد الأسماك الصدفية نسبة لوجود الأحياء الدقيقة وهذا إستنادا الي المعايير الدولية.

الكلمات الأساسية: التلوث بمياه الصرف الصحي، المواد الغذائية، كلوروفيل أ، بكتيريا ، الساحل السعودى للخليج العربى.

CHAPTER 1

INTRODUCTION

Coastal marine ecosystems provide a range of benefits for humans as they are exploited for seafood, recreational opportunities, aesthetic beauty etc. However, coastal waters also serve as recipients of vast quantities of human wastes. Municipal wastewater is characterized by constituent such as biodegradable organic matter, pathogenic organisms, and dissolved inorganic nutrients(Metcalf & Eddy, 2003). Consequently, introduction of pathogenic microorganisms and excessive nutrient loads via sewage outfalls constitute major concerns in coastal water quality management(Hendricks & Pool, 2012; Ho *et al*., 2008). Input of nutrients from sewage effluents can result into eutrophication of marine ecosystems. Eutrophication leads to environmental and water quality issues such as algal blooms and depletion of dissolved oxygen. The high photosynthetic rate associated with eutrophication can deplete dissolved inorganic carbon and raise pH to extreme levels in daylight (Van de Waal *et al*., 2011). Microbial decomposition of dead algal blooms creates hypoxic or anoxic water which causes fish kills (Ansari *et al*., 2011). Some algal blooms are toxigenic (i.e harmful algal blooms) and have been linked to seafood intoxication, destruction of commercial fisheries, and closure of recreational beaches (Patricia, 2006; Chorus & Bartram, 1999).

Sewage related microbial pollution of coastal waters portends health risks to humans. Direct contact with pathogenic microorganisms in seawater can occur due to occupational

and recreational exposure. During recreational activities such as swimming, surfing, boating and diving, infectious diseases may be contacted either by accidental ingestion or inhalation of contaminated water or through full body contact where the eyes, nose, ears, and wounded soft tissues are exposed (Soller *et al*., 2010). Filter feeding shellfish accumulate and act as carriers of pathogenic bacteria and human viruses, thus, serving as indirect source of infection when consumed (Prato *et al*., 2013).

1.1 Statement of the Problem

Sewage treatment plants (STPs) are designed to remove contaminants in form of suspended solids, biodegradable organics, pathogenic microorganisms, nutrients and toxic substances from large quantities of domestic sewage to varying extents (secondary or tertiary treatment levels) (Pescod, 1992). Nonetheless,contamination of coastal waters with pathogenic microorganisms via sewage effluents is a major public health risk concern(Al-Bahry *et al*., 2009). The long residence time of poorly flushed coastal bay systems implies that nutrients may accumulate in the system over time(Wazniak *et al*., 2005).However, there are only few published reports on the assessment of the influence of treated sewage effluents on microbiological and water quality characteristics in coastal Saudi waters of the Arabian Gulf.

1.2 Significance of the Study

The results of this study will be useful inunderstanding the water quality and the environmental status of the coastal ecosystems in the study area. The amount and types of human pathogens found in coastal waters is related to the epidemiological status of human (and/or animal) population (Belkin & Colwell, 2006). Thus, knowledge of the levels of indicator bacteria and the bacterial diversity in the marine environment can help

provide relevant insights in planning epidemiological studies of pathogenic microorganisms found in the coastal environment. The findings will also provide basis for evaluation of the effectiveness and/or shortfall of the different sewage treatment infrastructures that release effluents in to the coastal waters. Furthermore, the study will provide good baseline data for long-term studies aimed at evaluating the effects of anthropogenic perturbations, and the effectiveness of mitigation measures on microbiological and water quality parameters of the study area.

1.3 ResearchObjectives

The aim of this study was to assess the effects of effluent discharges from sewage treatment plants (STP) on Coastal Saudi Water of the Arabian Gulf from water quality and microbial aspects. The specific objectives were to:

i. assess the water quality (nutrients and chlorophyll *a*) of the sewage outfall locations,
ii. study the microbial diversity in relation to the effect of treated sewage effluents discharge in the coastal waters, and
iii. evaluate the risk of pathogenic bacterial contamination in the coastal waters using indicator bacteria.

CHAPTER 2

LITERATURE REVIEW

2.1 Municipal Wastewater

Municipal wastewater is composed of constituents that are deleterious to the marine environment such as suspended solids, biodegradable organic matter, toxic contaminants, dissolved inorganic nutrients and pathogenicorganisms (Table 2.1)(Metcalf & Eddy, 2003; Pescod, 1992). Conventional municipal wastewater treatment applies a number of methods for the removal of these constituents from sewage before disposal to the coastal waters. These methods are broadly classified into three namely (i) physical processes, including screening, comminution and sedimentation; (ii) chemical processes, including chemical precipitation, adsorption and disinfection; and (iii) biological processes such as in activated sludge process, and biological nutrient removal.These processes are configured into a variety of systems to achieve different of levels of treatment - primary, secondary and tertiary treatment levels(UNESCWA, 2003; Pescod, 1992).

In order to avoid pollution of the marine environment, coastal outfalls are designed to allow adequate dilution and dispersal of effluent in the receiving waters. This will ensure that the self-purification capacity of the natural water is not overwhelmed(Muhammetoglu *et al.*, 2012).Microbiological standards (e.g.Coliform levels) are generally a key consideration in municipal wastewater discharge. However, when the receiving water of sewage effluents is a shallow and poorly flushed system, other parameters such as nutrient loadings become equally important(Barrell *et al.*, 2000;

Salas, 2002).The Presidency of Meteorology and Environment (PME) is the regulatory agency responsible for protecting and monitoring the quality of Saudi water and marine environment. Selected standards for wastewater discharge are presented in Table 2.2.

Table 2.1: Some constituents of typical municipal wastewater

	Strong	**Medium**	**Weak**
Total solids (mg L^{-1})	1200	700	350
Dissolved solids (TDS) (mg L^{-1})	850	500	250
Suspended solids (mg L^{-1})	350	200	100
Nitrogen (as N) (mg L^{-1})	85	40	20
Phosphorus (as P) (mg L^{-1})	20	10	6
BOD5 (mg L^{-1})	300	200	100
[1]Total Coliform (CFU 100 mL^{-1})	10^8-10^9	10^7-10^8	10^6-10^7

Source: Pescod, 1992; Metcalf & Eddy, 2003

Table 2.2: Selected standards for municipal wastewater effluent discharge

	PME	EC directive	World Bank, 1998
BOD5 (mgL-1)	10 –25	25	50
COD (mgL-1)	50 –150	125	250
Total-N (mgL-1)	-	10 - 15	50
TKN (organic N)	5 –10	-	-
Total-P (mgL-1)	5	1 - 2	2
Total Coliform (counts/100mL)	5000	-	< 400[1]

[1] Unit in MPN/100 ml

2.2Water Quality

In subtropical systems, nutrient availability is the limiting factor of primary productivity(Hoch *et al*., 2008). Release of nutrients from sewage effluentscontributes greatly to eutrophication of marine ecosystems.Major environmental risks associated with eutrophication include development of hypoxic or anoxic conditions and occurrence of harmful algal blooms (Nogales *et al*., 2011).

Globally, eutrophication of marine environment has led to degradation of water quality, increase in the prevalence of harmful algal blooms - causing fish kills and seafood intoxication, anoxic conditions, alterations in the ecosystems and closure of recreational beaches (Patricia, 2006).High nutrient level discharged into coastal waters was a contributing factor that eventually led to the 2001 massive fish kill event in Kuwait Bay due to outbreaks of HABs (Glibert *et al*., 2002). In a study,Ed Parnell,(2003)compared the effects of primary and secondary treated sewage effluents on water quality of Hawai'ian coastal waters. Results of the study revealed that secondary effsluents exhibited no merits over primary effluents with regard to nutrient inputs into the coastal waters.Peña-García *et al*.,(2014) reported that Al-Khumra sewage outfall in Jeddah coast of the Red sea impact on the surrounding water by contributing to moderate increase in nutrient concentrations in surface water with maximum total nitrogen (TN) and total phosphorus (TP) values of 21 micromole/L and 2.1 micromole/L respectively. Likewise, Saleh,(2012)reported higher nutrient levels in seawater samples from a station impacted bysewage at Half Moon Bay compared to a control area in Saudi coast of the Arabian Gulf. Mean values of nitrate, ammonia and phosphate concentrations of 33microgram/L, 13 microgram/L and 10 microgram/L were recorded at the station respectively.

Reduction in chronic input of nutrients from STPs often helps control eutrophication. For instance, a 16-year water quality monitoring study in Moreton Bay, a shallow subtropical embayment in southeast coast of Queensland, Australia, revealed that (70%) reduction in nutrient inputsfrom a large STP resulted in lower mean concentrations of dissolved inorganic nitrogen and chlorophyll *a* in the Bay(Saeck *et al*., 2013).

2.3 Pathogenic Contamination of Coastal Waters

According to the World Health Organization, approximately88% of diarrheal diseases and 1.7 million deaths are attributable to unsafe water globally(WHO, 2008). Health risks posed by recreational water depend on factors such as nature of microbial hazard and the immune status of the user(Belkin & Colwell, 2006).Monitoring the presence and concentrations of microbes is a common means of ensuring the safety of water bodiesin situations where fecal contamination could pose serious health risks. Microbial indicators associated with human and animal excreta help in detection of such pollution (Barrell *et al*., 2000).

Total Coliforms are aerobic or facultative anaerobic, gram-negative, oxidase-negative, rod-shaped and non-spore-formingbacteria that ferment lactose with gas and acid formation within 48 h at 35 °C. Fecal Coliforms are members of Coliform group which can grow at an elevated temperature of 44.5 °C (WHO, 2008). Total Coliforms include several species of the *Enterobacteriaceae* family. Members of *Enterobacteriaceae*live in the human and animal intestine (Ashbolt, 2004). Fecal Coliforms include *E. coli* and some *Klebsiella species*, such as *K. oxytoca* and *K. pneumonia.E. coli*is exclusively found in human and animal excreta. Fecal Coliforms are therefore much better indicators of

fecal pollution than total Coliforms. There are however possibilities of false positive results in some cases due to the ubiquitous nature of *Klebsiella sp.* (Leclerc *et al.*,. 2001).

