

MICROBIAL AND CHEMICAL POLLUTION OF WATER-WELLS RELATIVE TO SEWAGE EFFLUENTS IN OMAN

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Abstract. During the last four decades in Oman there has been a rapid increase in the population, construction of housing, heavy industry and also an increase in agriculture. This rapid growth shows no signs of abatement. This has led to an ever increase in water demand. Based on these facts, the existing methods of sewage water treatment and chlorination process is not effective in eradicating microbial contamination. In this study, the reuse of sewage effluent was one of the major causes of underground water pollution. Cracked septic tanks, cesspits, fertilizers and recycled treated sewage effluent (TSE) also added to underground water pollution. Excessive use of underground water due to water shortage led to salinization of the wells. A total of 276, 305 and 290 water-wells were sampled in 1995, 2000 and 2010 respectively for biological and physiochemical water contamination in Muscat, Oman. Relative to that, 300 samples of TSE were taken from four main sewage treatment plants (STPs). These samples were analyzed and compared with well-water samples. The analyses involved electrical conductivity, total dissolved solid (TDS), iron concentration, heavy metals, trihalomethanes (THMs), nitrate NO₃ and microbial contamination. The dominant heavy metals in wells and TSE were Ni and Zn. In well-water, NO₃, TDS and microbial count were high. The above parameters declined significantly in 2010 because of heavy rain. Heavy metals, THMs and nitrates in some wells exceeded maximum permissible level even after the 2010 declined. Multiple antibiotic resistant bacteria (MARBs) were tested for 16 antibiotics and were found in both TSE and well-water. Resistance of *Escherichia coli* to antibiotics varied and multiple resistance was 2-8 antibiotics. Presence of THMs and MARBs in well-water is an indication of sewage contamination. A frequent analysis and stringent regulations must be implemented to avoid further environmental deterioration. Agencies need to begin implementing strict regulations to help in the prevention of the spread of pollution and disease.

Keywords: Well-water, Sewage effluents, Oman, Chemical pollutants, Antibiotic resistance

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1. Introduction

The water shortage and low rainfall is a major problem facing all aspects of life in Oman (Mahmoud *et al.*, 2013, Al-Musharafi *et al.*, 2014a). Due to rapid development which took place in the last 40 years, it is perfect model to study massive underground water pollution and environmental deterioration due to human activities (Al-Musharafi *et al.*, 2014b). Rapid growth has resulted in an increase in water consumption, creating an imbalance between recycling and excessive use of underground water. The severity of the arid condition has forced the authorities to desalinate brackish and sea water and use the recycled water mainly for irrigation in areas such as public parks and greeneries. Most of the greeneries along the main highways are irrigated by treated sewage effluent (TSE). The government estimated that water usage and sewage produced is steadily increasing (Fig. 1). Similar situations occur in many countries in the world especially in the Arabian Peninsula (UNEP, 2013).

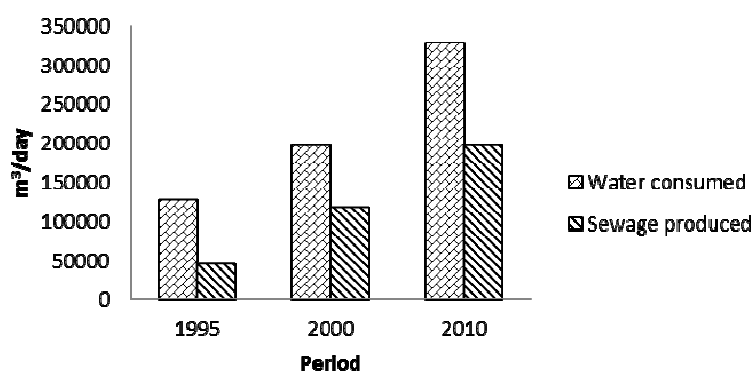


Figure 1. Water use and recycled treated sewage affluent. Estimated daily water demand in relation to sewage produced in Muscat during three periods

Due to excessive use of underground water for farming, water tables have fallen to lower levels, causing salinization which led to destruction of farmlands. As a result, many of the farms have been abandoned or converted to residential, commercial and industrial areas (UNEP 2013).

In order to solve the water-demand problem and at the same time meet the developmental needs, many countries in the arid zone have involved highly in costly desalination projects. The main municipal water source is desalinated sea water and the desalination program in Oman during the last 40 years has steadily increased to meet public demand. At the same time, recycling sewage water for irrigation of greeneries and agriculture has also increased. The common system for wastewater collection is septic tanks and cesspits. Recycling of sewage water for irrigation is becoming a common practice (Al-Bahry *et al.*, 2000).

The use of sludge as fertilizer, septic tanks, cesspits and soakaways allow seepage of effluents into the soil contaminating underground water (Al-Bahry *et al.*, 2000, Al-Bahry *et al.*, 2014, Al-Musharafi 2014 b). Moreover, soil texture in many areas of Oman is porous, allowing easy penetration of effluents through the soil strata and consequently reaching the underground water (Al-Busaidi and Cookson, 2003). Both the recycled water and septic tanks may play a major role in contaminating underground water and wells. One of the reasons may be the irrigation of public greeneries which

depends mainly on usage of recycled water that is mainly TSE (Dewalle, and Scaff, 1980). Some contaminated effluents are dumped in waste lagoons contributing to underground water pollution (Al-Musharafi *et al.*, 2014c). In addition, septic tanks and soakaways have risen substantially in housing, public buildings and industries. This may have resulted in a significant increase in the intrusion of sewage water from TSE, soakaways and cesspits through the soil and finally into water-wells (Al-Bahry *et al.*, 2000). This facilitates an increase in microbes, chemicals and physical contaminants of underground water.

High concentration of heavy metals in drinking water has been responsible for a variety of chronic diseases, such as chronic anemia, liver cirrhosis, renal failure and a variety of cancers (Duan *et al.*, 2011; Mishra *et al.*, 2010).

Some chemical indicators can be used for detection of underground water pollution by sewage, such as nitrate (NO₃) and trihalomethanes (THMs). Nitrate in groundwater indicates that contamination may be the results of cracked septic tanks, cesspits, sewage-treatment plants (STP)s, or in some cases, fertilizers (Al-Bahry *et al.*, 2000). Chlorination of drinking-water is used globally to disinfect microbial contamination. However, this process causes the formation of THM's which resulted in chlorine reaction with organic material in water-forming compounds such as chloroform, bromoform, bromodichloromethane and dibromochloromethane which may pose a health risk, include liver, colon, rectal, kidneys and bladder cancer. There is no unified international maximum limit of THMs in drinking-water (Government of Western Australia, 2009). Although THMs remain a public health concern, the International Agency for Research on Cancer and the World Health Organization reported there is insufficient evidence of health risks. However, *in vitro* studies on experimental animals revealed that THMs cause neurotoxicity, genotoxicity, acute toxicity, developmental toxicity, chronic toxicity, carcinogenicity and toxicity of the reproductive system (WHO, 2004). In Oman, stomach cancer is the most common reported, followed by non-Hodgkin lymphoma and leukemia (Al-Shereiqi 2008).

