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Impact of the reusing of food manufacturing wastewater for irrigation in a closed system on the microbiological quality of the food crops

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ABSTRACT

In order to evaluate if the reuse of food industry treated wastewater is compatible for irrigation of food crops, without increased health risk, in the present study a cropping system, in which ground water and treated wastewater were used for irrigation of tomato and broccoli, during consecutive crop seasons was monitored. Water, crop environment and final products were monitored for microbial indicators and pathogenic bacteria, by conventional and molecular methods. The microbial quality of the irrigation waters influenced sporadically the presence of microbial indicators in soil. No water sample was found positive for pathogenic bacteria, independently from the source. *Salmonella* spp. and *Listeria monocytogenes* were detected in soil samples, independently from the irrigation water source. No pathogen was found to contaminate tomato plants, while *Listeria monocytogenes* and *E. coli* O157:H7 were detected on broccoli plant, but when final produce were harvested, no pathogen was detected on edible part.

The level of microbial indicators and detection of pathogenic bacteria in field and plant was not dependent upon wastewater used. Our results, suggest that reuse of food industry wastewater for irrigation of agricultural crop can be applied without significant increase of potential health risk related to microbial quality.

1. Introduction

Wastewater reuse for irrigation in agriculture is considered a significant opportunity for arid and semi-arid countries, where shortage of freshwater resources is critical for agriculture and urban development. The need of increasing reuse of wastewater in agriculture is not only circumscribed by arid climate- and developing countries, but it is considered a primary objective also for countries where freshwater is still not considered a limiting resource, i.e. USA and EU (UKWIR, 2004; EU, 2000; Angelakis and Durham, 2008). Nevertheless, climate change and increased demand of urban and industrial water requirements is forcing the reuse of wastewater in agriculture (Dupont, 2013).

Notwithstanding the increasing reuse of wastewater worldwide, still many aspects about the potential environmental impact and the safety and risk assessment need to be investigated (Dickin et al., 2016). One of the major obstacles is the possible presence of pathogenic microorganisms in reused water that can survive in the field environment and contaminate crops, causing foodborne outbreaks. To protect consumers and avoid outbreaks of food borne diseases, efforts must focus on each point in the field-to-fork chain (Battilani et al., 2010). Since wastewaters of different sources (urban, greywater, industrial, etc.) may have different physico-chemical and biological characteristics, any evaluation of possible sustainable reuse must rely on the determination of their specific chemical and biological quality, their interaction with the field environment and with irrigated crops. Several researches focused on the evaluation of microbiological safety in the reuse of treated wastewater for crop irrigation: The microbiological quality of final production crops, which were irrigated with different methods and by using different types of wastewater was assessed demonstrating that the possible health risk due *E. coli and helminth eggs* was not directly correlated with the use of wastewater for irrigation (Forslund et al., 2010, 2012).

Similarly, Christou and colleagues reported that final crop productions, which were irrigated with wastewaters from two types of depuration technologies, were microbiologically as good as the crops irrigated with control well water, by assessing fecal indicator (*E. coli*) and pathogens like *Salmonella* spp. and *Listeria* spp. (Christou et al., 2014).

Orlofsky et al. (2016) conducted field experiments to monitor the different human pathogenic bacteria, protozoa, and viruses in parallel trials of tomatoes watered either with treated wastewaters or potable

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water using a combination of microscopic, cultivation-based, and molecular techniques. The results revealed that the microbial contamination on the surface of the tomatoes was not associated with the source of irrigation waters. However, in all the cited studies, urban wastewater was used for irrigation and in all except the last cited study, only cultivation-based methods were used to quantify microbial indicators and pathogens.

In this work, the impact of the irrigation of tomato and broccoli in succession planting with secondary treated food industry wastewater was assessed by evaluating the quality of the cropping whole environment, soil, plant and final yield. A secondary settler wastewater no subjected to tertiary treatment was intentionally used in order to increase the potential microbial load onto the field. The study was conducted in an agricultural-food industry that cultivates and processes the same products in a closed cycle system, in a water saving and recycling perspective.

Samples of water, soil, plant and produce (tomato and broccoli) were routinely analyzed for indicator microorganisms (*E. coli*, fecal enterococci) by standard methods and for three different pathogens of different potential sources (*Salmonella* spp., *Listeria monocytogenes*, *E. coli* O157:H7) by quantitative-real-time-PCR (qPCR). In parallel, conventional well water was used for irrigation and the same microbiological parameters were controlled, in order to compare the two types of irrigation strategies. To our knowledge, this is the first study in which industrial wastewater from food processing is considered for irrigation and both conventional and molecular methods are used to assess the presence of both microbial fecal indicators and pathogens.

2. Material and methods

2.1. Agronomic conditions and experimental design

The experimental site belongs to an agricultural and food manufacturing company, which produces and processes vegetables. The field was used in the previous years for the same crops used in the present study, namely tomato (*Lycopersicon esculentum* Mill.) cv. Manyla, and broccoli (*Brassica oleracea* L. var. *italica*, cv. Parthenon F_1), during consecutive growing seasons. Prior to the experimentation, crops were only irrigated with ground water. The soil characteristics were as follows: sand, 40.1%; loam 32.5%; clay 27.4%; organic matter 1.6%; Olsen P_2O_5, 80.1 mg kg^{-1}; Ac-extractable K_2O, 730 mg kg^{-1}; total N, 0.8‰ (Kjeldahl); mineral NO_3-N, 4.75 mg kg^{-1}; mineral NH_4-N, 7.50 mg kg^{-1}; pH 7.9; electrical conductivity, 0.49 dS m^{-1}. The tomato and broccoli plants were grown under a net house structure, which was covered with an anti-hail net and located close to the wastewater treatment plant of the company. The tomato seedlings were transplanted on April 12, 2012 in mulched paired rows (plant density 2.7 plants m^{-2}) and harvested from June to August. The broccoli seedlings were transplanted in the same field on October 12, 2012 in paired rows (plant density 3.2 plants m^{-2}) and harvested February 24, 2013. Crop water requirements were completely satisfied by drip irrigation system and standard agronomic practices for both crops were performed.

