Pathogens Assessment in Reclaimed Effluent Used for Industrial Crops Irrigation

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Received: 24 October 2005 / Accepted: 19 January 2007 / Published: 31 March 2007

Abstract: Reuse of treated effluent is a highly valued water source in Palestine, however with limited success due to public health concerns. This paper assesses the potential pathogens in raw, treated and reclaimed wastewater at Albireh urban wastewater treatment facility, and provides scientific knowledge to update the Palestinian reuse guidelines. Laboratory analyses of collected samples over a period of 4 months have indicated that the raw wastewater from Albireh city contained high numbers of fecal coliforms and worm eggs while 31% of the samples were *Salmonella* positive. Treated effluent suitable for restricted irrigation demonstrated that the plant was efficient in removing indicator bacteria, where fecal coliforms and fecal streptococci removal averaged 99.64% and 93.44%, respectively. Although not disinfected, treated effluent was free of *Salmonella* and parasites, hence safe for restricted agricultural purposes. All samples of the reclaimed effluent and three samples of irrigated grass were devoid of microbial pathogens indicating a safe use in unrestricted agricultural utilization. Adequate operation of wastewater treatment facilities, scientific updating of reuse guidelines and launching public awareness campaigns are core factors for successful and sustainable large-scale wastewater reuse schemes in Palestine.

Keywords: Agricultural effluent reuse; microbial pathogens; waterborne disease; wastewater reclamation

Introduction

The disposal of raw, partially and secondary treated domestic and municipal wastewater into receiving water bodies still continues in Palestine. However, in recent years there has been an increasing effort to utilize both stabilized biosolids and treated effluent on land for agricultural crops. This has arisen from concern about water scarcity, public health risk caused by microbial pathogens and serious degradation to soil and water environment caused by salt, heavy metals and nutrient pollution [1-5]. The newly enacted Palestinian guidelines on reuse of treated effluent have been developed based on limited results of a small research group or modified foreign guidelines [6]. Given the high cost of treatment and the lack of financial resources for treatment facilities, the municipality also discharges un-disinfected treated effluent into the nearby seasonal Wadi thereby incurring hidden environmental costs, which is not accounted for. The municipality is further encouraged to do so, as the main goal of wastewater management from the budgetary viewpoint, is the treatment costs reduction, rather than environmental quality improvement. There seems to be a lack of awareness of the public health impacts of wastewater irrigation on the part of the farmers and Albireh municipality as well.

The use of treated domestic and municipal effluents in agriculture is an efficient way to conserve water resources, recycle nutrients (N & P) and minimize pollution loads to receiving water bodies. There are crucial health and environmental risks associated with using partially or full treated wastewater and the official policy has therefore been to provide adequate treatment of sewage before use. However, in most cases the use is an unplanned practice of poor farmers in developing countries that lack resources to establish adequate wastewater treatment facilities. The first difficulty in wastewater reuse is the absence of reconnaissance of such water by the Palestinian public as a non-conventional resource. All reuse pilot-scale projects were failure as no rational use of this water is organized in most cases. Lack of large-scale water reuse projects is due to bad effluent quality from the existing old sewage works.

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About 35% of the total population in Palestine is served by central sewerage systems and about 17% of the collected municipal wastewater is being partially treated. Thus establishing new sewerage infrastructures is an environmental protection measure that is superior to discharging treated effluents into receiving water bodies. Moreover, Palestine as a semi-arid region has very few flowing streams with sufficient capacity to serve as natural reservoirs for treated sewage effluents. The main disadvantage of using treated wastewater for agricultural purposes is the presence of pathogens as bacteria, viruses, and parasites that can pose health risks for the farmers, soil, nearby located communities, and also to the consumers of product irrigated with treated sewage. To reduce these health risks the Palestinian Water Authority has newly developed national guidelines based on recommended rules issued by the World Health Organization [7-8]. If the wastewater contains industrial effluent, chemical pollutants such as heavy metals might pose additional public health and environmental problems [9]. Recently, Kamizoulis et al., [10] underlined the needs for additional research on uncertainties of potential impacts on public health and environment of the various effluent reuse options, as there is no scientific data that the WHO reuse guidelines failed to protect public health. The aim of this paper is to make assessment on microbial pathogens and fecal indicator bacteria occurrence and removal at Albireh wastewater treatment plant (AWWTP) and effluent reclamation site.