Although most faecal microorganisms are eliminated by sewage treatment, considerable numbers are released in the treated sewage effluents (Toze, 1997). Concentrations of indicator bacteria including total Coliforms, faecal Coliforms, *Escherichia coli*, and *Enterococcus species* are used as proxy measures to determine the relative risks of the presence of pathogens in water. Faecal Coliforms are the major indicator bacteria used in testing for faecal pollution and effectiveness of water and wastewater treatment. This is partly due to their being thermotolerant; and also because majority of microbial pathogens found in water and wastewater are enteric in origin and thus excreted in faecal matter.(Nagvenkar & Ramaiah, 2009; Rodrigues *et al*,. 2011). However, thermotolerant bacteria in the aquatic environment may include other bacteria that are not necessarily of faecal origin, such as thermotolerant *Klebsiella* species. On the other hand,the use of *E. coli* has the added advantage that its environmental characteristics and behaviour are well-known and is the only organism considered to be primarily of faecal origin. Furthermore, drawbacks associated with the use of thermotolerant Coliforms include their higher sensitivity to environmental stress and treatment processes compared to most resistant pathogenic bacteria, protozoan cysts and viruses as well as their inability to distinguish between human and animal sources of faecal contamination (Costán-Longares *et al*,. 2008; Wen *et al*,. 2009). Oocysts and cysts of *Cryptosporidium* and *Giardia* respectively exhibit high stability in the environment and resistance to disinfectants such as chlorine (Kitajima *et al*., 2014). Levantesi *et al.*, (2010) found out that there was a good correlation between presence of *Giardia* cysts and resistant microbial indicators

such as *Clostridium* spores and coliphages in Managed Aquifer Recharge (MAR) systems operated with reclaimed wastewater.

The survival of bacteria indicators is influenced by biotic and abiotic factors in the water which vary significantly based on the type of coastal waters(Pote *et al.*, 2009).*E. coli* is a good indicator of non-halophilic pathogens (particularly,*Salmonella*) in coastal waters. In contrast however, due to its lower persistence when compared to that of *Vibrio parahaemolyticus* in seawater,*E. coli* is a poor indicator for halophilic pathogens(Lee *et al.*, 2011). High levels of bacterial indicators in water column may not only be dueto recent contribution of point and non-point contamination sources such as sewage treatment plants (STPs) and rivers, but also as a result of the persistence and removal from sediment, and multiplication of the bacteria in the sediment and water column(Pote *et al*., 2009).

CHAPTER 3

MATERIALS AND METHODS

3.1 StudyArea

Saudi coast of the Arabian Gulf lies in the western shoreline of the Gulf(Figure 3.1). The Arabian Gulf is a shallow subtropical embayment of the Indian Ocean covering an area of 240 000 km^2 with a mean depth of 35m. It is a poorly flushed system with an estimated water residence time of over 3 years along the Arabian coast(Alosairi *et al*., 2011). The Gulf's climate is characterized by year round aridity, high summer temperature with occasional rainfall only in the winter months (October and April). The tidal range rises from less than 0.6m to 1-2m inshore. The surface water is characterized by temperature extremes with wide daily and seasonal variations (ranging from less 10^oC in winter to over 35^oC in summer) and high salinities. Saudi Arabian shores of the Gulf have high evaporation rates, low fresh water input with seawater flowing southward. Salinity of fresh seawater (36.5 - 37 ppt) from the Indian Ocean increases southward along the Saudi coast. Salinities ranging between 38 and 42 ppt are found in areas north of Al-Khobar and this was found to greatly influence the abundance of plankton in the coastal waters(Barth & Khan, 2008).

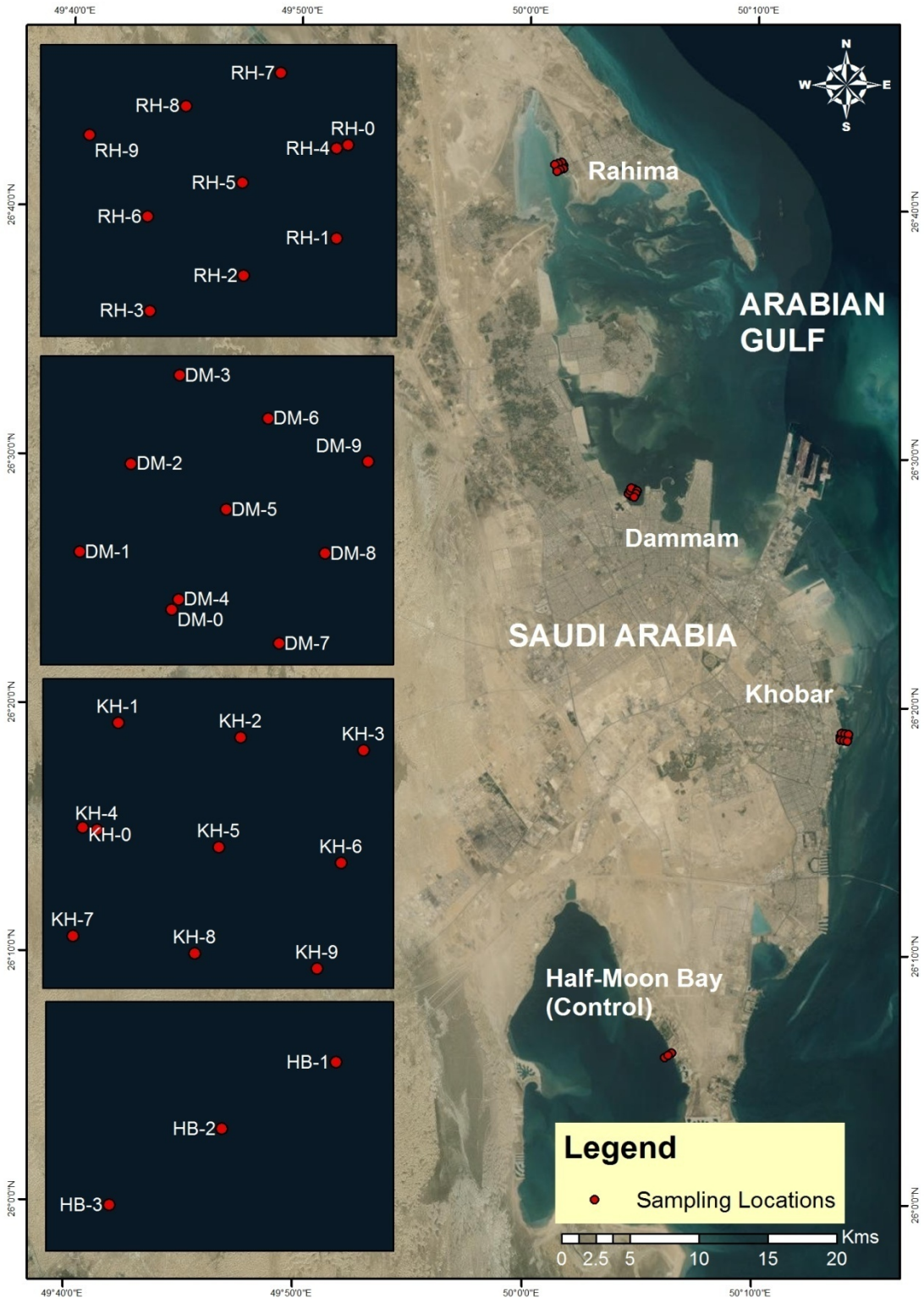

Figure 3.1: Map of study area showing sampling stations

3.2 Sample Collection

Water samples (15-20cm below the water surface) were collected from three coastal sewage outfall locations (i.e. Al-Khobar, Dammam and Rahima) and a control site (Half-Moon Bay) (Figure 3.1). Water samples were collected in triplicates from nine sampling stations in three transects for each location. In each transect, the stations were located at distances near-outfall, 250m and 500m seaward from the outfall as shown on the map in Figure 3.2. Triplicate samples from the discharge points were also collected to characterize the effluent quality.

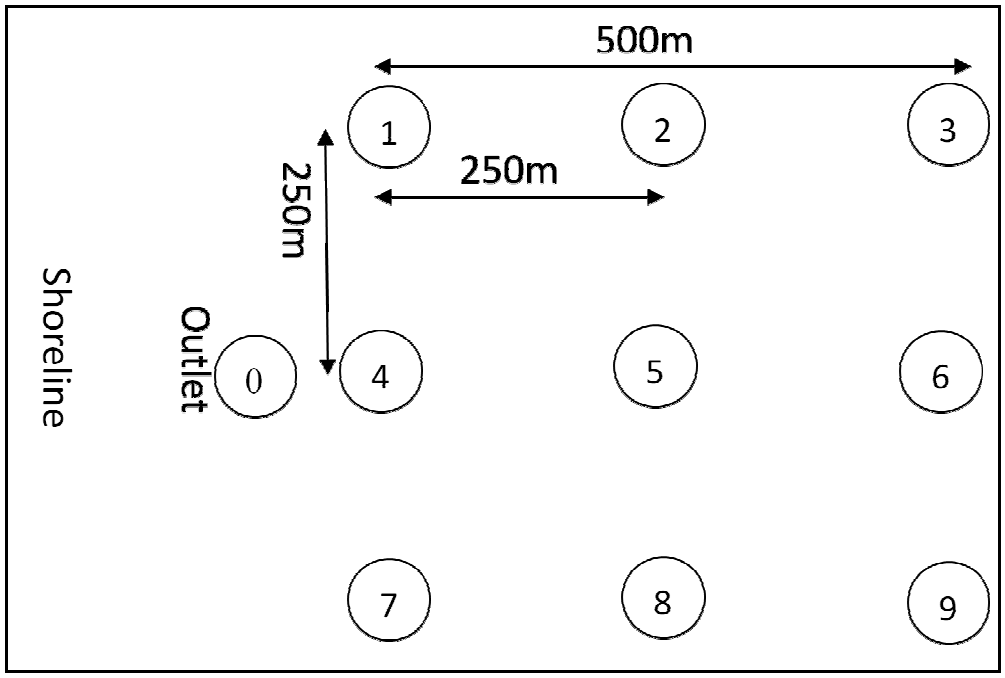

Figure 3.2: Sampling scheme

3.3 Physical and chemical Parameters

3.3.1 In-situ Measurement of Environmental Parameters

Environmental parameters in the water column were measured *in situ* at each sampling station. Temperature, pH, and dissolved oxygen were measured using hand-held Eutech CyberScan PC 650 Water Quality Monitoring Instrument. Salinity was measured using ATAGO Hand Refractometer.

