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Effects of irrigation with secondary treated wastewater on physicochemical and microbial properties of soil and produce safety in a semi-arid area

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A B S T R A C T

Water scarcity is becoming one of the largest problems worldwide. Agricultural reuse of wastewater has been considered a valuable and reliable alternative, alleviating the pressure on freshwater resources in arid and semi-arid regions such as the Middle East. Inadequate microbial quality of treated wastewater is a challenge for developing countries, which limits agricultural reuse of wastewater. This study assessed the impact of irrigation with secondary treated wastewater (STWW) on soil properties as well as the safety of various types of crops as compared with tap water (TW) irrigation through a furrow system. Total and fecal coliforms and *Escherichia coli* were monitored as indicator bacteria in STWW, irrigated soil and harvested crops. The presence of pathogenic *E. coli* O157, *Salmonella* and *Shigella* was also monitored in all samples using a combination of culture and molecular methods. The microbial quality of wastewater in terms of *E. coli* concentration (4.18 Log MPN/100 ml) failed to meet the world health organization (WHO) recommendation for irrigation of root and leafy crops ($\leq 10^3$ and $\leq 10^4$ *E. coli* per 100 ml for root and leafy crops, respectively). No significant effects on physicochemical properties of the soil irrigated with STWW was found in comparison with control plots, except for electrical conductivity (EC) and sodium adsorption ratio (SAR), which were slightly higher in STWW soil samples. Although the microbial quality of soil was affected by STWW irrigation, a relatively low concentration of *E. coli* was detected in soil. No microbial contamination in terms of *E. coli* was found on harvested maize and onion. *E. coli* contamination of lettuce and spring onion was found for both irrigation schemes. No STWW, soil or crop samples were found positive for pathogenic bacteria. According to the analyzed parameters, STWW could be safely used as an alternative source for irrigation of root and leafy crops.

1. Introduction

Fresh water scarcity is one of the greatest environmental challenges in the 21st century, and is caused in part by an uneven distribution of available water resources around the world (Becerra-Castro et al., 2015; Jasim et al., 2016). As a consequence, 40% of the total land area is classified as arid, semi-arid and dry sub-humid (Becerra-Castro et al., 2015). Several factors including population growth, surface water and groundwater pollution, droughts and climate changes are expected to increase stress on water sources in many regions of the world in the future decades. It is estimated that more than 40% of the world's population will face water stress or scarcity within the next few decades, with significant impact on socio-economic, water and food security (Becerra-Castro et al., 2015; Elgallal et al., 2016; WHO, 2006). Iran, with an average annual precipitation less than one third of the global average (250 mm), is located in one of the most arid regions in the

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world, is facing a serious and protracted water crisis. It is anticipated that the level of water resources in Iran will decrease from 2025 to 816 m^3 per capita from 1990 to 2025 (Jasim et al., 2016; Lehane, 2014). The agricultural sector, with about 70% of the total water usage, is the largest user of freshwater worldwide (Becerra-Castro et al., 2015; Elgallal et al., 2016; Jasim et al., 2016). In some of the countries in the Middle East such as Iran, more than 90% of the total water withdrawal is used for agricultural activities (Jasim et al., 2016; Lehane, 2014). As the communities are increasingly facing water scarcity, wastewater reuse is becoming an essential and reliable component of integrated and sustainable water resource management worldwide, especially in arid and semi-arid areas. In other words, the growing water shortage and its impact on water and food security emphasize the necessity for wastewater reuse as a valuable water resource for agricultural activities (Becerra-Castro et al., 2015; Elgallal et al., 2016). Irrigation with wastewater has many economic and environmental benefits such as reduction of utilization of natural water resources, reduction of chemical fertilizer usage, protection of aquatic ecosystems from contamination and improvement of crop yield due to nutrient delivery; however, it may also result in environmental and health problems. Irrigation with wastewater may alter the physicochemical properties of soil and promote soil salinization. Salinization increases the osmotic pressure in the root zone, constituting a limiting factor for the use of land for cultivation and plant growth and productivity (Elgallal et al., 2016). Salinity is evaluated via electrical conductivity (EC) and sodium adsorption ratio (SAR), which refers to infiltration problems and degree of saturation of the soil with sodium (Gharaibeh et al., 2016). Furthermore, wastewater may contain pathogens that pose a threat to human health and increase the risk for bacterial, parasitic and viral infections in consumers of wastewater-irrigated crops (Bahri et al., 2009; Elgallal et al., 2016; WHO, 2006).