*Hygienic Safety Guidelines of Treated Effluent for Agricultural Production*

Angelakis et al. [11] reported that one of the major constraints for water reuse and its public acceptance is the lack of relevant and unified regulatory guidelines and legislation worldwide and at EU level in particular. More realistic water policies and reuse guidelines of domestic and municipal wastewater are urgently needed. Numerical values of fecal coliforms that are endorsed in present water quality standards are based mainly on philosophy and experience rather than applied scientific knowledge. In Palestine, the discussion on health risks caused by reuse of reclaimed effluent for food crops production often does not go beyond the level of vague generalizations. The outcome of similar discussions has been newly reflected in the suggested Palestinian guidelines on wastewater reuse [6] due to lack of solid scientific data and absence of field practical knowledge. Several research projects have underlined the benefits, potential health risks and environmental impacts resulting from use of reclaimed wastewater and suggested management measures in order to use treated effluents within acceptable levels of risk for the public health and the receiving water bodies [5, 12-14]. Worldwide, most epidemiological evidence is gained from a limited number of pilot-scale studies made by small research groups. Furthermore, the focus has been on localized negative health impacts of wastewater irrigation.

*Case Study: Albireh Wastewater Treatment Plant*

Albireh City and portions of the nearby dwellings of Ramallah city, domestic septage and some commercial and small family owned industrial enterprises generate the wastewater, which enters Albireh Wastewater Treatment Plant (AWWTP). The greater volume (about 90%) is from residential homes and apartment buildings. A lesser portion of the wastewater (about 7%) is generated by local industries. The wastewater from industries is periodically tested for the presence of toxic materials such as heavy metals and solvents.

With German financial and technical assistance, AWWTP comprised of two oxidation ditches to serve about 50,000 capita for the first planning phase, one oxidation ditch with two secondary settling tanks were put into operation in February 2000 to serve 25,000 capita (Fig. 1). At present, AWWTP treats an average of 3200 m$^3/d$ of wastewater, but flow may exceed 6000 m$^3/d$ during wet weather periods such as snow melt in December coupled with heavy rainfall. The purpose of wastewater treatment at AWWTP is to reduce pollution loads discharged into receiving water bodies, protect public health and produce an effluent of international quality suitable for agricultural purposes. Albireh municipality operates the WWTP under a permit from the Palestinian Water Authority (PWA) and monitors daily effluents. To reduce pollution loads (BOD, nutrients and suspended solids), AWWTP utilizes two main types of processes; physical (screening and two aerated grit chambers) and biological (low rate oxidation ditch with aerobic sludge stabilization).

*Figure 1: Overview of Albireh wastewater treatment plant (AWWTP)*

The sludge line (Fig. 1) entails a gravity thickener and two filter presses for sludge dewatering, where the stabilized and dewatered sludge is landfilled. During the last three years, the plant produces an effluent of international quality standards. About 95% of the BOD, 92% COD, 90% of the suspended solids and about 70% of total nitrogen during normal plant operation are achieved [15-16]. At present, as the reuse facilities are not yet in operation, treated effluent is discharged without

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disinfecting into nearby seasonal wadis, however, UV-disinfecting units are installed but non-functional.

Wastewater Reuse Demonstration Project

One of the aims of this project was to utilize the reclaimed effluent using a pressurized sand filter and chlorine disinfecting unit (Fig. 1) for irrigation of a wide range of processed vegetables; fruit trees, and fodder [8]. Initial results of this demonstration project showed that the quality of the reclaimed effluent complied with prescribed national standards and the Israeli guidelines (BOD/TSS= 20/30 mg/l; fecal coliforms (FC) = less than 1000 FC/100 ml. Chemical analysis of reclaimed effluent revealed that BOD values ranged between 3-4 mg/l, and microbiologically free of fecal coliforms with no human intestinal nematode eggs. Results on microbial tests of industrial corps (egg plants) and fodder (Alfalfa) drip irrigated with reclaimed effluent were also pathogens free [8].