3.3.2 Biochemical Oxygen Demand (BOD)

BOD measurements were done within 24 hof sample collection using standard method for 5-Day BOD test. Temperatures of samples were adjusted to 20 ± 3 °C. Appropriate dilutions of each sample were prepared in overflowing airtight 300 ml BOD bottles. Initial dissolved oxygen (DO) contents were measured using Mantech PC BOD DO probe. After incubation at 20 ± 1 °C for 5 d ± 6h, the final DO readings were taken. BOD values were calculated as the differences between DO readings of samples before and after sample incubation with the dilution factor considered (APHA, 2012)

3.3.3 Chemical Oxygen Demand (COD)

Chemical Oxygen Demand (COD) of samples was determined using the closed reflux, titrimetric standard method(APHA, 2012). 2.5 mL of each sample was placed in a culture tube, followed by addition of 2.5 mL of dichromate digestion solution. 3.5 mL sulfuric acid reagent was carefully run down inside the tube so that an acid layer is formed under the sample-digestion solution. The tubes were placed in preheated block digester and refluxed for 2 h at 150 °C. The tubes were then allowed to cool to room temperature. Tubes which turned greenish were repeated with smaller sample volumes. Contents of each tube were transferred and rinsed with distilled water into a 100 mL beaker with a

magnetic stirrer. 1 to 2 drops of ferroin indicator were added followed by titration with standardized 0.10M ferrous ammonium sulphate (FAS) solution. COD was calculated using Equation (3.1).

$$COD\ as\ mg\ O_2/L\ =\ \frac{(A-B)\times 8000}{volume\ of\ sample\ (ml)} \qquad \text{Equation (3.1)}$$

Where:

A = volume in mL of FAS used for blankA

B = volume in mL of FAS used for sample

M = molarity of FAS

3.3.4 Determination of Nutrient Concentrations

Water samples for analysis of nitrate (NO_3^-), nitrite (NO_2^-) and phosphate (PO_4^-) were preserved by freezing at -15 ^oC. Phosphate, nitrite and nitrite+nitrate were simultaneously analyzed using the Skalar San++ auto-analyzer which works based on the principle of continuous flow analysis.Nitrite present in water sample was quantified by the auto-analyzer by diazotizing it with sulphanilamide followed by coupling with α-naphthylethylenediamine dihydrochloride. The absorbance of the reddish-purple color of the resulting water-soluble dye was measured at 540nm. Nitrate+Nitrite was determined based on the principle of cadmium reduction method. Nitrate present in sample is reduced to nitrite by passing the sample through copperized cadmium column. Then the resulting nitrite plus the originally present nitrite was measured. Nitrite concentrations were subtracted from corresponding nitrate+nitrite to obtain concentrations for nitrate.

The automated procedure for the determination of phosphate is based on the reaction where ammonium molybdate and potassium antimony tartrate react under acidic conditions with diluted solutions of phosphate. A complex (i.e. antimony-phospho-

molybdate complex) is formed which is then reduced with ascorbic acid to an intensely blue-coloured complex. Light absorbance of the blue complex at 880 nm was measured to determine the phosphate concentration in the sample.Silicate measurement was based on reaction where the sample is acidified and mixed with ammonium molybdate solution to form molybdosilicic acid. The acid formed is reduced to a blue dye by addition of ascorbic acid. Oxalic acid was added to control phosphate interference. Light absorbance of the blue dye at 810 nm was measured to determine the silicate concentration in the sample.

3.3.4.1 Ammonia

Manual fluorometric method as described by Holmes *et al*., (1999) was used for measurement of ammonium concentrations in the water samples. The method utilizes a stable working reagent (WR) made of orthophthaldialdehyde (OPA), sodium sulfite, and a borate buffer at concentrations of 50 $mL.L^{-1}$, 40 $mg.L^{-1}$, and 40 $g.L^{-1}$ in the WR respectively. 10mL of WR was added to 40mL of each sample in a 100 mL (acid-washed) sample bottle. Standards were prepared from ammonium stock solution and WR. The bottles were incubated in the dark for 2-3 h at ambient temperature. After incubation, samples and standards were poured into test tubes and the OPA-ammonium flourescence for each tube was immediately scanned on the fluorometer with excitation bandwidth of 340-360 nm. Corrected sample fluorescence ($F_{sample\ cor}$) was determined using the Equations (3.2), (3.3) and (3.4) below and the sample's ammonium concentration was computed using standard regression.

$$F_{sample\ NH4} = F_{sample\ obs} - F_{sample\ BF} \quad \text{Equation (3.2)}$$

$$ME = \frac{(F_{std\ spike} - F_{std\ zero}) - (F_{sample\ spike} - F_{sample\ obs})}{(F_{std\ spike} - F_{std\ zero})} \times 100\% \quad \text{Equation (3.3)}$$

$$F_{sample\ cor} = F_{sample\ NH4} + F_{sample\ NH4}\left({}^{ME}/_{100}\right) \quad \text{Equation (3.4)}$$

Where:

$F_{sample\ NH4}$ = Sample's ammonium luorescence

$F_{sample\ obs}$ = Fluorescence of sample when incubated with WR

$F_{sample\ BF}$ = Background luorescence (sample combined with borate buffer)

$F_{sample\ cor}$ = Corrected sample luorescence

ME = Matrix effects

3.3.5Chlorophyll*a*

Chlorophyll *a* measurement was done via fluorescence detection on a Turner Designs bench fluorometer Trilogy calibrated with pure chlorophyll *a* of known concentration according tomethod developed by Lorenzen (1966).Water samples (1000mL) were filtered at low vacuum (pressure < 20 kPa) through 45µm glass fiber filters. The filters were soaked in 10mL of 90% acetone, grinded with the aid of a sonicator and allowed to stay for 2-24 h at 4 ℃ in the dark for sufficient extraction of chlorophyll *a*. An aliquot (5ml) of the supernatant was then transferred to a glass cuvette and fluorescence was measured before and after acidification with 0.15ml of 0.1N HCl solution.

3.4 Microbial Analyses

3.4.1 Enumeration of Bacteria

Water samples for bacteria analysis were collected in sterile bottles, kept in ice box and transported to laboratory for processing within 3-5 h after sampling. Heterotrophic plate counts were done on nutrient agar using standard serial dilution and spread plate methods. Samples and dilutions were thoroughly mixed on a mechanical-vortex shaker. 0.1 mL and 0.5 mL of each sample/dilution were aseptically transferred using sterile pipette on agar plate in duplicates. Total Coliforms were enumerated on Levine EMB agar using membrane filter technique. Inoculated plates along with sterility controls were incubated at 37 ^{o}C for 24-48 h. After incubation, colonies on nutrient agar plates were counted on Quebec Colony Counter while colonies on membrane filter (i.e. EMB plates) were counted using a low power binocular wide-field dissecting microscope. Levine EMB agar is a selective medium for Gram-negative bacteria and help differentiate bacteria that ferment lactose (lac + colonies). Coliforms were counted as the number of lactose fermenting organisms visualized as purple-black colonies.

3.4.2 Characterization and Identification of Bacteria

A total of 50 colonies were isolated, sub-cultured, and used for identification. Eight isolates were identified (table 4.4) based on their cultural, morphological and biochemical characteristics. The standardized API 20 E and API 20 NE strips (manufactured by Biomerieux Sa, France) were used for the biochemical characterization. Identification was done by comparing the API profiles with the Analytical Profile Index database.

3.5 Statistical Analysis

Data obtained from the study were analyzed using R programme and SPSS v.20(IBM Corp., USA). Description of the distribution of environmental variables, nutrients and indicator bacteria among the sampling locations and stations was done using descriptive statistics. Tests of normality and homogeneity were performed on the data distribution obtained for each parameter. The differences in the mean concentration of nutrients, chlorophyll *a* and indicator bacteria among sampling locations were assessed using Analysis of Variance (ANOVA).Generalized linear model (glm) was used to determine the relationships between bacteria counts and distance from discharge points and selected nutrients.

CHAPTER 4

RESULTS

4.1 Physical and Chemical Parameters

Table 4.1: Summary of physical and chemical variables in the study sites

Location		Temp. (°C)	pH	Salinity (‰)	DO (mg/L)	BOD (mg/L)	COD (mg/L)
Al-Khobar	Seawater	21.4 (±0.4)	8.2 (±0.1)	46.7 (±4.4)	6.5 (±0.6)	3.2 (±1.7)	54.97(±22.7)
	Effluent	-	-	-	-	-	
Dammam	Seawater	22.1 (±0.7)	8.3 (±0.1)	32.3 (±9.9)	4.5 (±0.9)	10 (±2.5)	155.48 (±55)
	Effluent	23.4	7.6	8	4.3	27.55	91.26(±7.3)
Rahima	Seawater	21.5 (±0.4)	8.9 (±0.4)	42.3 (±5.6)	6.2 (±0.9)	5.4 (±1.2)	105.86 (±34)
	Effluent	23	7.99	8	6.1	1.29	45.71 (±3.5)
Half Moon Bay (Control)	Seawater	21.4 (±0.2)	8.0 (±0.02)	39	7.2 (±0.2)	2.5 (±1.2)	60.1(±18)

The temperature values of surface waters were generally higher at stations closer to the outfalls. All sampling were done around noon time. Seawater temperature ranged from 20.90 to 23.40 ^{o}C in all the stations. The saliniity of effluents at the discharge point were as low as 8.00 for Dammam and Rahima. The salinity and pH of seawater samples generally increased with increasing distance away from the outlets. The average dissolved oxygen content in the outfall locations (Dammam 4.51±0.81 mg/L; Al-khobar 6.38±0.71mg/L; Rahima 6.20±0.89mg/L) were lower compared to that of the control location (7.74±0.71).High COD and BOD were recorded at the outlets compared to the surrounding water except for Rahima diffuser. The value ranges of temperature, pH,

salinity, dissolved oxygen, chemical oxygen demand and biochemical oxygen demand for each study location are presented in Table 4.1.

4.2 Water quality: Nutrients and Chlorophyll*a*

High nutrient concentrations were associated with all discharge points studied.Elevated chlorophyll *a* concentrations were recorded at the outfall locations compared to the reference site (Half Moon Bay).