Water contamination is the most widespread health risk. Turbidity is an important factor for evaluation of water quality. Higher levels of total dissolved solids and turbidity in drinking water were found to be a major factor in causing gastrointestinal illnesses and infections (Egorov *et al.*, 2003). *Escherichia coli* and other fecal microbes are commonly used as fecal contamination indicators. However, the absence of microbial indicator does not necessarily mean the absence of fecal pathogens (Van Lieverloo *et al.*, 2007). Cases of hepatitis-B in Oman reached maximum levels in 1995 (Ministry of Health, Sultanate of Oman, 2008). Some nuisance microbes in water cause corrosion and changes in color, odor and taste, making water aesthetically unacceptable (Al-Bahry *et al.*, 2011).

Heavy metal pollution in Oman caused by contaminated sewage effluents was reported by Al-Musharafi *et al.*, (2012; 2013a; b, 2014a, b, c). Bacteria that are resistant to antibiotics are also resistant to heavy metals. Antimicrobial resistance mechanisms are linked to heavy metal tolerance mechanisms (Akinbowale *et al.*, 2007; Bass *et al.*, 1999). In another investigation, Hölzel *et al.*, (2012), revealed that antibiotic resistant bacteria from pig manure have increased tolerance to heavy metals.

Bacteria in sewage sludge remain viable for several weeks. If such sludge is used as fertilizer, microbial contamination will increase in the environment consequently (Al-Bahry *et al.*, 2014). Antibiotic-resistant bacteria were isolated from different environmental samples were used as bioindicators of contaminated effluents. Resis-

tant bacteria were isolated from treated sewage effluent, fish, marine turtles and fowls (Al-Bahry *et al.*, 1999; 2006; 2007; 2009a; b; c; 2010; 2012). Antibiotic-resistant bacteria from treated sewage occasionally remained viable after chlorination (Al-Bahry *et al.*, 2009a).

In this investigation, the data obtained from TSE and wells, relative to chemical, biological and physical contamination, were analyzed. The significant changes in vegetation were measured along with the changes of NO₃, using Remote Sensing (RS) and Geographical Information System (GIS) techniques.

This investigation is of value for conservation and environmental strategies to minimize pollution in Oman.

2. Materials and Methods

2.1. Selection of the study area and remote sensing analyses

The study area is located at longitudes of Eastern 2612000 and 2622000 and Northern 624000 and 608000 in Muscat, Oman. Landsat Thematic MapperTM data from 1995-2010 was used to provide land cover information of Seeb, a suburb of Muscat. The method of Vanoverstreten and Trefois (1993) was used for Unsupervised classification maps. According to the method of Thomson (1992) Supervised classification was conducted. Normalized Difference Vegetation Index (NDVI) image was used to analyze vegetation changes. The area field of vegetation changes was estimated using Arc Map software version 10.5 in the study area. The feature classes of area fields were added in geodatabase automatically to estimate vegetation changes (Davenport and Nicholson 1993). False Color composite Landsat Image was used to identify urban areas, cities and towns (Williams 1983).

2.2. Well-water and TSE samples collection

A total of 276, 305 and 289 samples were collected from water wells in 1995, 2000 and 2010 respectfully. Three hundred sewage effluent samples from four main sewage-treatment plants (STPs) were also collected (100 samples per period). Sample collection and handling were analyzed following the standard methods (APHA/AWWA/WEF 1998; OS 52/1; OS 8/2006). Samples were collected aseptically every two weeks in sterile 500 mL glass and analyzed immediately after collection. Sodium thiosulphate pentahydrate (Na₂S₂O₃·5H₂O, 100 mg/L; SIGMA, USA) was added to sample containers for water collection as a de-chlorinating agent to avoid bactericidal activity (WHO 1995). Oligodynamic activity of heavy metals was avoided by adding a chelating agent di-sodium salt of phenylenediaminetetracetic acid (EDTA, 372mg L⁻¹) and pH was adjusted to 6.5 (ISO 1994).

2.3. Bacteriological analysis of well-water and TSE

Glassware and sample bottles were washed with detergent, soaked in 0.1% free chlorine hypochlorite solution for 1 h and then rinsed several times with distilled water. Chlorine-free water was used for the final rinse. Sterilization of the glassware was conducted in a hot-air oven at 170 °C for 1 h.

Enumeration and isolation of heterotrophic bacteria was conducted following the method of Collins *et al.*, 1995; British Standard BS 6068 1995. Enumeration of total coliforms and fecal coliforms, was conducted using membrane filters with a pore size of $0.45 \pm 0.02 \mu\text{m}$ and a diameter of 47 mm (Millipore, Bedford, UK) according to Augoustinos *et al.*, 1993; Aulicino and Orsini 1996; ASTM 1992.

2.4. Antibiotic susceptibility test

Luria Bertani broth was used to grow *E. coli* isolates to late logarithmic phase for 4–6 h at 37 °C. The isolates were inoculated on DST agar (Oxoid, UK) to make lawn cultures, using sterile swabs following the standards disk diffusion method of Bauer *et al.*, (1966). The isolates were exposed to 16 antibiotics on DST agar and incubated at 37 °C for 24 h (National Committee for Clinical Laboratory Standards, NCCLS, 1997). Measurements of Inhibition zones were done after incubation for 18–24 h at 37 °C according to the international standards of disk diffusion method. *E. coli* (ATCC 25922) was used as a control. The antibiotics used were: Amikacin (Ak) 30 μg , Ampicillin (Amp) 10 μg , Carbenicillin (Cn) 100 μg , Cephalexin (Ctx) 30 μg , Chloramphenicol (C) 30 μg , Ciprofloxacin (Cip) 30 μg , Gentamicin (Gm) 10 μg , Kanamycin (K) 30 μg , Minocycline (Min) 30 μg , Nalidixic acid (Na) 30 μg , Neomycin (N) 30 μg , Sulfamethoxazole (Smx) 30 μg , Streptomycin (S) 10 μg , Tetracycline (Te) 30 μg , Trimethoprim (Tmp) 5 μg , and Tobramycin (Tob) 10 μg .

2.5. Physicochemical analysis of sewage and water

Free chlorine in water samples was measured using a chlorine test kit (CHEMetrics, Inc., USA). A bluish-violet color indicates positive results for free chlorine. Chlorine concentration was determined by the color produced compared with the provided standards of the kit.

A conductivity meter was used to measure water salinity on site. Pre-calibration of the conductivity meter was done according to the manufacturer's instructions.

Ion chromatography was used to measure the nitrate concentration. 1 mL of water sample was added to a volumetric flask containing 25 mL of double de-ionized water and then injected in the ion chromatography apparatus (Long & McClenny 2006).

Detection of chloro-compounds was analyzed according to the methods of Wolska *et al.*, 1998. Physicochemical analysis of samples was carried out using distilled and deionized water (British Standard BS 6068 1995). The glassware was cleaned with detergent and treated with HNO_3 (0.1 N) then rinsed thoroughly with deionized water. Free and total chlorine concentrations were conducted using the HACH free and total chlorine test kit (HACH Company, USA). The detection range was from 0.0 to 0.7 mg L^{-1} for the free chlorine and from 0.0 to 3.5 mg L^{-1} for the total chlorine. Lovibond drinking-water test kit (Lovibond, USA) was used to examine the color, then measured in Hazen units with a detection range of 5 to 70 units.