The short-term results are thought to encourage and enhance the promotion of reclaimed effluent use for crops irrigation on a wide scale. However, the gained data were not sufficient enough to develop reliable and adequate microbiological guidelines, since the monitoring program did not analyze other important microbial pathogens. Furthermore, the site location for the establishment of a large scale wastewater reuse scheme in Ramallah-Albireh district was not socially and economically well selected, severe public rejection in the area for the reuse idea was the outcome.

Materials and Methods

Sampling and Analysis of Potential Pathogens

The study was designed on the basis of sampling and analysis (30/3/2004 - 15/09/2004) for microbial indicators of human and animal origin and potential pathogens. Water samples and industrial crops were obtained from the following sources; wastewater treatment plant influent (raw sewage); secondary effluent (treated effluent); reclaimed effluent (disinfected) and vegetable samples that can be obtained during the irrigation season. As no industrial vegetables were harvested (available) during the study period, samples from greenhouse grass and irrigated loan of the wastewater treatment plant were taken and analyzed. Laboratory personnel of the Water Studies Institute and the Palestinian Water Authority obtained and analyzed the samples according to detailed procedures. These procedures are documented in various sections of Standard Methods [17], Ayres and Mara [18] and UNRWA [19]. As indicated, four types of samples were taken from different sources. A sampling frequency of once every week was chosen.

A 6-month occurrence study was performed to detect and enumerate indicator bacteria (Fecal coliforms, total coliforms, and fecal streptococcus) and pathogens (Salmonella, Giardia, Trichomonas, Enterobius vermicularis, Ascaris, Balantidium coli, Strongyloides stereoralis, Ameba cysts, Entameba histolytica, and Entameba coli) before and after the current treatment. Water samples of influent and tertiary treated effluent were collected once a week. Two liters were used for parasite enumeration and the remaining volume for bacteriological analysis. Fecal coliforms (m-FC) and fecal streptococci (m-Enterococcus) were enumerated by membrane filtration methods according to Standard Methods [17]. The water samples used for the enumeration of cultivable pathogenic parasites were concentrated by sedimentation for 1 hour and 0.8 L of settled water was then centrifuged at 1500 RPM for 5 min. One drop of sediment was taken on a slide and microscopically examined using ZEISSZ microscope (10x and x40). No attempt was made to differentiate viable from non-viable parasites [18-19].

Results and Discussion

The results obtained are presented and discussed in the following Tables and Figures. Data on wastewater characteristics and removal efficiencies, relevant for wastewater reuse and public health are presented in average values. Fecal indicator bacteria were detected in numbers similar to those reported for sanitary wastewater [20-21]. Fecal coliforms were the most numerous of the indicator bacteria and their removal was 25% on average, the same range as fecal streptococci (29%), while E. coli removal was slightly lower (12%). Removal percentages are primarily caused by the settlement of bacteria, which are adsorbed to, or entrapped within biosolids particles in the aeration tank. The reported bacterial die-off in sewage suggest that only a very small reduction (<10%) would be expected due to natural death in short hydraulic retention times (HRT) in the oxidation ditch [21].

Fecal Coliforms

Even when the BOD₃ is reduced to low levels (<20mg/l), the treated effluents may still contain large amount of pathogenic bacteria, protozoa, and helminthes ova. From the perspective of effluent reuse, these water characteristic are at least as, if not more important as the conventional BOD and COD values. Nevertheless, these effluents comply with the WHO guidelines for restricted irrigation but not with those for unrestricted irrigation (Table 1 and Fig. 2). About 99.64% of fecal coliforms were removed in the oxidation ditch with aerobic sludge stabilization. Therefore, tertiary or advanced treatment should be applied to improve the pathogenic removal especially in those areas where the effluent is to use for unrestricted irrigation, which is less than 1000 CFU/100ml and less one egg/l of helminthes [7]. Putting the UV-disinfecting units into operation, which are currently non-functional at AWWTP, use of waste stabilization ponds, soil aquifer treatment or conventional treatment processes (filtration and disinfecting) can produce a reclaimed effluent meeting the WHO microbiological guidelines for agricultural irrigation.
**Table 1:** Fecal coliforms and Fecal streptococcus in AWWTP influent and effluent*