4.2.1 Nutrients

The average nutrient concentrations found in the study locations are presented in Figure 4.1a-d. The discharge points were important sources of inorganic nutrients. However, higher mean ammonia concentrations in seawater samples were obtained compared to the effluent samples. Nutrient concentrations ranges recorded in the outletsare (unit in µmol/L); NO3: 2.1 - 29.05, NO2: 0.71 - 10.71, NH4: 0.34 - 1.33, PO4: 18.29 - 36.08 and Si: 51.05 - 79.39. The mean nutrient concentrations in seawater of sample stations around the outlets were (unit in µmol/L): Al-Khobar – $NO_3$1.21, $NO_2$0.27, $NH_4$3.07, $PO_4$2.43, Si 9.48L; Dammam - $NO_3$17.89, $NO_2$6.45, $NH_4$12.35, $PO_4$7.78, Si 22.70; and Rahima - $NO_3$14.30, $NO_2$0.63, $NH_4$0.74, $PO_4$4.30. The concentrations of nitrate and nitrite in Dammam and Rahima; ammonia in Al-Khobar and Dammam; and phosphate in all three outfall locations were significantly higher compared to the control (Table 4.2 and 4.3).

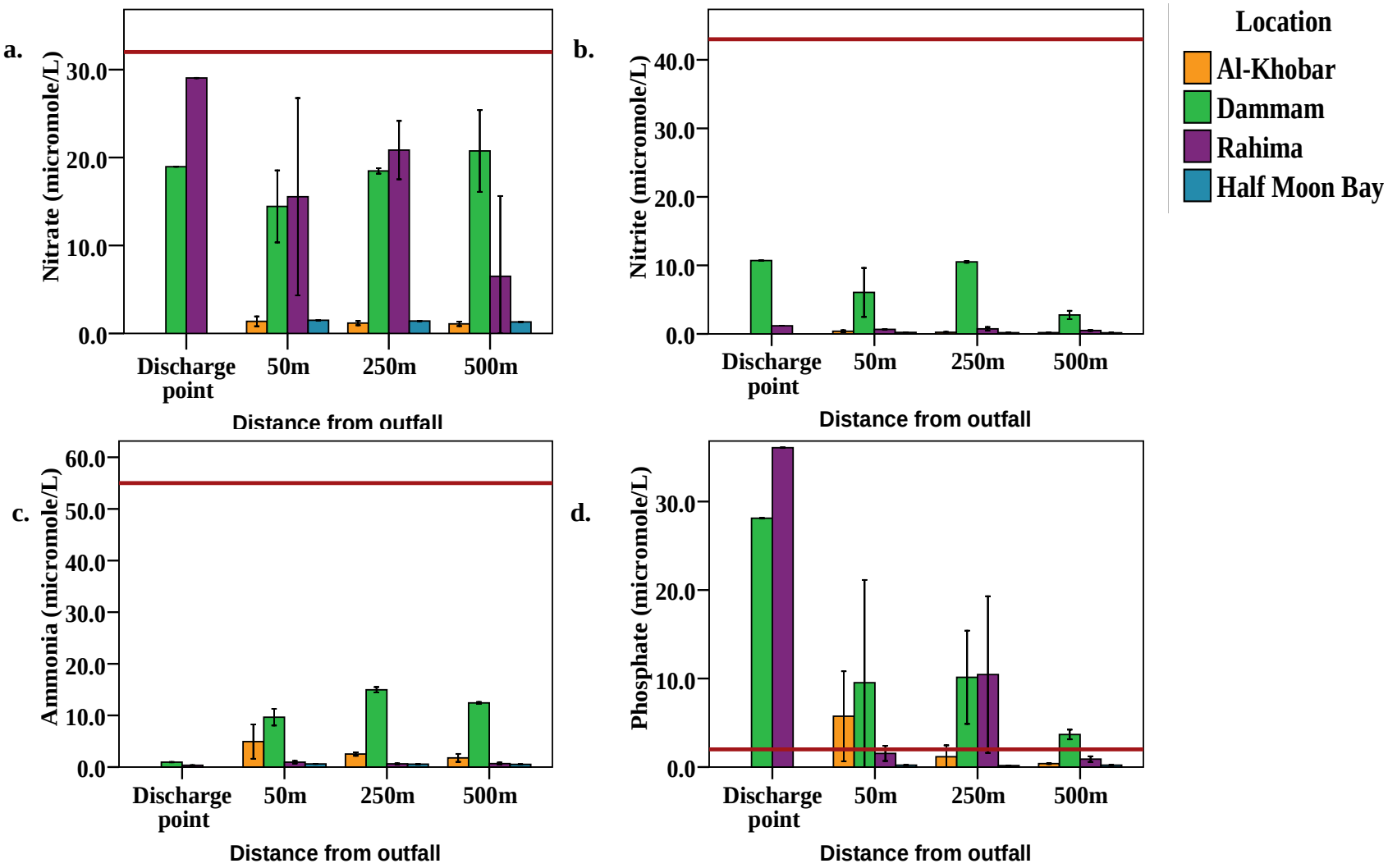

Figure 4.1 (a-d): Distribution of mean nutrient concentrations among stations in the study locations (a) Nitrates (b) Nitrites (c) Ammonia and (d) Phosphate. Red lines represent PME standards for Saudi coastal water of the Arabian Gulf

4.2.2 Chlorophyll*a*

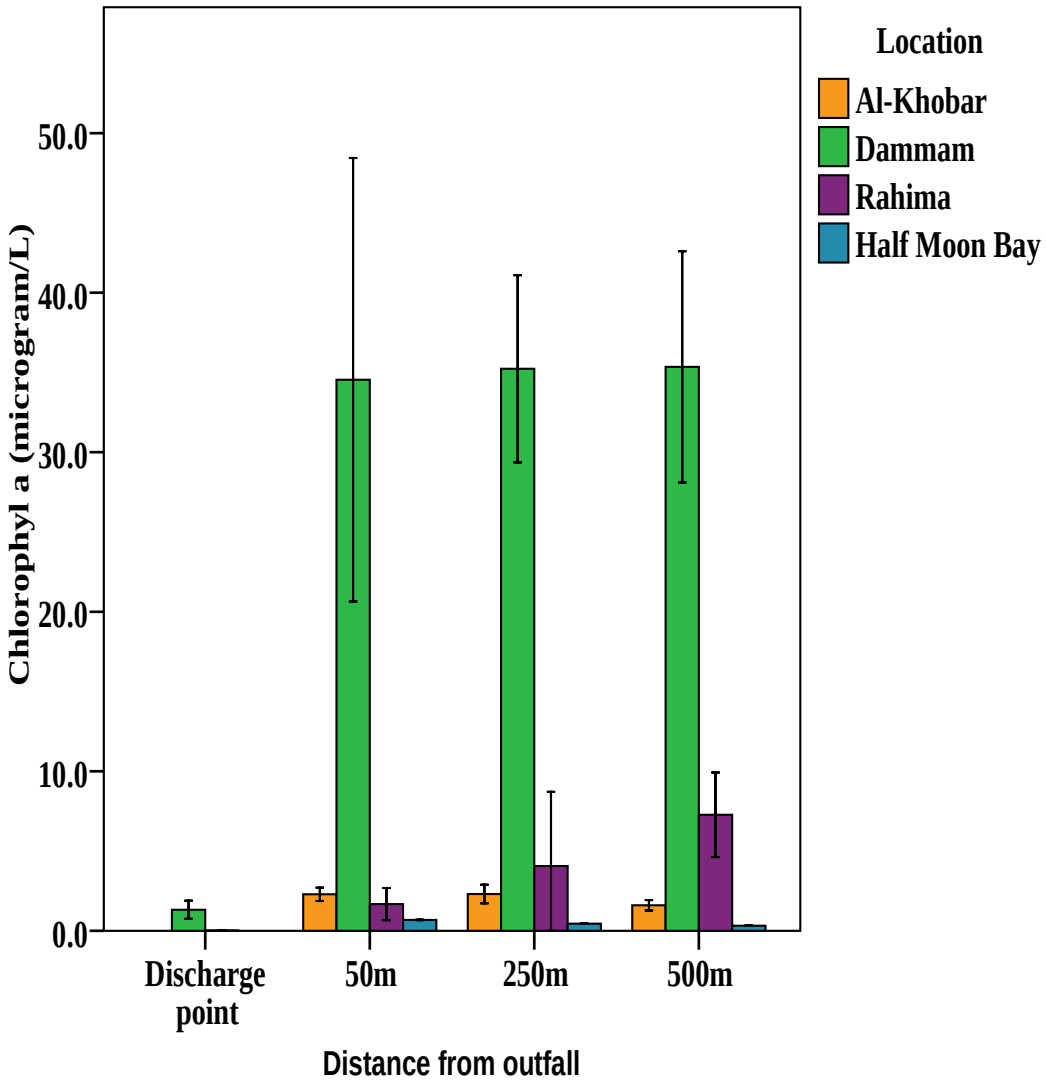

Figure 4.2: Mean chlorophyll *a*in sampling stations at study sites

Chlorophyll *a* concentrations were higher in the vicinity of the sewage outfalls relative to the reference site as shown in Figure 4.2. The highest concentrations were recorded in sampling stationsat Dammam outfall with a mean concentration of 35.04 µg/L. Stations in Al-Khobar and Rahima had mean concentration of 1.39 µg/L and 0.24 µg/L respectively. Lower concentrations were obtained at discharge points compared to seawater samples. This indicates that the sewage outlets were not significantly a direct source of Chl *a*. The differences in chlorophyll *a* concentrations between outfall locations and control were significant in Dammam and not statistically significant for Al-Khobar and Rahima(Table 4.2 and 4.3).

Table 4.2: Results of Kruskal-Wallis One Way Analysis of Variance (Ranks). Normality Test (Shapiro-Wilk) failed (P < 0.050) for all selected parameters.

	NO_3	NO_2	NH_3	PO_4	Chl *a*	BOD	TCC	HPC
H	45.227	82.480	72.887	45.531	53.912	68.792	28.057	24.237
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Table 4.3: Results of pair-wise comparison test (Dunn's Method) of selected parameters among study locations. Statistically significant differenceswere obtained between locations at *P*< 0.05.