The water pH was measured during sampling using a pH meter (Orion, USA).

TDS, EC, and salinity measurements were conducted using field-equipped electrodes (Orion, USA).

Water and sewage samples were analyzed for heavy-metals; Al, As, B, Cd, Co, Cr, Cu, Fe, Hg, Mn, Mo, Ni, Pb, V and Zn using Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) type Perkin Elmer 3300 DV ICP (USA).

3. Results

The data were based on the examination of 276, 305 and 290 wells in 1995, 2000 and 2010 respectively. A total of 300 TSE samples were collected from four main STPs and were compared with the well-water samples.

The meteorological records show that the annual rainfall between 1995-2010 was very low with the exception of 2004, 2006 and 2007 with an unusual high rainfall. In 2007 the cyclone Guno produced a substantial rainfall within 48-hours, causing serious damage and floods. In addition, an extraordinary rainfall was recorded in 2004 and 2006 (Fig 2).

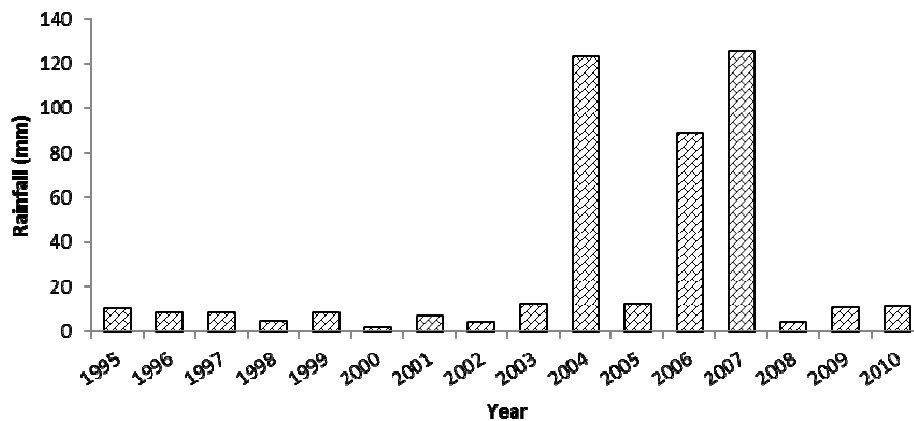


Figure 2. Annual rainfall 1995-2010. The annual rainfall in Oman is minimal except for the year 2004, 2007 and 2007

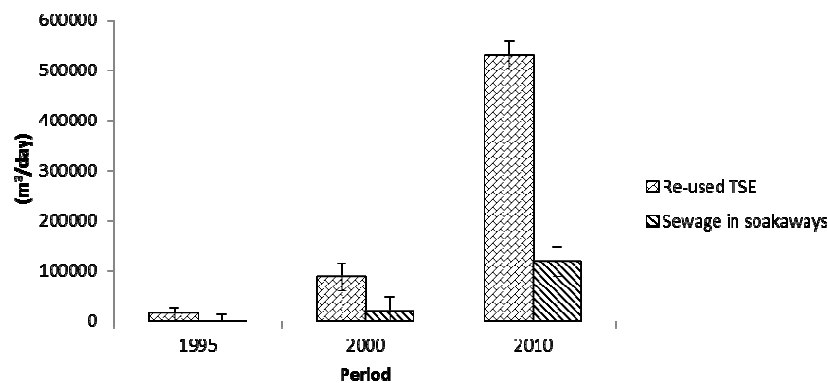


Figure 3. Sewage reuse and sewage in soakaways in Muscat during three periods

The estimated average of both treated sewage effluent and untreated sewage in soakaways significantly increased ($P < 0.05$) during 1995-2010 (Fig 3). The NDVI of Landsat TM satellite image showed vegetation changes in the study area with the colors green, yellow, and red represent vegetation distribution in 1995, 2000 and 2010 respectively (Fig 4). Image analysis revealed that there was a steady decrease and deterioration in vegetation due to depletion and drastic decrease of well-water levels. Most of the damaged areas of vegetation were replaced by housing, public buildings and industries (Fig 5). Based on NDVI of Landsat TM satellite image of vegetation changes in 1995 was 9025 m² but increased to 12160 m² in 2000. However, the vegetation decreased

drastically in 2010 to 4650 m². Water salinity as indicated by EC values was the highest during 1995 and 2000, However, EC dropped significantly ($P < 0.01$) in 2010 (Fig 6).