Several studies have assessed the microbial health risks of wastewater reuse in agricultural activities. Most of these studies have estimated the risk of infection based on the microbial concentration of reused wastewater without investigation into the fate of pathogenic or indicator microorganisms in the field (Bahri et al., 2009; Moazeni et al., 2017; Mok et al., 2014; WHO, 2006). Although, the microbiological characteristics of wastewater for crop irrigation is important from a public health point of view; several factors potentially influence the microbial load of wastewater-irrigated soil and crops and associated health risks. Ambient temperature and humidity, rate of ultraviolet radiation, soil moisture and pH, antagonism with indigenous soil microorganisms, method of irrigation, and finally the type of plant could impact the fate and population of microorganisms in soil and on crop surfaces (Becerra-Castro et al., 2015; WHO, 2006). In the environment, in particular in arid and semi-arid areas, UV radiation from natural sunlight could be an important factor in the process of inactivation of microorganisms (Bichai et al., 2012). Therefore, for appropriate management of wastewater reuse and in order to assess the health risks associated with agricultural application of wastewater; it is essential to track the fate of indicator and pathogenic microorganisms through in-field experiments; especially in arid and semi-arid regions facing water scarcity. Some studies have already been published on the effect of agricultural reuse of treated municipal wastewater on the physicochemical and microbial characteristics of soil and microbial safety of the produce based on field experiments. These studies reported no significant microbial effect of wastewater irrigation on crops and indicated that irrigation with treated wastewater presents no microbial risk for consumers (Christou et al., 2014, 2016; Cirelli et al., 2012; Gatta et al., 2016; Li and Wen, 2016; Lonigro et al., 2016; Orlofsky et al., 2016; Shock et al., 2016; Urbano et al., 2017). However, in most of the studies, tertiary wastewater or wastewater with good microbial quality according to the world health organization (WHO) guideline ($\leq 10^3$ *E. coli* per 100 ml) (WHO, 2006) has been used through surface or subsurface drip system for crop irrigation, which may be different from real-field conditions in developing countries. Based on our knowledge, very few studies have applied furrow irrigation as well as various types of crops for impact assessment of wastewater reuse (Lonigro et al., 2016; Shock et al., 2016; Song et al., 2006). Since the microbial safety of produce is affected by the type and concentration of microorganisms in wastewater, climate condition, irrigation method and type of plant; it is crucial that the impact of wastewater reuse on soil and irrigated crops be properly evaluated through field experiments.

The present study was undertaken to investigate 1) the effect of irrigation with secondary treated municipal wastewater in comparison with tap water on the microbial quality of soil and crops based on in-field experiments, 2) the influence of environmental factors including ultraviolet index, ambient temperature, soil moisture and pH on *E. coli* die-off in soil, 3) the effect of application of wastewater on the physicochemical properties of soil, and 4) the microbial quality and safety of four types of crops including spring onion and onion as root crops, maize as a fast growing crop, and lettuce as a high risk-concern leafy vegetable (Mok et al., 2014) in a semi-arid area that is facing a water crisis.

2. Material and methods

2.1. Experimental field description

The experiment was conducted in an open site of a wastewater treatment plant which had not already been used for agricultural activity. The wastewater treatment plant was located in Isfahan (32° 37′ 6.024″ N, 51° 43′ 38.983″ W; elevation, 1564.8 m), in the central part of Iran. Isfahan with a semi-arid climate has an average annual temperature of 16.7 °C and an average annual rainfall of 130 mm/year. Climate parameters were recorded during the experimental period.

2.2. Experimental design

In order to assess the effect of various parameters in the reuse of wastewater for agriculture, the experiment was carried out over two consecutive cropping seasons from July to November 2016 (cycle 1) for cultivation of lettuce and maize as summer crops, and from December 2016 to June 2017 (cycle 2) for cultivation of spring onion and onion as winter crops, according to the common practice used in the study region.

The experimental design was based on the randomized use of two water qualities: secondary treated wastewater (STWW) and tap water (TW). Three types of plants (lettuce and maize in cycle 1 and onion in cycle 2) were used, each in three replications as presented in Fig. 1. Each plot comprised 7.5 m^2 (2.5 m × 3 m) and consisted of three rows with a distance of 0.4 m. Seeds of lettuce and maize were implanted in rows with a distance of about 0.15 m and 0.2 m, respectively. Seeds of onion were directly sown at the soil surface. STWW was the effluent of a municipal treatment plant after screening and grit removal, primary clarification followed by activated sludge process and finally chlorination. Tap water was provided through the municipal water supply system, with no microbial pollution, for irrigation of control plots. The soil was characterized as silty-loam (51% silt, 30% sand, 19% clay). A furrow irrigation system, as a common practice in the study region, with an irrigation volume of 0.5 m^3/plot per each irrigation event was applied. Irrigation of plants was performed once a weak except for the first two weeks of planting in cycle 1, in which watering was