|                        | Influent (n=15) | Effluent (n=13) | Removal efficiency (%) (n=13) |
|------------------------|-----------------|-----------------|-------------------------------|
| **Fecal coliforms**    |                 |                 |                               |
| Influent               | 7.32 (±0.61)    | 4.42 (±0.27)    | 99.64 (±0.52)                |
| Effluent               |                 |                 |                               |
| **Fecal streptococcus**|                 |                 |                               |
| Influent               | 6.32 (±0.64)    | 4.81 (±0.64)    | 93.44 (±8.39)                |
| Effluent               |                 |                 |                               |

*Data are presented as average values in log_{10} term (± standard deviation); n = number of samples.

According Angelakis *et al.*, [11], the FC guidelines vary from one country to another (Jordan < 1000; Israel < 250; Kuwait < 1000 CFU/100ml. The variations in fecal coliforms values in the different countries reflect the variations in the treatment, climatological conditions and reclamation technologies applied as well as the different reuse guidelines adopted in each respective country. Payment *et al.*, [22] demonstrated that an urban physical-chemical primary treatment plant in America was not efficient at removing indicator bacteria and the pathogens tested (*Giardia* and *Cryptosporidium*). Fecal coliforms were the most numerous of the indicator bacteria and their removal averaged 25% while fecal streptococci removal was 29%. Higher removal percentage of FC at Albireh sewage works can be attributed to both long hydraulic and solids retention times (sludge age) in the aeration tank.

The behavior of fecal coliform and fecal streptococcus is the same in terms of concentration (log_{10}) and removal (Fig. 2 and 3). It has been reported that the quantities of fecal coliforms and fecal streptococci that are discharged by human beings are significantly different from the quantities discharged by animals. Geldreich and Kenner [23] reported that FC/FS ratio for domestic animals was less than 1, whereas the ratio for human beings was more than 4. This indicates that FC was originating from the contamination of human waste when greater than FS, as in our case with an average FC/FS of 1.20. However, this is not a rule according to published literature [24], where the ratios varied widely and averaged 0.51 for pulp waste with no animal waste of any kind. These ratios cannot be taken seriously due to the long hydraulic retention (1-2 days) and sludge age (25 days) prevailing in the aeration basin, die-off of fecal coliforms might be greater than that of fecal streptococci. The results obtained are difficult to interpret as the ratio FC/FS will decrease based on the survival time of microbial pathogens [21].

**Figure 2:** Fecal coliforms result for influent, effluent of oxidation ditch

**Figure 3:** Fecal streptococcus in AWWTP influent and effluent

**Fecal Streptococcus**

*Streptococci* have an interesting history and as a single genus of bacteria have probably caused disease that is more widespread and morbidity in man over the centuries than almost any other bacteria. The relationship between *Streptococci* and rheumatic fever is well established, however, not as indicators of the bacteriological quality of wastewater irrigated crops [5]. The removal efficiency of fecal streptococcus was about 93.44%, indicating further reclamation needs (Table 1). The pattern of removal rates in terms of log_{10} is presented in Fig. 3.

Worldwide, the health impacts of inadequate water supplies and sanitation services are behind 15% and 7% of all deaths respectively. The global incidence and prevalence of two of the most important groups of water- and excreta-related diseases are namely diarrhea and the geohelminthiases, where young children bear most of the burden of diarrhea, and older children and adults the burden of the geohelminthiases [25].

The results shown in Figure 4 reveal a wide variation reflecting the behavior of FS concentration in the same sequences since in some time it is equal or greater than the influent, this make the removal value negative value. Samhan and Al-Sa’ed [1] reported negative removal efficiency of microbial pathogens of anaerobically pre-
treated municipal effluent. Fecal streptococcus is considered as a pathogenic indicator for agricultural reuse purposes, since fecal streptococcus infection on the area of mouth and throat and it is normal flora on the stomach and intestinal.