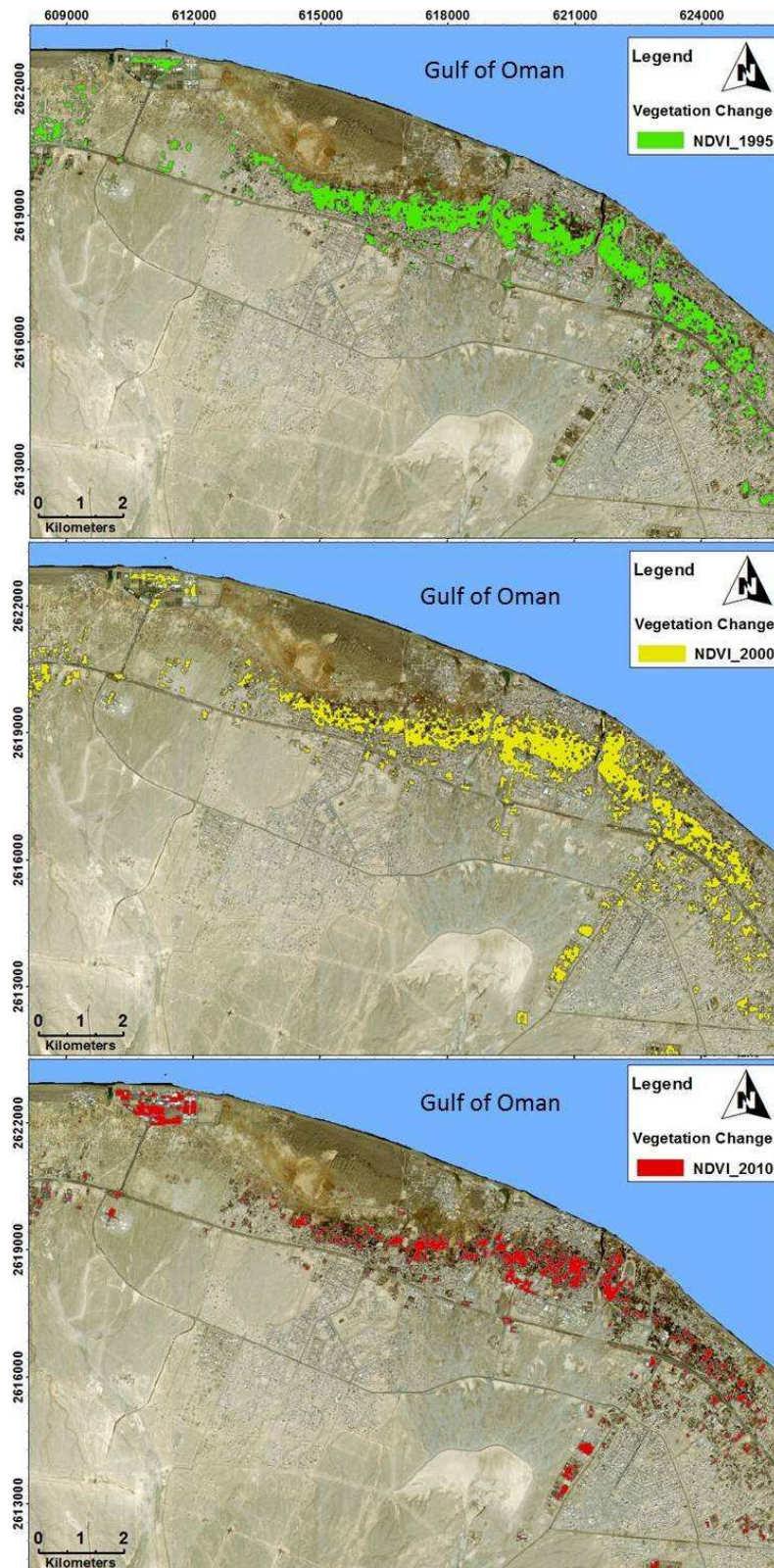


Figure 4. NDVI of Landsat TM satellite image of vegetation changes in Seeb-Muscat. Data acquisition for 1995, 2000 and 2010. Image IKONOS 1m resolution

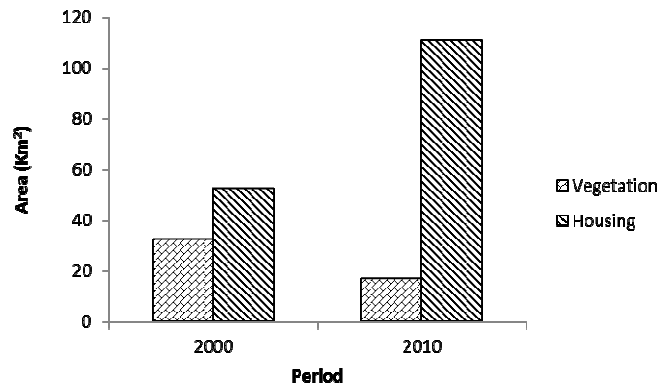


Figure 5. Changes in vegetation in relation to housing development, based on remote sensing image data acquisition in 2000 and 2010. See also Fig 4.

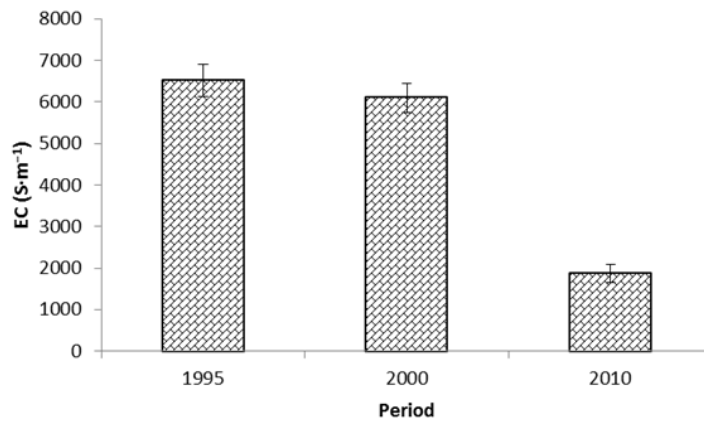


Figure 6. Electrical conductivity of well-water (SE±) during three periods.

The highest ion concentration of both well-water and TSE water was Na and Cl. Also the concentration of SO₄ was similarly high in wells in 2010. However, Mg and Ca were at low levels in both well-water and TSE (Fig 7). The dominant heavy metals in both well-water and TSE were Ni and Zn followed by Cr. These elements were significantly higher in TSE than in wells ($P < 0.05$). The rest of heavy-metals were at lower levels (Fig 8).

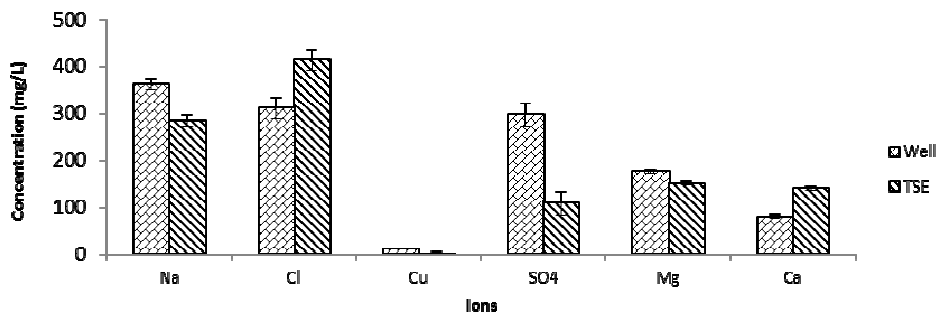


Figure 7. Ion concentration (SE±) in wells and TSE (2010).

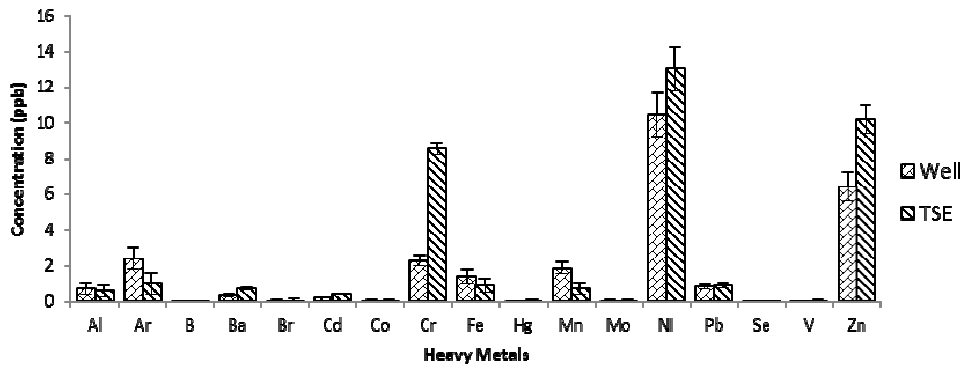


Figure 8. Heavy metals concentration (SE±) in wells and TSE (2010).

Based on Omani standards for TSE (OS 52/1) and drinking water (OS 8/2006) few of the wells exceeded the minimum permissible levels (MPL) for heavy metals analyses in 2010, Overall they were higher in TSE than well-water (Table 1).

Trihalomethanes concentration, which included bromoform, chloroform, bromodichloromethane and chlorodibromomethane in wells (1995 to 2010) and in TSE (2010), showed variation throughout the study period (Fig 9). There was a significant decrease in the four trihalomethanes in 2010. However, chlorodibromomethane was highly significant ($P < 0.01$) in 1995 and 2000 over the others during the same periods. In TSE, chloroform concentration was the highest and there was no significant difference between the four compounds ($P < 0.01$). The percentage of wells with THMs above the MPL varied, (Fig. 9 and 10). Most of the wells that exceeded the MPL were in 2000 and the lowest in 2010.