Locations		**Parameters**							
		NO3	NO2	NH4	PO4	Chl *a*	BOD	TCC	HPC
Half Moon Bay (Control)	Al-Khobar	NS	NS	*P*< 0.05	*P*< 0.05	NS	*P*< 0.05	*P*< 0.05	NS
	Dammam	*P*< 0.05	*P*< 0.05	*P*< 0.05	*P*< 0.05	*P*< 0.05	*P*< 0.05	*P*< 0.05	*P*< 0.05
	Rahima	*P*< 0.05	*P*< 0.05	NS	*P*< 0.05	NA	NS	*P*< 0.05	NA
Al-Khobar	Dammam	*P*< 0.05	*P*< 0.05	*P*< 0.05	*P*< 0.05	*P*< 0.05	*P*< 0.05	NA	NS
	Rahima	*P*< 0.05	*P*< 0.05	*P*< 0.05	*P*< 0.05	NA	*P*< 0.05	NA	*P*< 0.05
Dammam	Rahima	NS	*P*< 0.05	*P*< 0.05	NS	*P*< 0.05	*P*< 0.05	NS	NA

Key: NS = Not significant, NA = Not applicable

4.3Microbiological Parameters: Total Coliformsand Heterotrophic Plate Counts

Mean total Coliform counts (TCC) and heterotrophic plate counts (HPC) in discharge points and surface water sample stations are presented in Figure 4.3(a-b). Statistically significant differences in total Coliform counts between each of the outfall locations and control were obtained (Table 4.2 and 4.3).Bacteria densities were generally higher in seawater near the outfalls (within 250 m seaward) in all the outfall locations. The lowest bacterial count (TCC 40-100 CFU/ml; HPC $1.5 \times 10^2 - 2 \times 10^2$ CFU/ml) were recorded at Rahima where the diffuser apparently releases tertiary treated sewage effluents. In addition, it was observed that the mean total Coliform count in seawater samples within 250 m distance from the diffuser in Rahima were higher than that obtained from the effluent. The sampling was done during low tide when the dilution influence of the seawater was minimal in stations close to the diffuser. In addition, the increased Coliform densities in the water samples may also be due to bacterial re-suspension from sediment and contribution from seabirds(Pote *et al*., 2009). However, the mean Coliform density dropped back to lower values at 500 m distance seaward. Although, the highest bacteria densities were found at the discharge point in Al-Khobar (TCC 3.1×10^3 CFU/ml; HPC 2.1×10^5 CFU/ml), the concentration however became greatly reduced in seawater with increasing distance away from the discharge. In Dammam, high bacterial counts were recorded in seawater samples (TCC $2.8 \times 10^2 - 7 \times 10^2$ CFU/ml; HPC $2.9 \times 10^4 - 5 \times 10^4$ CFU/ml CFU/ml) even at distance 500 m seaward from the outfall.

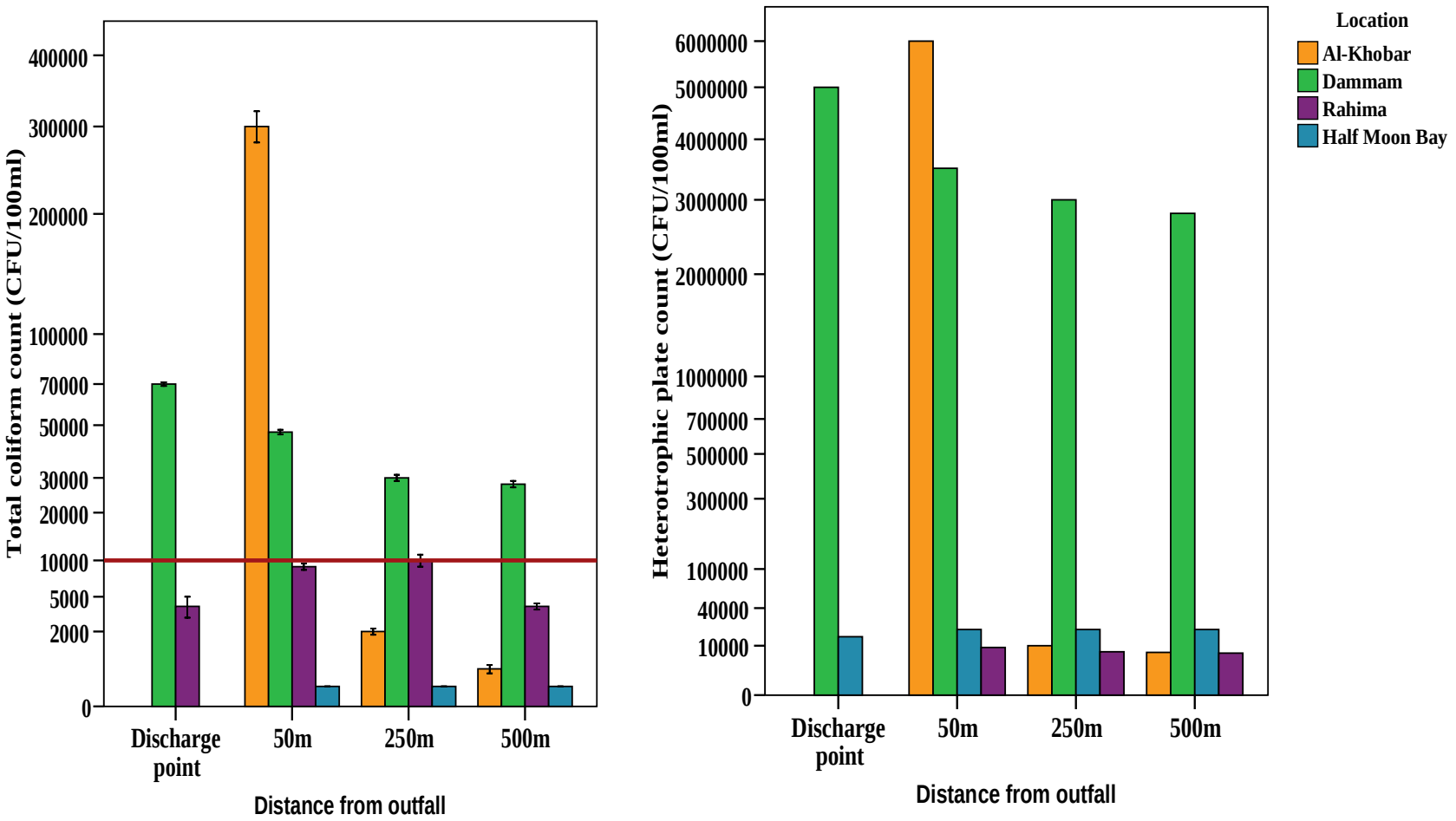

Figure 4.3 (a-b): Distribution of mean(a) total Coliform counts(b) heterotrophic plate countsin sampling stations at outfall locations. Red line representsEuropean Community (EC) Bathing Water Directive (76/160/EEC) mandatory standard for total Coliform

4.4 Relationship between bacteria counts and distance from outfall, and selected nutrient parameters

Table 4.4: Results of generalized linear model that best fit the relationship between total Coliform counts and distance, and selected nutrients in Al-khobar. Gamma probability was used. Significant relationship is obtained at *P*< 0.05. (-) sign on the estimate value means negative relationship, otherwise there exist positive relationship.

	Predictor variables	Estimate	*P*
Total Coliform counts (CFU/100ml)	Distance from outfall(m)	-5.785e-06	0.00
	Nitrate (µmol/L)	1.810e-08	0.997
	Phosphate (µmol/L)	3.416e-07	0.764
	Biochemical Oxygen Demand (BOD) (mg/L)	-2.854e-04	0.01

The result of glm in Table 4.4 shows that total Coliform counts in stations at Al-Khobar have negative and significant relationship ($p<0.05$) with distance from outfall and biochemical oxygen demand. This indicate that bacteria counts significantly decrease as we move farther away from the outfall. The negative relationship between Coliform counts maybe as a result rapid attenuation of Coliform bateria by prevailing environmental factors such as high salinity and solar insolation. On the other hand, Coliform counts have a positive relationship with nitrate and phosphate but not significant ($P>0.05$).

Table 4.5: Results of generalized linear model that best fit the relationship between total Coliform counts and distance, and selected nutrients in Dammam. Gamma probability was used. Significant relationship is obtained at *P*< 0.05. (-) sign on the estimate value means negative relationship, otherwise there exist positive relationship.

	Predictor variables	**Estimate**	***P***
Total Coliform counts (CFU/100ml)	Distance from outfall(m)	-2.650e-07	0.00
	Nitrate (µmol/L)	-5.125e-07	0.007
	Phosphate (µmol/L)	4.847e-06	0.00
	Biochemical Oxygen Demand (BOD) (mg/L)	-4.812e-07	0.00

Table 4.5 shows that in Dammam, Coliform counts have significant relationship with distance from outfall and all selected nutrient parameters. It has a negative relationship with distance, nitrate, and BOD but a positive relationship with phosphate.

Table 4.6: Results of generalized linear model that best fit the relationship between total Coliform counts and distance, and selected nutrients in Rahima. Gamma probability was used. Significant relationship is obtained at *P*< 0.05. (-) sign on the estimate value means negative relationship, otherwise there exist positive relationship.

	Predictor variables	Estimate	*P*
Total Coliform counts (CFU/100ml)	Distance from outfall(m)	7.440e-07	0.62
	Nitrate (µmol/L)	-1.895e-05	0.59
	Phosphate (µmol/L)	-6.084e-05	0.079
	Biochemical Oxygen Demand (BOD) (mg/L)	-5.282e-04	0.027

In Rahima, Coliform counts have positive but not significant relationship with distance from outfall (Table 4.6). This is not unexpected because the effluent from Rahima outfall releases only minimal amount of Coliform bacteria into the coastal waters. similar to the situation in Al-Khobar and Dammam, there is significantly negative relationship between Coliform counts and BOD. While its relationships with both nitrate and phosphate is insignificant.

4.5Bacterial diversity

A total of 50 colonies were purified and sub-cultured for identification. Eight species were identified, those are, *E. coli, Pseudomonas aeruginosa,Vibrio cholera,Aeromonas hydrophila, Salmonella typhi, Shigella spp., Campylobacter jejuni,*and *Yersinia enterocolitica*. The profile of biochemical test results used in the identification of each bacteria type is presented in Table 4.7.