Two irrigation treatments were applied to the crops: irrigation with ground water (GW) from a water source, which is located in the experimental area, and irrigation with secondary treated industrial wastewater (SW). The SW was taken from the wastewater treatment plant that manages all of the sewage produced by the company, including toilet wastewater (about 40 workers using the facilities) and water from industrial processing of vegetables (i.e., tomatoes, broccoli, egg plants, peppers). For this study, part of the treated wastewater from secondary settler, prior the chlorine treatment, was directed into the experimental area through PVC pipe, and stored in a 10,000 l tank; subsequently, it was used for the tomato and broccoli irrigation. A second 10,000 l tank was used to store and distribute the ground water. The experiment was carried out in a randomized complete block design (Fig. 1) with the two irrigation treatments, each one replicated three times. The amount of irrigation water applied to the tomato crop during the whole crop cycle was 4957 m^3 ha^{-1}, with the water volume at each irrigation varying from 100 m^3 ha^{-1} to 300 m^3 ha^{-1}, depending on the growth stage of the crop and the natural precipitation event. In the winter crop season (broccoli) the applied irrigation water was of 922 m^3 ha^{-1} also in this case with a water volume for single treatment in the range of 100–300 m^3 ha^{-1}.

2.2. Sampling strategy

Water, soil, plant and tomato and broccoli samples were collected monthly during the whole cultural cycle (from plant seeding to final harvest of the product). Water samples (1 l) from secondary settler effluent (SW) and ground water from the well (GW) were transferred in sterile glass flasks, which were then transported to the laboratory in a refrigerated bag and stored at 4 °C before analyses (within 5 h from the sampling event). Soil samples (triplicate), which were irrigated either with SW or GW, were collected as follows: same amount of soil surrounding roots (obtained by scrubbing soil particles that were adhering to the plant root) and free soil, collected with a cork in the first 20 cm of depth about 10 cm far from the dripping point, were pooled together. Then the soil samples were put in a pre-sterilized 50 ml tube, stored and transported as reported above. Each soil sample unit consisted of about 25 g homogenized soil from three sites of a single plot and replicates consisted of one sample from each of the three field plots. Three plants were sampled from the same sites, harvested and transported in a sterile plastic bag in a refrigerated container. Before plate counting and DNA extraction from plant material, a portion of basal leaf, distal leaf, stem and eventual shoot or flower was cut, pooled and homogenized in a single plant sample unit (25 g). Triplicate tomatoes and broccoli florets samples were collected only at ripening and final harvest stage, respectively.

2.3. Microbiological and physico-chemical characterization of irrigation waters

All water samples were analyzed for the enumeration of the fecal indicators *Escherichia coli* and fecal enterococci. Membrane filtration method was used for bacterial enumeration in water samples following the Italian APAT-IRSA standard methods (APAT, IRSA-CNR, 2003) that refers to the APHA methods (APHA, 2005). Serial dilutions of each sample were made and filtered through 0.45-μm-pore-sized (47-mm-diameter) nitrocellulose membranes (Whatman, Maidstone UK). Membranes were then placed on TBX agar (Oxoid, London, UK) and incubated at 37 °C for 24 h and on Slanetz & Bartley Agar (Oxoid, UK) and incubated at 44 °C for 48 h for the isolation and enumeration of *E. coli* and enterococci, respectively.

Determination of total heterotrophic count (THC), fecal coliforms (FC) and *E. coli* was performed for soil, plants and produce samples, by spread plate method. Briefly, 25 g of each sample was diluted in 225 ml of buffered peptone water (BPW), placed in a blender and homogenized for 180 s. Then, serial dilution in BPW were made and spread onto agar plates containing TSA for THC, CEC agar (Biolife) for FC, and TBX for *E. coli*. Plates were then incubated for 24 h with different incubation temperature as follows: 22 °C for TMC, 44 °C for FC and 37 °C for *E. coli*.

The irrigation water samples were analyzed in triplicate according to the Italian standard methods (APAT, IRSA-CNR, 2003). The analysis included the physico-chemical parameters of pH, electrical conductivity (ECw; dS m^{-1}), ammonium-nitrogen (NH_4-N; mg l^{-1}), nitrate-nitrogen (NO_3-N; mg l^{-1}), phosphorus (PO_4-P; mg l^{-1}), biological oxygen demand over 5 days (BOD_5; mg l^{-1}), chemical oxygen demand (COD; mg l^{-1}), sodium (Na^+; mg l^{-1}), calcium (Ca^{2+}; mg l^{-1}), magnesium (Mg^{2+}; mg l^{-1}), potassium (K^+; mg l^{-1}), sulfate (SO_4^{2-}; mg l^{-1}).

The pH was measured using a GLP 22 + pH & Ion Meter (CRISON Instruments SA, Barcelona, Spain) and the electrical conductivity with a