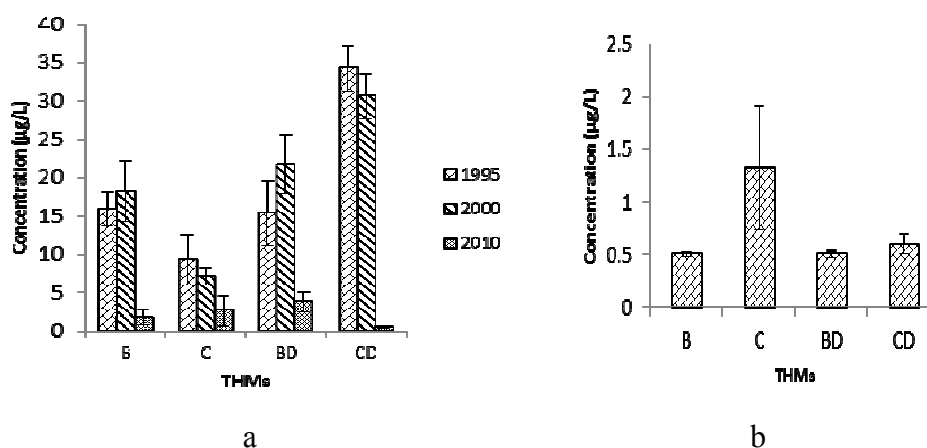
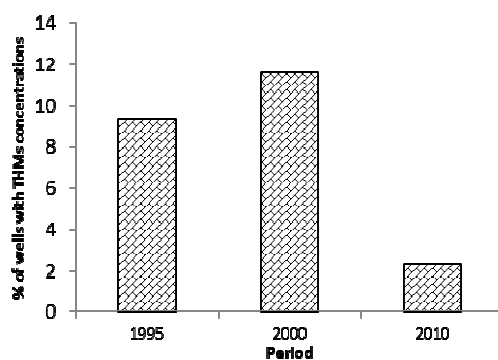
Also, the maximum nitrate concentrations in wells during the three periods were in 1995 and 2000 (Fig 11a). Based on the data presented in Fig 11a the percentages of wells with high nitrate concentrations exceeded the MPL according to the Omani Standard Drinking-Water (Fig 11b). The lowest percentage of wells with nitrate was in 2010. Relative to Fig 11a, nitrate concentration in wells, using satellite image distribution in the study area in 1995, 2000 and 2010, varied significantly (Fig 12). Red dots represent the highest concentrations in wells.

Similarly, the highest values for microbial count (cfu/ml) of coliforms, enterococci and *E. coli*, were recorded in 1995 and 2000. The lowest counts were in 2010. *E. coli* remained low throughout the three periods (Fig 14). In TSE the coliform and enterococci data in 2010 were not significantly different. However, colony forming units for *E. coli* was significantly lower than the other two (Fig 15). The *E. coli* isolates varied in their antibiotic resistance. Out of the sixteen antibiotics, most of the strains were resistant to more than one antibiotic (Fig 16). Isolates from TSE were more resistant to antibiotics than the isolates from the wells. In general, the dominant resistance to antibiotics from wells and TSE isolates was to ampicillin, followed by tetracycline, minocycline, and streptomycin and sulphamethoxazole. None of the well-isolates were resistant to amikacin, chloramphenicol, neomycin and nalidixic acid.

There was insignificant difference in TDS values in 1995 and 2000. However, TDS decreased significantly in all examined wells in 2010 (Fig 13).

Table 1. Percentages of TSE and wells with heavy metal concentrations in 2010 relative to minimum permissible levels (MPL) according to Omani Standards for TSE (OS 52/1) and for drinking water (8/2006).

Element	Minimum Permissible Levels (MPL)		% Exceeded MPL	
	TSE (mg L ⁻¹) OS 52/1	Drinking water (mg L ⁻¹) OS 8/2006	TSE	Drinking water (wells)
Al	5	0.1	1.8	0.4
As	0.1	0.01	0.2	0.5
B	1	0.5	0.3	0.04
Ba	2	0.7	0.1	0
Br	0.01	0.01	0	0
Cd	0.01	0.003	0.7	0.9
Cr	0.05	0.05	0.6	0.5
Co	0.05	0.05	0.2	0.1
Fe	5	1	0	0.07
Hg	0.001	0.001	0.4	0.3
Mn	0.5	0.1	0	0.07
Mo	0.05	0.07	0	0
Ni	0.1	0.02	8.3	4.4
Pb	0.2	0.01	1.5	1.1
Se	0.02	0.01	0	0
V	0.01	0.01	4.1	3.9
Zn	5	3	7.2	4.9

**Figure 9.** Trihalomethanes (B=bromoform, C=chloroform, BD= bromodichloromethane, CD= chlorodi-bromomethane) concentrations (SE±) a: in wells during three periods (1995, 2000 and 2010); b: in TSE (2010).**Figure 10.** Percentage of wells with THMs above the allowed minimum permissible levels (MPL).

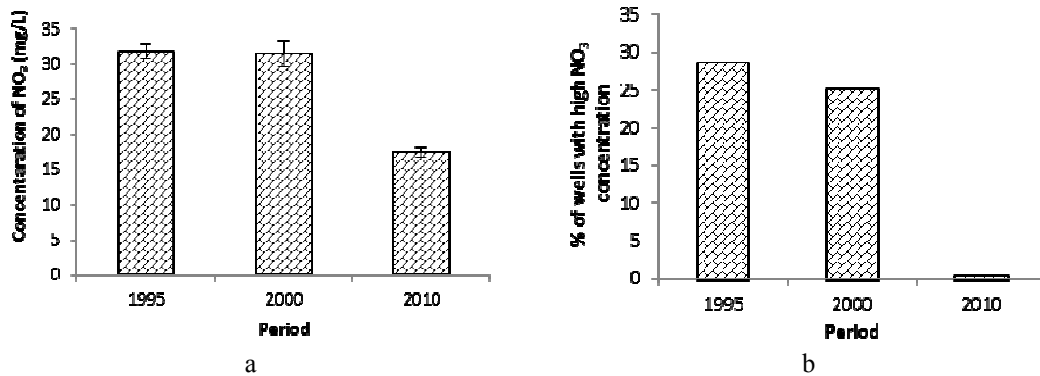


Figure 11. Nitrate concentration (SE±) in well-water during three periods, **a** = nitrate concentration, **b** = percentage of wells with nitrate concentration exceeding the maximum permissible level (MPL) of the Omani-Standard Drinking-Water (50 mg L⁻¹).