Salmonella

Salmonella transmission takes place when the infected feces of man or animal are ingested by a susceptible person. Food-borne outbreaks of typhoid and other salmonelloses are well documented in the literature. In some cases they are due to contaminated raw materials, such as poultry, tinned meat, or eggs. In the case of typhoid fever, transmission is only from human feces or urine to mouth. This fecal-oral (or occasionally urinary-oral) transmission may be direct where personal cleanliness is poor, or it may be via contaminated food or water, case of salmonelloses or enteric fever may excrete up to $10^{10}$ Salmonella per gram of feces. The monitoring of sewage for Salmonella in general, or for S. typhi in particular, is of practical public health value in epidemiological surveillance.

As the identification of Salmonella is beyond the scope of this research study, only confirmation tests made on 13 influent and effluent samples (01.06.-15.09.2004) revealed about 31% of the raw wastewater (influent) showed positive results (Salmonella presence), where all effluent samples were negative (devoid of Salmonella). Methods for pathogen detection are still being developed and issues of comparability and infectivity remain to be resolved. Pathogen enumeration in treated effluent can provide information complementary to classical indicators in order to establish the level of reclamation to achieve. Furthermore, results obtained from routine monitoring or research studies are not easy to verify in the absence of detectable public health effects.

Pathogens Occurrence in Raw Wastewater of Albireh WWTP

All pathogens found during the study period of 190 days where the presence is not regular and some of them found 2-5 time only. All of the pathogens found in the influent were not detected in the treated effluent of AWWTP (Fig 4). For example, Ascaris lumbriciodes was absent and not detected during the time of analysis, this indicates that inhabitants of Albireh city are not Ascarisis infected. It is well known, that only the eggs form of Ascaris are pathogenic to humans.

Unlike Amoebae of variable shape, flagellates are more rigid and tend to retain distinctive shapes. Trichomonas can be identified by its motion as quick, jerky, and darting, however its identification is difficult as it is fragile and does not stain well. Trichomonas was detected the influent of AWWTP in 13 samples with an average of 18 eggs/l (±8). Samhan and Al-Sa’ed [1] reported that the raw wastewater contained a small amount Trichomonas compared to numbers (577-708 eggs/l; n=13) found in the anaerobic UASB-septic tank, where they assumed that sufficient HRT (2-4 days) is enough for regrowth. After the tertiary treatment, all samples analyzed were free of parasites and probably removed through the sludge line via attachment and settlement. Results depicted in figure 4 shows a wide range in the detection of parasites eggs in raw samples. Methodological difficulties often encountered with the methods of concentration and microscopic enumeration of eggs might explain these observations [26]. Similar difficulties were reported by Payment et al., [22] while enumerating some pathogenic parasites (Giardia and Cryptosporidium) in both raw and treated effluent of a primary physical-chemical treatment plant. After coagulant addition (ferric chloride or aluminum) Giardia remained in the 2-log range while Cryptosporidium oocyte counts were erratic and variably detected with 29% removal.

Pathogens in the influent

![Figure 4: Pathogenic parasites and helminthes found in Albireh WWTP influent](https://ssrn.com/abstract=1676705)

Pathogens ability to infect depends on a large number of factors and both host and parasite are living creatures and therefore do not respond to environmental factors in a similar mode. So the analysis for wastewater in term of pathogens must be done in a regular basis for the influent by monitoring the endemic parasite and control the disease associated with. It is also important to mention that it is difficult to determine the infectious dose, as there are several factors that should be take into consideration.

Microbial Criteria for Wastewater Reuse in Agricultural Irrigation

Wastewater treatment should reduce the number of microorganisms to an acceptable level. This level can vary according to the envisaged use of the receiving water bodies, such recreational activities, drinking water source, irrigation, and aquaculture. The guidelines that were set until now might not be sufficient to satisfy today’s needs for a better quality of life, free of waterborne diseases. Municipal sewage contains a large variety of pathogenic organisms, including, bacteria, protozoa and helminthes.
Based on pathogens contents (fecal coliforms) the PSI [6] entails three quality categories within the Palestinian guidelines on wastewater treatment, disposal and reuse; category A (high quality), B (good quality), and C (fair quality) and D (bad quality). Treated effluent should have less than 200 CFU/100 ml to comply with Category A) quality criteria, whereas categories B-D should have less than 1000 CFU/100 ml and can only be utilized under certain conditions.