Table 4.7 : Some biochemical reactions used to differentiate isolated bacteria species

Species	GRAM	OX	CAT	ONPG	LDC	CIT	H2S	URE	IND	VP	GLU	MAN	LACT	SUC	MOT
Escherichia coli	-	-	+	+	+	-	-	-	+	-	+	+	+	d	+
Shigella spp.	-	-	+	-	-	-	-	-	-	-	+	+	-	-	-
Salmonella typhi	-	-	+	-	+	-	+	-	-	-	+	+	-	-	+
Yersinia enterocolitica	-	-	+	+	-	-	-	+	-	-	+	+	-	+	+
Vibrio cholera	-	+	+	+	+	d	-	-	+	-	+	+	-	+	+
Campylobacter jejuni	-	+	+	-	-	-	+	-	+	-	+	+	+	+	+
Aeromonas hydrophila	-	+	+	+	-	+	-	-	+	-	+	+	-	+	+
Pseudomonas aeruginosa	-	+	+	+	+	+	-	+	+	+	-	+	+	+	+

GRAM = Gram reaction, OX = Oxidase test, CAT = Catalase test, ONPG = beta-galactosidase, LD C= Lysine decarboxylase, CIT = Citrate test, H2S= Hydrogen sulphide, URE = Urease, IND = Indole test, VP = Voges-Proskauer, GLU = Glucose, MAN = Mannitol, SUC = Sucrose

CHAPTER5

DISCUSSION

The potential influence of sewage effluents on thewater qualityandmicrobial characteristics of receiving coastal waters in the Saudi Arabian coast of the Arabian Gulf was investigated in three outfall locations. Analysis of physico-chemical characteristics of effluent samples shows that, with regards to temperature and pH, effluent samples collected from Rahima and Dammam met the standards prescribed by the Presidency of Meteorology and Environment (PME)(Table 4.1). Unfortunately, we could not sample effluent from Al-khobar outlet due to the fact that the sewage outlet was submerged under the tide and immediately mixed with seawater. Salinity of effluents was as low as 8 ppt and was considerably lower than that of the surrounding seawater in the outfall locations. Unlike in Rahima, where the mean BOD values (1.29 mg/L) of effluent samples was very low, that of Dammam (27.55 mg/L) was slightly above PME standard limit (of 25 mg/L) at the time of sampling.

Sewage plants are major sources of nitrogen and phosphorus into water. Substantial amount of inorganic nutrients generally remains in secondary treated sewage (Xu, Lee*et al.*, 2011). Results of this study (Figure 4.1 (a-d)) indicate that effluents from STPs were important sources of nitrate, nitrite and phosphate into the coastal waters. Mean phosphate concentrations in the effluent samples were above the PME prescribed limit (10 µmol/L).Chlorophyll *a* concentrations in effluent samples were low. This indicates

that the sewage outlets were not directly releasing significant quantity of Chlorophyll *a* into the coastal waters. From microbiological aspect, lower Coliform counts were obtained from Rahima diffuser apparently due to effective tertiary treatment prior to disposal. The mean total Coliform counts (4000 /100ml) of the effluent samples was within the PME prescribed limit of 5000 counts/100 ml for discharge into coastal waters. In contrast, mean total Coliform counts of effluent samples from Dammam was very high compared to PME standard. Apparently, effluent from Al-Khobar outfall was also above the standardas indicated by high total Coliform counts obtained from water samples near the outlet.

Figure 4.2 (a-d) shows that elevated nutrient concentrations were recorded in stations at the three outfall locations. The mean concentrations of inorganic nitrogen (nitrate, nitrite and ammonium) varied among stations; however, values obtained were generally below PME prescribed limits for (industrial) coastal waters (i.e NO3 - 32 µmol/L; NO2 - 43 µmol/L, and NH3 - 55 µmol/L). High seawater ammonium concentrations in some stations especially at Dammam may be attributable to mineralization of organic matter derived from both BOD coming from the sewage effluent and the increased growth of phytoplanktonas indicated by elevated levels of chlorophyll *a* in the area. Mean nitrate concentrations in some stations in Dammam and Rahima were similar to values reported in enriched waters of Kuwait bay (nitrate - >20 µmol/L) (Glibert *et al*., 2002). The maximum limit for phosphate concentrations was exceeded in some stations from all three outfall locations. With regards to nutrient input, tertiary effluent in Rahima exhibited no advantages over secondary effluent in Al-Khobar and Dammam outfalls.

Generally, higher chlorophyll *a* concentrations were obtained in all the three outfall locations compared to the control location, it was however statistically significant only for Dammam (Table 4.2 and 4.3). High Chlorophyll *a*concentrations reflect symptoms of eutrophication and are indicative of deterioration of water quality. The mean values of chlorophyll *a* concentrations obtained in the three outfall areas are similar to those classified to be of poor environmental quality in the Gulf of California based on the Arid Zone Coastal Water Quality Index (AZCI) (Vargas-González *et al*., 2014). The poor water quality in Dammam was also reflected in the high concentrations of ammonium and nitrite obtained in the area. Re-mineralization of organic matter introduced by increased algal biomass may have contributed significantly to the inorganic nutrients in the water(Buchan *et al*., 2014), besides the fraction coming from sewage effluents.

Total Coliform counts were significantly higher in stations at outfall areas relative to the control stations (Table 4.2 and 4.3). The lowest bacterial counts (TCC 4000-10000 CFU/ml; HPC 1.5 x 10^2 – 2 x 10^2 CFU/ml) were recorded at Rahima where the diffuser apparently releases tertiary treated sewage effluents. Bacterial re-suspension from sediment (Pote *et al*., 2009) and contribution from excreta of sea birds (Pond, 2005) are factors that could be responsible for the higher mean total Coliform counts recorded in some seawater samples near the outfall relative to that of the effluent samples. Although, the highest bacteria densities were found at stations near discharge point in Al-Khobar (TCC 3 x 105 CFU/100ml; HPC 6 x 10^5 CFU/ml), the concentration became greatly diluted in seawater with increasing distance away from the discharge. In Dammam, high bacteria counts were recorded in seawater samples (TCC range 2.8 x 10^4 – 7 x 10^4 CFU/100ml; HPC range 2.9 x 10^4 – 5 x 10^4 CFU/ml CFU/ml) even at distance 500 m

seaward from the outfall. However, the relationships between Coliform counts and distance from outfall in Al-Khobar and Dammam were both statistically significant in the negative direction (Table 4.4 and 4.5).European Community (EC) Bathing Water Directive (76/160/EEC) set a mandatory standard 10000 counts/100ml for total Coliforms. In addition, according to Mueller *et al*., (1987), total Coliform count in shellfish harvesting areas should not exceed 70 per 100mL. Consequently, microbiological quality of stations close to the outfalls is generally not safe for activities such as recreation and shellfish harvesting that may expose one directly or indirectly to pathogenic microorganisms. Identified bacteria isolates from stations near the outfalls confirm the presence of human pathogens in the coastal waters including those that are primarily of faecal origin. Eight types of pathogenic bacteria were identified; those are *Escherichia coli, Pseudomonas aeruginosa, Vibrio cholera, Aeromonas hydrophila, Salmonella typhi, Shigella spp., Campylobacter jejuni and Yersinia enterocolitica*. *E. coli* is a bacterium species which belongs to the total Coliform group. It is the only member of the group that is found exclusively in faeces of humans and other animals (Pond, 2005). *E. coli* and other Coliform bacteria are less resistant to disinfection compared to intestinal viruses and protozoa.Health risks associated to each of the bacteria types are presented in Table 5.1.

Table 5.1: Identified bacteria species and their associated health risks

Pathogen	Health risk(s)	Remarks
E. coli	Dysentery-like infections	Though most *E. coli* found in human intestine are harmless but five groups are recognised as human pathogens. Reported incidence is highest among children aged under 15 years. Infectious dose of E. coli O157:H7 may be be less than 50 organisms (Pond, 2005).
Pseudomonas spp.	opportunistic infections including pneumonia, infections of wounds, eyes, ears and skin	Pseudomonads are non-fecally derived microbial hazards that are ubiquitous in the einvironment.*P. aeruginosa* and *P.maltophilia* account for approximately 80 percent of pseudomonads recovered from clinical specimens(Iglewski 1996).*P. aeruginosa* is a major cause of folliculitis and ear infections acquired by exposure to contaminated recreational waters(Mena & Gerba, 2009).
Vibrio cholera	Cholera	Vibrio species are indigenous pathogenic microorganisms in marine aquatic environments. They can be introduced into waters receiving human wastes from areas with cholera outbreaks. Typical Infective dose of V. cholerae is upto 106 organisms or more. It is often unlikely that persons involved in recreational water activities would ingest vibrios in numbers high enough to cause gastroenteritis. However, the risk of wound and ear infection by pathogenic Vibrio species is also of health importance(WHO, 2003).
Aeromonas hydrophila	Gastroenteritis, Wound infections and Pneumonia	Aeromonas spp. are natural inhabitants of marine waters. However, they can also be found in sewage (106 - 108 cells per ml of aeromonads). Cases of wound infections and pneumonia due to

		aeromonads in healthy persons during recreational water activities have been reported(WHO, 2003).
Salmonella typhi	Typhoid	Typhoid fever affects all age groups. Approximately, 16 million cases and 600,000 deaths occur annually worldwide. Infectious dose is around 10,000 organisms(Pond, 2005).
Shigella spp.	Bacillary dysentery (Shigellosis)	Apparently, humans and gorillas are the only natural hosts of *shigellae*. Shigellosis is responsible for 1.1 million deaths and over 164 million cases each year, with the majority of cases occurring in the children of developing nations. Estimated 448,240 cases (mosly due to *S. sonneri* infection) occur in the United States each year. Around 100 organisms of *S. sonneri* or *S. flexneri* can sufficiently cause infection while as few as 10 *S. dysenteriae* bacilli can elicit clinical disease(Pond, 2005).
Campylobacter jejuni	Acute enterocolitis	It is believed that campylobacter is the leading cause of acute infectious diarrhoea in most developed countries.Campylobacter is capable of entering into a viable but dormant state to overcome adverse conditions.Gulls are believed to be a major reservoir of *Campylobacter spp*. Infectious dose is between 500 and 1000 organisms(Pond, 2005).
Yersinia enterocolitica	Gastroenteritis	*Y. enterocolitica* is a relatively uncommon cause of diarrhea. Infection occurs mainly in young children (CDC 2008). Some strains are invasive and toxigenic, producing an inflammatory reaction with dysentery(Cheesbrough, 2006).