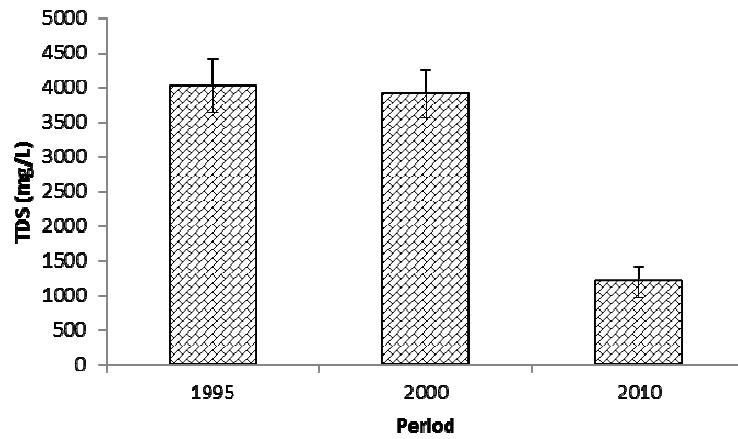


Figure 13. Total dissolved solids (TDS) (SE±) in well-water during three periods.

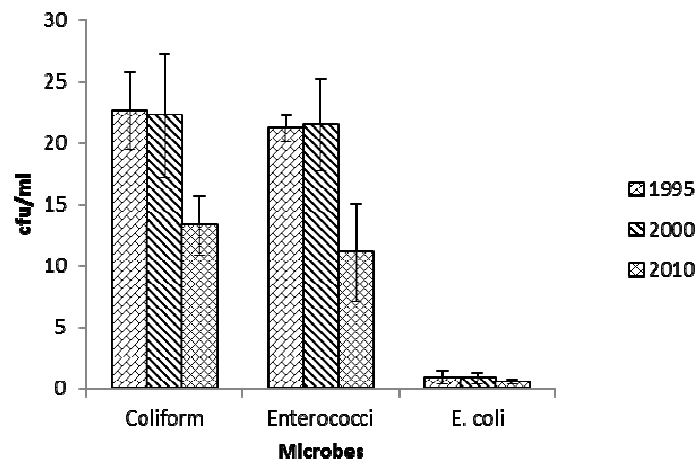


Figure 14. Microbial count (cfu/ml) (SE±) in well-water during the three periods.

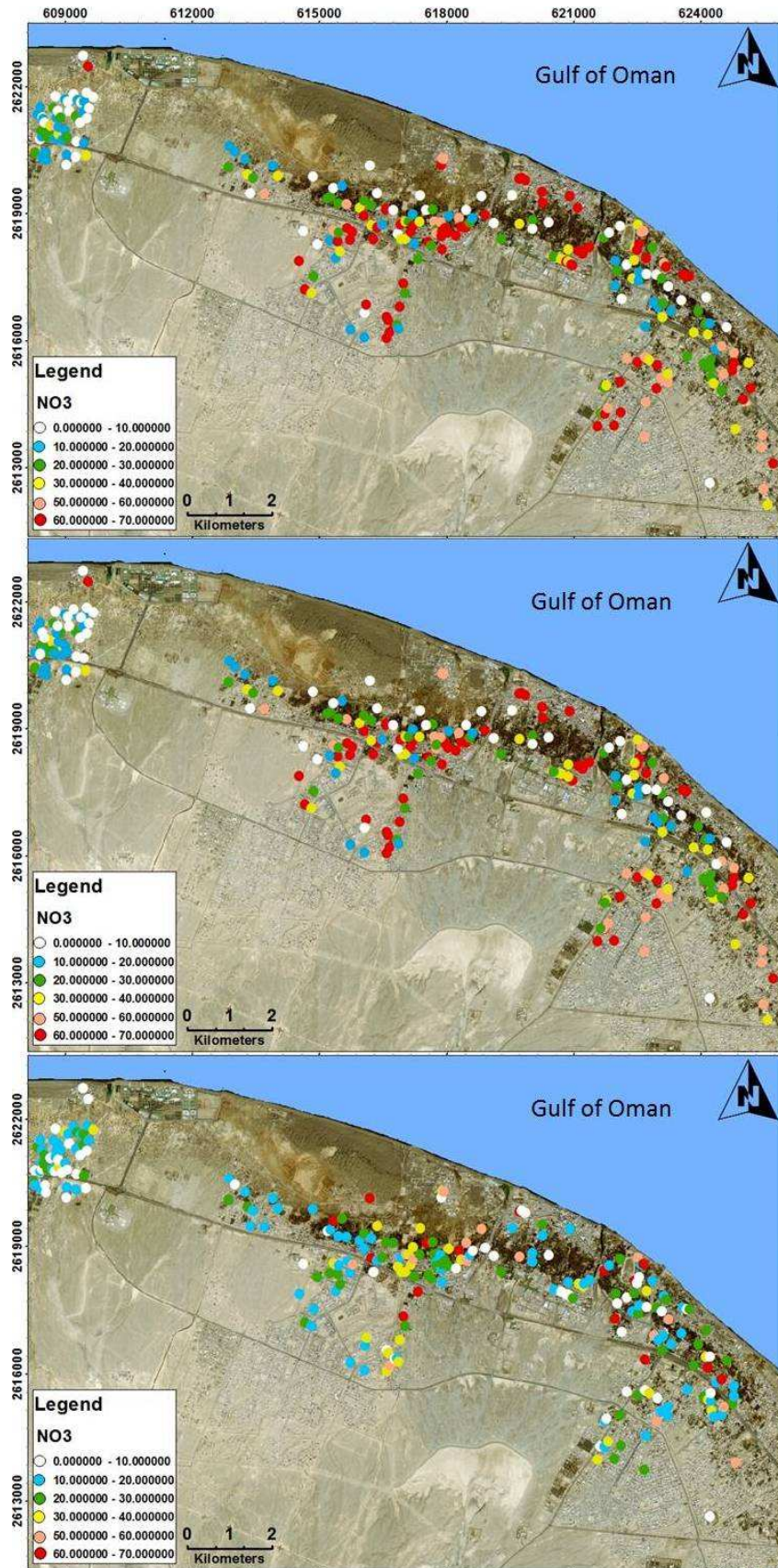


Figure 12. Satellite image of nitrate distribution in Seeb-Muscat wells. Data acquisition for 1995, 2000 and 2010. Image IKONOS 1m resolution.

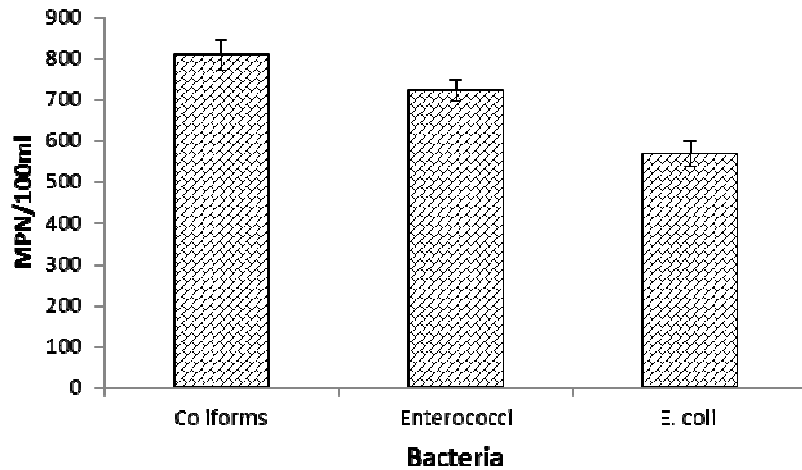


Figure 15. Microbial count (cfu/ml) (SE±) in TSE in 2010.