The results in Table 2 show that the treated effluent from AWWTP is pathogens free and suitable for restricted agricultural irrigation. Reclaimed effluent and irrigated grass within the greenhouse showed high quality that meets the prescribed PSI national quality for unrestricted agricultural irrigation. Similar results were published by Sheikh et al., [27] where pathogens were not found in any of the samples of tertiary recycled water from the Monterey County Water Recycling Projects. Bahri and Brissaud [13] proposed regional guidelines on wastewater reuse for the Mediterranean zone. These guidelines were based on several considerations as agricultural market, tourism, public concern and fair competition among farmers.

No bacterial guideline was proposed for restricted irrigation in the WHO guidelines [7], due to the lack of evidence of risks of bacterial and viral infections to farm workers and nearby residents. A recent review [28] on reuse standards for agricultural irrigation demonstrated that contact with partially treated irrigation effluent (10^3 - 10^6 FC/100 ml) was associated with an increased risk of diarrhea disease among 5-14 year children in Mexico City. In situations where insufficient treatment prevailed to reach 10^5 FC/100 ml, then a relaxed guideline of 10^4 FC/100 ml was suggested, provided that other supplementary health protection measures are met for children. In situations where no children were involved in farm work, there was insufficient evidence to justify a Fecal coliforms guideline as there was only limited evidence of a risk to adults from enteric infections [29]. Similarly, within the Mediterranean region, as non potable reuse will remain the main goal of wastewater reuse schemes, suggested regional guidelines for agricultural water reuse should focus on the microbiological hazards and long term impacts of chemical pollutants.

Agricultural effluent utilization and prospective recreational uses of Ramallah-Albireh district are prompting a review of the practiced wastewater management in the area. This implies the necessity of putting disinfection units in operation before treated effluent discharge in the nearby seasonal wadis. However, there is little scientific data on the dilution effect of the wadi flow during wet weather periods as well as of the survival of potential pathogens in these waters. The long term effects of tertiary effluent discharge and re-installation of disinfection units have to be investigated to quantify the impact on groundwater, cultivated industrial crops and to predict the health risks associated.
Conclusions and Recommendations

From a thorough revision of the collected literature and overall assessment of the potential pathogenic parameters made within this study, which is the main focus of the article, the following conclusions emerge:

- This study on hygienic quality aspects did not detect any Salmonella or pathogenic parasite in any of the samples of the tertiary treated effluent of Albireh WWTP. Hence, the treated effluent is safe for only restricted reuse in agricultural irrigation.
- Though potential pathogens were not present in the treated effluent; however, adequate reclamation including disinfection using either UV light or chlorination is of utmost necessarily, if unrestricted effluent reuse in agricultural irrigation is planned.
- The microbial analysis of irrigated grass within the greenhouse at Albireh sewage works revealed re-growth of pathogen indicators despite effluent reclamation using slow sand filter and chlorine disinfecting units within the reuse demonstration project.

The final reclaimed effluent quality of Albireh wastewater treatment plant met all relevant local and WHO health standards for unrestricted non-potable use, including playground irrigation, agricultural irrigation of all crops and contact recreation. Hence the following recommendations can be made:

- Use of non-disinfected effluent for unrestricted irrigation may impose serious health risks, hence pathogens long-tem monitoring and prohibition of raw municipal discharges are highly recommended.
- Establishment of large reuse schemes to serve protection of receiving water bodies, public health, ecosystems and landscape.
- Public awareness campaigns and conduction of local and regional workshops on research needs in the field of wastewater reuse are recommended. These will identify priority projects, mind repetition, and facilitate cooperation between relevant stakeholders at local and regional levels.

Acknowledgements: The author acknowledges the financial support made by the EU within the Medreunet II program coordinated by the Spanish Aqbar Foundation. The help provided by Albireh City Mayor and the treatment plant personnel are highly appreciated.

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