CHAPTER 6
CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Results of the study indicated that all three outfalls studied served as important sources of inorganic nutrients into the coastal waters. However, nutrient levels in sampling stations were generally below the PME limits except for phosphate concentrations in some of the stations. Significantly high phosphate concentrations were recorded in all three outfall locations studied (Al-Khobar, Dammam and Rahima) relative to the control location (Half Moon Bay). Concentrations of nitrate and nitrite were significantly higher in Dammam and Rahima compared to the control.With regards to organic nutrients in form of biochemical oxygen demand (BOD), unlike in Rahima where sewage effluent was not an important source of BOD, significantly higher BOD levels were generally obtained in both Dammam and Al-Khobar compared to the control location.

Though, higher concentrations of chlorophyll*a* were obtained in stations at Al-Khobar and Rahima compared to the control, the differences were not significant. In contrast, significantly high levels of chlorophyll*a* indicative of poor water quality were found in stations at Dammam. Results from all other water quality indicator parameters analyzed also show that stations at Dammam appeared to be the most impacted by nutrient input from the outfall. Relatively higher tidal influence and/or deeper water column and thus

greater flushing capacity seemed to have prevented the same level of deterioration in water quality at the other locations especially in Al-Khobar.

Furthermore, findings from this study revealed that sewage outfalls not only influence the water quality of the Saudi Arabian coast of the Gulf but also the microbiological characteristics. Differences in total Coliform counts between each of the outfall locations and control were significant. High Coliform bacteria counts above safe limits for recreational activities and shell-fish harvesting based on international standards were recorded in stations close to Al-Khobar Outfall. Effluent samples from Dammam outfall were foundtocontain high total Coliform counts above PME standard for municipal wastewater effluent discharge. In addition, all water samples from stations in Dammam contained Coliform bacteria counts above safe limits. Rahima outfall was observed to release lownumber of microbes below the PME standard.Coliform counts recorded in Water samples from Rahima were mostly within the mandatory limit set by European Community (EC) Bathing Water Directive (76/160/EEC). We however isolated and identified bacteria species such as *E. coli* that are known to cause diseases to humans from the each of the three outfall stations.

6.2 Recommendations

It is recommended that effective tertiary treatment of sewage similar to that of Rahima be adopted in all STPs to control the level of microbial pollution introduced into the coastal waters.Regular and proper maintenance of existing treatment plants should be of priority to ensure that the treatment capacity of the plants to remove organic materials and other constituents of sewage is fully utilized at all times. Sewage treatment technology that can

help to further reduce the level of inorganic nutrients released into the coastal waters should be adopted. Meanwhile, there should be regular monitoring of the trophic state and pathogenic contamination levels of the coastal waters. Shellfish harvesting and recreational activities in coastal waters around sewage outfalls should be controlled to forestall public health risks.

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APPENDICES

APPENDIX A

Coordinates of Sampling Stations

Table A1: Coordinates for Stations at Al-KhobarOutfall

S/N	Station ID	Coordinates	
		X	Y
1.	KH-00	26° 18' 51.517" N	50° 13' 45.486" E
2.	KH-01	26° 18' 59.345" N	50° 13' 48.110" E
3.	KH-02	26° 18' 58.240" N	50° 13' 57.043" E
4.	KH-03	26° 18' 57.268" N	50° 14' 5.995" E
5.	KH-04	26° 18' 51.342" N	50° 13' 46.550" E
6.	KH-05	26° 18' 50.059" N	50° 13' 55.454" E
7.	KH-06	26° 18' 48.891" N	50° 14' 4.376" E
8.	KH-07	26° 18' 43.373" N	50° 13' 44.788" E
9.	KH-08	26° 18' 42.094" N	50° 13' 53.692" E
10.	KH-09	26° 18' 40.959" N	50° 14' 2.619" E

Table A2: Coordinates for Stations at DammamOutfall

S/N	Station ID	Coordinates	
		X	Y
1.	DM-00	26° 28' 29.675" N	50° 4' 31.743" E
2.	DM-01	26° 28' 34.418" N	50° 4' 24.339" E
3.	DM-02	26° 28' 41.645" N	50° 4' 28.465" E
4.	DM-03	26° 28' 48.971" N	50° 4' 32.372" E
5.	DM-04	26° 28' 30.528" N	50° 4' 32.267" E
6.	DM-05	26° 28' 37.887" N	50° 4' 36.096" E
7.	DM-06	26° 28' 45.422" N	50° 4' 39.474" E
8.	DM-07	26° 28' 26.888" N	50° 4' 40.339" E
9.	DM-08	26° 28' 34.295" N	50° 4' 44.050" E
10.	DM-09	26° 28' 41.795" N	50° 4' 47.525" E

Table A3: Coordinates for Stations at RahimaOutfall

S/N	Station ID	Coordinates	
		X	Y
1.	RH-00	26° 41' 48.025" N	50° 1' 28.044" E
2.	RH-01	26° 41' 39.589" N	50° 1' 27.008" E
3.	RH-02	26° 41' 36.192" N	50° 1' 18.791" E
4.	RH-03	26° 41' 33.010" N	50° 1' 10.468" E
5.	RH-04	26° 41' 47.715" N	50° 1' 27.015" E
6.	RH-05	26° 41' 44.599" N	50° 1' 18.661" E
7.	RH-06	26° 41' 41.534" N	50° 1' 10.283" E
8.	RH-07	26° 41' 54.529" N	50° 1' 22.088" E
9.	RH-08	26° 41' 51.569" N	50° 1' 13.664" E
10.	RH-09	26° 41' 48.959" N	50° 1' 5.097" E

Table A4: Coordinates for Stations at Half Moon Bay (Control)

S/N	Station ID	Coordinates	
		X	Y
1.	HB-00	26° 54.441'N	50° 03.191'E
2.	HB-01	26° 54.436'N	50° 02.868'E
3.	HB-02	26° 54.599'N	50° 02.974'E

APPENDIX B

Physical and Chemical Parameters of Sampling Stations

Table B1: Physical and Chemical Parameters for stations at Al-Khobar Outfall

Station ID	Temp. (°C)	pH	Salinity(‰)	DO ($mg.L^{-1}$)	COD ($mg.L^{-1}$) Mean(±SD)	BOD ($mg.L^{-1}$) Mean(±SD)
KH-00	-	-	-	-	-	-
KH-01	21.5	8.22	50	6.9	31.92 (±10.45)	1.37
KH-02	21.3	8.16	50	7.1	93.47 (±10.44)	6.14
KH-03	21	8.18	50	7.3	77.52 (±7.90)	4.91
KH-04	22.1	7.9	41	5.53	38.76 (±10.45)	1.9
KH-05	21.9	7.97	50	5.7	68.4 (±18.10)	4.2
KH-06	21.3	8.33	47	6.8	43.32 (±10.45)	2.26
KH-07	21.5	8.11	40	6.1	34.2	1.55
KH-08	21.1	8.3	42	6.5	66.12 (±7.90)	4.02
KH-09	21	8.25	50	6.7	41.04 (±6.84)	2.08

Table B2: Physical and Chemical Parameters for stations at Dammam Outfall

Station ID	Temp. (°C)	pH	Salinity(‰)	DO ($mg.L^{-1}$)	COD ($mg.L^{-1}$) Mean(±SD)	BOD ($mg.L^{-1}$) Mean(±SD)
DM-00	23.4	7.6	8	4.3	91.26 (±7.26)	27.55 (±0.58)
DM-01	22.9	8.2	40	3.9	166.21 (±23.75)	6.53
DM-02	22.1	8.24	25	3.62	117.14 (±2.74)	10.08
DM-03	20.9	8.22	36	5.67	101.47 (±1.25)	12.24
DM-04	23	8.28	15	3.5	258.32 (±8.43)	9.24
DM-05	22.3	8.32	23	4.6	221.61 (±9.88)	15
DM-06	21.5	8.37	39	4.76	104.47 (±4.75)	9.54
DM-07	22	8.35	42	3.87	185.2 (±4.75)	9.3
DM-08	22.3	8.41	28	5.2	132.96 (±20.70)	10.74
DM-09	21.9	8.31	43	5.7	111.96 (±3.36)	7.26

Table B3: Physical and Chemical Parameters for stations at Rahima Outfall

Station ID	Temp. (°C)	pH	Salinity(‰)	DO ($mg.L^{-1}$)	COD ($mg.L^{-1}$) Mean(±SD)	BOD ($mg.L^{-1}$) Mean(±SD)
RH-00	23	7.99	8	6.1	45.71 (±3.52)	1.29 (±0.08)
RH-01	21.8	9.01	41	6.8	80.86 (±3.51)	5.17
RH-02	21.5	8.92	45	5.3	108.99 (±3.52)	7.34
RH-03	21.6	8.32	45	6.9	101.96 (±3.52)	6.07
RH-04	22.1	9.14	40	4.8	105.47 (±14.07)	5.8
RH-05	22	8.93	29	4.82	56.25 (±7.03)	3.26
RH-06	21	8.33	45	6.7	126.57 (±7.00)	5.71
RH-07	21.5	9.12	44	6.7	70.32 (±7.04)	4.35
RH-08	21.1	9.51	43	6.9	172.27 (±3.52)	4.95
RH-09	21.3	8.49	49	7	130.08 (±3.52)	5.98

Table B4: Physical and Chemical Parameters for stations at Half Moon Bay (Control) Outfall

Station ID	Temp. (°C)	pH	Salinity(‰)	DO ($mg.L^{-1}$)	COD ($mg.L^{-1}$) Mean(±SD)	BOD ($mg.L^{-1}$) Mean(±SD)
HB-01	21.60	7.99	39	6.97	69.50 (±1.14)	3.21 (±0.10)
HB-02	21.3	8	39	7.32	67.78 (±2.62)	2.99 (±0.08)
HB-03	21.3	8.02	39	7.2	39.07 (±1.29)	1.28 (±0.06)

APPENDIX C

Nutrient and Chlorophyll *a*concentrations in surface waters at Sampling Stations

Table C1: Nutrient and chlorophyll *a*concentrations for stations in Al-Khobar Outfall