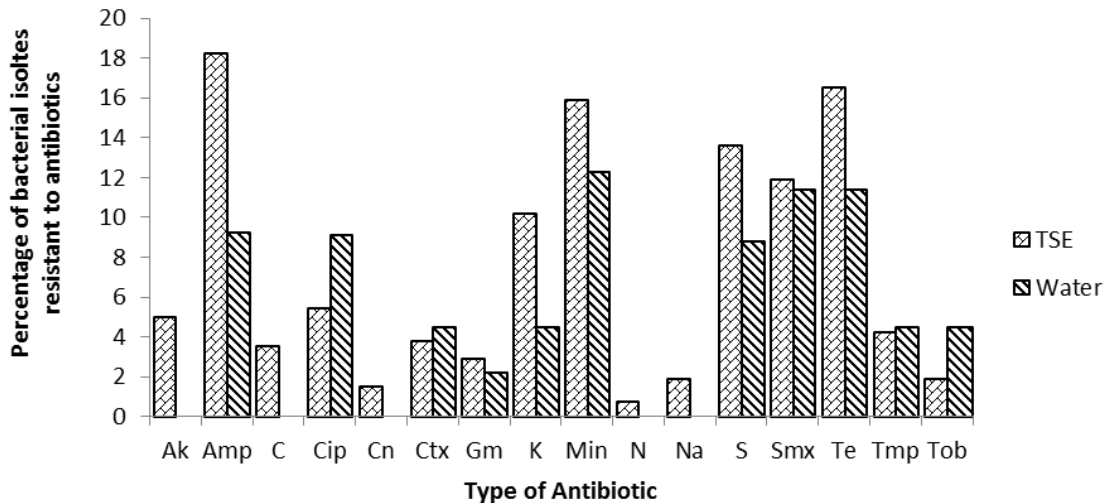


Figure 16. Antibiotic-resistant *Escherichia coli* in TSE and well-water.

4. Discussion

A survey of chemical, biological and physical factors which had an environmental impact in the Seeb area was investigated during three periods. During these periods there was a gradual deterioration in environmental and ecological conditions which threatened Seeb.

Approximately 200 sewage treatment plants are located in Muscat and the area in which this study was conducted. However, a small percentage of the populated area is connected to the sewage system, with most using septic systems, holding tanks, and cesspits. Due to poor construction, untrained work force, along with infrequent inspections, the older systems are breaking down and inadequate, thus allowing wastewater to discharge into the surrounding soil which is also porous (MEMWR 1998). A large amount of wastewater is discharged directly into the soil. Therefore, holding-tanks should be installed in areas where sandy soil, high water table, and dense housing are

characteristic. Various public health and environmental pollution will take place if this is not done, ranging from food poisoning to disease causing pollutants such as cancer. To avoid contaminated sewage intrusion into the environment, the authorities have taken a major step in planning to construct one of the largest membrane bioreactor (MBR) in the world, serving 90% of the Muscat residents. The project is expected to be finalized in 2017, connecting more than 18 thousand houses and public institutions with 330 km of pipeline of which 70km of pipeline will be used to distribute treated effluents for agriculture and public use (Water technology 2014).

Due to severe shortage of rainfall in Oman, TSE is used for irrigation or discharged to soakaways, wadis, boreholes and some to the sea. In addition, the underground water is polluted by infiltration of untreated sewage from cracked holding tanks, septic tanks, and cesspits.

Two types of STPs are operated either by government or private agencies, most of which are in Muscat. According to Government regulations, STP inspection in Muscat is supposed to be conducted monthly. However, STPs inspection is irregular. These regulations need to be vigorously enforced (MEMWR 1998). Sewage treatment plants in Muscat have been privatized. HAYA-Water is a new establishment for wastewater services which is planning to use MBR technology. It is expected to produce cleaner sewage treated effluent with effective inspection systems.

In this study, based on microbiological and physicochemical data, it is evident that well-water is contaminated by sewage effluents. Several factors influenced the environmental and ecological conditions which resulted in major deterioration of well-water quality in affected farmlands, loss of natural vegetation, and may have an impact on public health in the region. All these factors directly or indirectly, could be responsible for economic loss (Raina and Sangar 2002, Olmstead 2010, WHO 2001). For example, thousands are spent for treatment of diseases which could possibly be prevented (WHO 2001). Overuse of underground water was probably one of the major factors that led to high salinity of most wells and caused significant vegetation changes in the area, which then led to abandonment of most farms. This was followed by extensive urbanization of farmlands which was another environmental and ecological factor that led to substantial underground water pollution. A similar trend of water pollution was also reported by Kodarkar (2004) in Hyderabad, India.

Due to having little rainfall, and with extensive use of groundwater, the salinity and chemical pollution rendered the farmland unfit for farming and led to its abandonment and being used for urbanization. The urbanization then led to a significant increase in construction of septic tanks, holding tanks and cesspits. Deterioration in ecological conditions was also related to dramatic population increase. This major increase in industry and population growth has consequently led to an ever increasing demand for water.

High concentrations of THMs were detected in samples near the wells close to the irrigated greeneries and public parks. This suggests that THMs are good indicators for TSE infiltration into the ground water. According to the Omani standards, OS 52/1 and OS 8/2006, several wells were found to exceed MPL which is in agreement with standards of some major health organizations (WHO 1982; NSF International 2003; The soil profile 2006; Mahadev *et al.*, 2010). These standards must be followed to avoid further THMs pollution, even though there is no unified international maximum limit of THMs in drinking-water (Government of Western Australia 2009). The WHO (1982), NSF International (2003) and The Soil Profile (2006) standards could be used as basis

for THM maximum limit in water to avoid health risks of different types of toxicities leading to physiological toxicity of organ systems and cancers (WHO 2004).

TDS, NO₃, and fecal coliforms are the main parameters chosen to determine the quality of the treated wastewater. High levels of nitrate were found in many samples. Sewage from nearby septic and holding-tank leakage may be the main factor contributing to nitrate contamination. Nitrate concentration was recorded at maximum levels in 1995-2000. More than 25% of the wells exceeded the permissible levels according to the Omani-Standards Drinking-Water 8/2006 (OS 8/2006). Nitrate contamination of underground water may also occur from fertilizers. Nitrate has also been used as an indicator of microbial activity and growth. Al-Bahry *et al.*, (2009a) reported that nitrate concentrations are related positively to microbial growth in TSE distribution and irrigation lines. They reported that viable bacteria and nitrate concentrations in the distribution lines increased significantly farther from the STP storage reservoir.

TDS is considered an important factor for assessing water quality. TDS in well-water during the three periods reached maximum values during 1995-2000 but decreased significantly in 2010. High TDS levels probably originated from contaminated effluents with organic and inorganic contents in water such as industrial wastewater and agricultural run-off, and possibly from natural environments. Its levels in a natural environment may vary, depending on geological regions. Higher TDS levels in drinking water may be related to cancer and cardiovascular problems, such as coronary and arteriosclerotic heart disease, as well as gallstones and inflammation of the gallbladder. Higher concentrations lead to corrosion and scaling of water distribution lines and house hold appliances (WHO 2003). Higher turbidity level is an indication of high microbial contents in water. Turbidity protects microorganisms and stimulates microbial regrowth even after disinfection (Al-Bahry *et al.*, 2009a, 2011, WHO 1997,). Egorov *et al.*, (2003) reported that high turbidity concentration in drinking-water was a major factor for gastrointestinal illnesses.