Station ID	Replicates	NO_3 ($\mu mol.L^{-1}$)	NO_2 ($\mu mol.L^{-1}$)	NH_4 ($\mu mol.L^{-1}$)	PO_4($\mu mol.L^{-1}$)	Chl *a* ($\mu g.L^{-1}$)
KH-00	KH-00a	-	-	-	-	-
	KH-00b	-	-	-	-	-
	KH-00c	-	-	-	-	-
KH-01	KH-01a	1.35	0.23	2.33	0.47	2.6
	KH-01b	0.07	0.22	2.93	0.49	2.14
	KH-01c	1.17	0.22	2.45	0.43	2.39
KH-02	KH-02a	0.89	0.18	2.55	0.36	2.31
	KH-02b	1.34	0.22	2.89	0.5	1.94
	KH-02c	1.51	0.24	3.12	0.51	2.43
KH-03	KH-03a	1.37	0.2	2.31	0.39	2.01
	KH-03b	1.29	0.19	2.28	0.34	2.05
	KH-03c	1.44	0.2	2.35	0.44	1.74
KH-04	KH-04a	1.49	0.54	3.16	11.97	1.68
	KH-04b	1.8	0.58	2.65	11.37	1.91
	KH-04c	1.54	0.56	2.86	12.69	1.79
KH-05	KH-05a	0.86	0.23	2.62	0.7	1.99
	KH-05b	1.3	0.23	2.57	0.54	1.65

	KH-05c	1.15	0.23	2.45	0.51	1.9
KH-06	KH-06a	0.86	0.17	0.55	0.41	1.21
	KH-06b	0.9	0.16	0.77	0.34	1.3
	KH-06c	0.7	0.17	0.93	0.3	1.17
KH-07	KH-07a	1.62	0.38	8.95	5.67	2.71
	KH-07b	1.28	0.31	9.82	4.42	2.7
	KH-07c	2.04	0.4	9.23	4.14	2.69
KH-08	KH-08a	1.32	0.34	2.26	3.54	2.15
	KH-08b	1.28	0.31	2.2	3.32	2.84
	KH-08c	0.88	0.17	1.98	0.45	3.55
KH-09	KH-09a	1.09	0.19	2.14	0.37	1.76
	KH-09b	0.94	0.2	2.29	0.42	1.67
	KH-09c	1.18	0.21	2.32	0.44	1.52

Table C2: Nutrient and chlorophyll *a* concentrations for stations inDammam Outfall

Station ID	Replicates	NO_3 ($\mu mol.L^{-1}$)	NO_2 ($\mu mol.L^{-1}$)	NH_4 ($\mu mol.L^{-1}$)	PO_4($\mu mol.L^{-1}$)	Chl *a* ($\mu g.L^{-1}$)
DM-00	DM-00a	18.95	10.73	0.93	28.07	0.67
	DM-00b	18.96	10.7	1	28.12	1.72
	DM-00c	18.96	10.69	0.92	28.16	1.58
DM-01	DM-01a	9.33	2.7	9.81	1.28	32.61
	DM-01b	9.52	2.61	12.83	1.21	34.27
	DM-01c	9.61	2.6	11.48	1.12	29.86
DM-02	DM-02a	18.59	10.53	15.18	7.37	34
	DM-02b	18.56	10.54	14.83	7.28	26.92
	DM-02c	18.56	10.53	15.43	7.41	25.95
DM-03	DM-03a	16.11	2.18	12.33	3.61	25.26
	DM-03b	15.15	2.02	12.15	3.8	35.79
	DM-03c	17.01	2.26	12.38	3.14	44.94
DM-04	DM-04a	18.91	10.65	8.75	24.97	19.48
	DM-04b	18.9	10.64	8.59	25	48.56
	DM-04c	18.88	10.65	9.96	25.03	26.57
DM-05	DM-05a	18.8	10.66	15.75	17.11	37.14
	DM-05b	18.77	10.67	14.73	17.14	35.94
	DM-05c	18.77	10.66	14.92	17.1	45.06
DM-06	DM-06a	29.57	3.92	12.83	4.56	37.34
	DM-06b	22.93	3.06	12.41	3.73	31.31
	DM-06c	23.67	3.15	12.35	4	35.63

DM-07	DM-07a	14.8	4.93	8.2	2.38	27.7
	DM-07b	14.96	4.86	7.86	2.4	64.98
	DM-07c	15.1	4.85	9.43	2.36	26.85
DM-08	DM-08a	18.1	10.35	13.91	5.95	36.92
	DM-08b	18.09	10.35	14.71	5.96	39
	DM-08c	18.09	10.35	15.27	5.94	36.13
DM-09	DM-09a	19.32	2.57	12.41	3.69	44.08
	DM-09b	23.93	3.14	12.56	3.95	39.19
	DM-09c	19.06	2.6	12.37	2.63	24.61

Table C3: Nutrient and chlorophyll *a* concentrations for stations in Rahima Outfall

Station ID	Replicates	NO_3 ($\mu mol.L^{-1}$)	NO_2 ($\mu mol.L^{-1}$)	NH_4 ($\mu mol.L^{-1}$)	PO_4($\mu mol.L^{-1}$)	Chl *a* ($\mu g.L^{-1}$)
RH-00	RH-00a	29.07	1.18	0.32	36.07	0.01
	RH-00b	29.04	1.18	0.36	36.05	0.02
	RH-00c	29.03	1.19	0.35	36.13	0.02
RH-01	RH-01a	3.29	0.65	0.73	1.04	2.3
	RH-01b	2.05	0.66	0.65	0.91	1.05
	RH-01c	1.88	0.66	0.72	0.92	0.11
RH-02	RH-02a	24.47	0.52	0.61	4.03	1.24
	RH-02b	24.57	0.52	0.6	3.97	0.55
	RH-02c	24.59	0.53	0.65	3.96	0.09
RH-03	RH-03a	18.51	0.42	0.49	1.28	5.25
	RH-03b	18.69	0.4	0.52	1.26	2.49
	RH-03c	18.79	0.4	0.51	1.25	9.09
RH-04	RH-04a	21.13	0.63	1.22	0.99	2.08
	RH-04b	21.09	0.64	1.26	1.17	0.91
	RH-04c	21.07	0.62	1.25	1.23	1.19
RH-05	RH-05a	16.92	1.1	0.52	22.18	0.18
	RH-05b	16.88	1.11	0.51	22.28	0.71
	RH-05c	16.87	1.09	0.53	22.23	6.11
RH-06	RH-06a	1.01	0.53	0.62	0.62	8.62
	RH-06b	0.22	0.53	0.49	0.43	10.17
	RH-06c	0.33	0.53	0.53	0.59	7.79

RH-07	RH-07a	23.97	0.7	0.88	2.31	3.49
	RH-07b	12.16	0.75	0.86	1.85	2.44
	RH-07c	33.25	0.68	0.89	3.43	1.53
RH-08	RH-08a	21.14	0.58	0.7	5.17	13.26
	RH-08b	21.11	0.6	0.73	5.11	6.31
	RH-08c	21.1	0.59	0.7	5.11	8.13
RH-09	RH-09a	0.32	0.53	0.93	0.82	10.43
	RH-09b	0.35	0.55	0.93	0.86	5.03
	RH-09c	0.24	0.57	0.96	0.96	6.58

Table C4: Nutrient and chlorophyll *a* concentrations for stations in Half Moon Bay (Control)

Station ID	Replicates	NO_3 ($\mu mol.L^{-1}$)	NO_2 ($\mu mol.L^{-1}$)	NH_4 ($\mu mol.L^{-1}$)	PO_4($\mu mol.L^{-1}$)	Chl *a* ($\mu g.L^{-1}$)
HB-01	HB-01a	1.5	0.22	0.62	0.21	0.69
	HB-01b	1.48	0.21	0.6	0.21	0.67
	HB-01c	1.51	0.23	0.59	0.22	0.7
HB-02	HB-02a	1.41	0.17	0.56	0.16	0.46
	HB-02b	1.4	0.17	0.55	0.17	0.44
	HB-02c	1.42	0.19	0.56	0.17	0.44
HB-03	HB-03a	1.32	0.18	0.51	0.21	0.33
	HB-03b	1.3	0.16	0.52	0.22	0.32
	HB-03c	1.29	0.16	0.51	0.21	0.32

APPENDIX D

Bacteria counts in Sampling Stations

Table D1: Bacteria counts for stations at Al-Khobar Outfall

Station ID	Replicates	Total Coliform counts (CFU/100ml)	Heterotrophic plate counts (CFU/ml)
KH-00	KH-00a	-	-
	KH-00b	-	-
	KH-00c	-	-
KH-04	KH-04a	300000	60000
	KH-04b	320000	59000
	KH-04c	280000	61000
KH-05	KH-05a	2200	100
	KH-05b	1800	97
	KH-05c	2000	103
KH-06	KH-06a	400	72
	KH-06b	300	68
	KH-06c	500	70

Table D2: Bacteria counts for stations at Dammam Outfall

Station ID	Replicates	Total Coliform counts (CFU/100ml)	Heterotrophic plate counts (CFU/ml)
DM-00	DM-00a	70000	49000
	DM-00b	69000	52000
	DM-00c	71000	49000
DM-04	DM-04a	48000	35000
	DM-04b	46000	34000
	DM-04c	47000	36000
DM-05	DM-05a	30000	30000
	DM-05b	29000	30000
	DM-05c	31000	30000
DM-06	DM-06a	28000	29000
	DM-06b	29000	27000
	DM-06c	27000	28000

Table D3: Bacteria counts for stations at Rahima

Station ID	Replicates	Total Coliform counts (CFU/100ml)	Heterotrophic plate counts (CFU/ml)
RH-00	RH-00a	3000	154
	RH-00b	5000	160
	RH-00c	4000	136
RH-04	RH-04a	9000	200
	RH-04b	8500	190
	RH-04c	9500	210
RH-05	RH-05a	10000	200
	RH-05b	9000	200
	RH-05c	11000	200
RH-06	RH-06a	4000	190
	RH-06b	3700	190
	RH-06c	4300	220

Table D4: Bacteria counts for stations at Half Moon Bay (Control)

Station ID	Replicates	Total Coliform counts (CFU/100ml)	Heterotrophic plate counts (CFU/ml)
HB-00	HB-00a	100	95
	HB-00b	100	90
	HB-00c	100	90
HB-04	HB-04a	100	75
	HB-04b	100	73
	HB-04c	100	70
HB-05	HB-05a	100	70
	HB-05b	100	67
	HB-05c	100	65
HB-06	HB-06a	100	95
	HB-06b	100	90
	HB-06c	100	90

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