Microbial counts of coliforms, enterococci and *E. coli* were much higher in TSE than in well-water during the three periods. Many of the wells were contaminated with *E. coli*. Detection of *E. coli* in water also indicates the presence of pathogenic protozoans, bacteria and viruses. Due to these conditions, microbial diseases such as cholera, typhoid and hepatitis are serious illnesses associated with water contamination (Sonnenwirth and Jarett 1988). According to Calderon & Mood (1998, 1991) potential pathogens occasionally exist in large numbers in water; however, when they are in small numbers they are unable to cause infection. There was a significant increase of chemical and microbial food poisoning in recent years in Oman (Community Health Disease Surveillance Newsletter 1998, Ministry of Health, Oman 2005) while the endemic viral hepatitis cases in Oman reported in the same period was fluctuating (Ministry of Health, Oman 2005). Probably, the infection is due to fecal contamination of well-water (Sonnenwirth and Jarett 1988).

In this study, the highest heavy metal concentration is probably related to sewage infiltration. Also, porous soil conditions may contribute to the infiltration of pollutants in well-water. The frequent heavy metals found in both well-water and TSE were Ni, Zn, and Cr. Many of the detected heavy metals in this study are commonly used in industries (Iqbal and Gupta 2009). In addition, the composition of ground water is affected by the lithology of rocks and quantity of infiltrating rainfall. Biological and human factors can alter water composition (Salem *et al.*, 2000). Skeat (1969) reported that heavy metal impurities penetrate the soil to reach underground water through sewage

and industrial effluents. A number of international organizations recommended safe levels of heavy-metals in drinking water (Adefemi and Awokunmi 2010; Krasniqi *et al.*, 2010; Lungu *et al.*, 2010; NSF International 2003; OS 8/2006; The soil profile 2006). However, with the exception of Ba, Br, Mo and Se, other heavy-metals in wells surpassed the safety levels which made drinking-water unsafe. Heavy metals and its distribution in wells in the study area also depend on the nature of soil and underground rocks. However, their level from natural sources is usually low (EPA 2012). The source of heavy metal in the well which exceeded MPL is probably from the surrounding industries or the overuse of underground water in arid regions, such as Oman (Al-Musharafi *et al.*, 2012, 2014a). With this in mind, there is a gradual accumulation of heavy metals in well-water which may lead, in time, to higher percentages of contaminated wells. Due to the availability of fresh water provided from the governmental sources in recent years, most of the wells are not being used for drinking purposes.

Many of *E. coli* isolates from both well-water and TSE were multiple-resistant to antibiotics and their resistance varied to the 16 antibiotics. The isolates were highly resistant to ampicillin, minocycline, streptomycin and tetracycline. Al-Bahry *et al.*, (2009a) reported that the majority of the isolates from TSE distribution lines were multiple-resistant to several antibiotics. The isolates resisted the chlorination process and remained viable in TSE and distribution lines. They reported that the isolates exhibited maximum resistance to ampicillin followed by sulphamethoxazole, carbenicillin, streptomycin, and minocycline. They also observed that viability of MARB TSE used for irrigation may have serious complications for public health and wildlife where inhabitants can be infected by MARBs. Al-Bahry *et al.*, (2009b; c; 2010; 2012) used multiple antibiotic resistant bacteria for biomonitoring of environment and bioindicators of pollutions caused by contaminated effluents. The overuse of antibiotics for treatment of diseases, prophylaxis and growth promotion in Oman caused the emergence of multiple antibiotic resistant microbes and led to contamination of soil and aquatic environments via sewage and agricultural runoff (Al-Bahry 2009b, c, 2010, 2012, Mahmoud *et al.*, 2013).

Globally, microbial infections were the most common diseases before the World War II and infections became insignificant due to antibiotic treatment. However, emergence of antibiotic resistant strains became very common and new generations of antibiotics are continuously being introduced. WHO (2014) published comprehensive data collected from 114 countries which reveals the major threat of MARBs and that antibiotics are no longer effective in treatment of infections. Some common infections which were curable are now becoming untreatable (WHO 2014).

Based on the present data, the highest concentrations of salinity and all contaminants associated with leakage of effluent from cesspits, septic and holding-tanks were recorded between 1995 and 2000. Salinity and contaminants concentrations dropped significantly in 2010. This is probably attributed to a significant amount of rainfall in 2004, 2006, and during the cyclone Guno in 2007. The heavy rainfall during these years has significantly diluted contaminants of underground and well-water.

The Omani population in 1995, 2000, and 2010 was 2.155, 2.193, and 2.8 million respectively, excluding expatriates. It is estimated that Omani population will increase to approximately 5 million in 2030 (The Demographic Profile of Oman, 2014). Based on population growth and the data presented above, there is a serious health problem due to leakage of wastewater from the cesspits, septic, and holding-tanks to well-water. This problem will escalate with dramatic increase in population which leads to an

increase in water usage and expanding urbanization using septic and holding-tanks. These conditions will result in a steady increase of sewage, containing health-threatening contaminants such as pathogenic microbes, chemicals and changes in the physical characteristic of ground water. The most frequent limiting factors for potable use of water is total salinity estimated as (TDS), or more as electrical conductivity (EC). Incidence of cancer on the rise may be related to the high concentration of nitrates and heavy metals. Also, water hardness is one of the common causes of kidney stones (Bellizzi *et al.*, 1999).

The regional governments have undertaken research to reduce the cost of desalination and enacted environmental legislation to minimize the environmental impact of TSE (MEMWR 1998). These regulations affect wastewater reuse and discharge of effluents, and the construction of septic tanks and holding tanks. It is imperative that the reused quantity of TSE in irrigation should be in conformity with the physicochemical and microbiological standards set by the Government authorities (WHO 2005). With the establishment of new institutes dedicated for the management of STPs and the treatment of sewage effluents, it is imperative that the physicochemical and microbiological standards will be followed.

If this trend continues without stringent environmental regulations and scientific investigations, it will lead to gradual deterioration in ecological and environmental conditions. During the last few years, there was a steady decrease in water resources mainly due to the lack of rainfall. In the study area alone, there has been a substantial increase in population and greeneries up to six-fold between 1980 and 1990. Only 45% of sewage effluent was treated, with the remaining going to sea and soakaways (MEMWR 1997). With the steady increase in population, the demand for fresh-water usage has increased significantly from 12000 m³ to 34000 m³ daily. During the last forty years, rapid development in all aspects, including population increase, social, industrial and agricultural sectors resulted in higher demand for water consumption.

5. Conclusions

In conclusion, the data presented in this study are alarming. In this study, MARBs were used as biological indicators of sewage effluent pollution. In all surveyed pollutants, MARBs and heavy metals are the main public health concerns. Heavy rainfall in 2010 diluted the pollutants. The necessity of reusing recycle water in this region caused accumulation of several chemical pollutants. Stringent governmental regulations and institutional monitoring are urgently needed.

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