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The physico-chemical and bacteriological effects of UV-treated wastewater irrigation on soil and turfgrass quality: A case study in a coastal golf course

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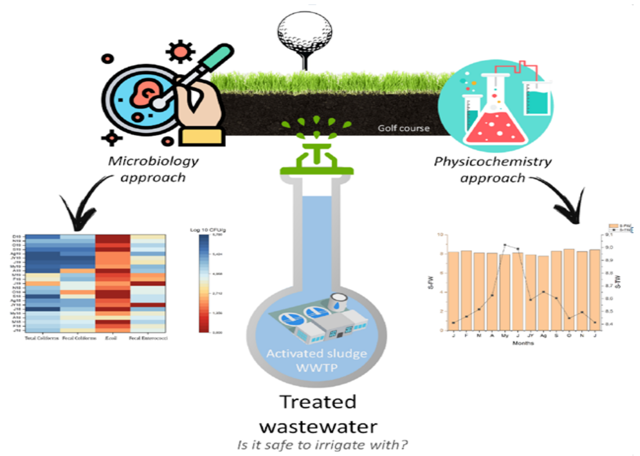
Graphical abstract



Abstract

This research describes the bacteriological and physicochemical impact of two types of irrigation water on soil and turfgrass quality during 2018 and 2019. Wastewater treated by an activated sludge process coupled with UV disinfection UV-TW was compared to fresh water FW. The first cycle (2018) was devoted to monitoring soil and turfgrass irrigated by FW, and the second cycle (2019) for UV-TW. Our results showed that the mean concentration of fecal indicators of treated wastewater UV-TW is about 2.17, 1.74, 1.77, and 1.52 log10 CFU/100ml for total coliforms, fecal coliforms, *E. coli* and fecal enterococci, respectively. The physicochemical characteristics showed no significant difference between soil irrigated with UV-TW and soil irrigated with FW except for pH and electrical conductivity. No significant difference was recorded comparing the fecal contamination of soil and turfgrass between the two irrigation cycles, except for fecal coliforms. Overall, the outputs of this work reported that the irrigation with UV-TW presents advantages not only on the quality of the soil and the vegetation, but also on the management of water scarcity. Thus, a highly controlled process of treatment and irrigation must be conducted to assure a safe hydric resource and to avoid any potential risk to human health.

Key words: Fecal contamination, irrigation, risk, safety, sustainable resource, wastewater

1. Introduction

Wastewater reuse has been considered a common procedure in many countries around the world and an Amount of research have recognized its efficiency (Mujeriego & Sala, 1991; Mcheik *et al.*, 2017; Bihadassen *et al.*, 2020; Ofori *et al.*, 2021). Wastewater recovery and reuse has been an attractive alternative source of water destined to irrigation (Candela *et al.*, 2007). Treated sewage is used exponentially for agriculture in areas suffering from water scarcity (Ofori *et al.*, 2021). This could be an economical way to reduce surface water pollution and allow groundwater recharge for other agricultural areas (Asano, 2006; Yuan *et al.*, 2016; Ventura *et al.*, 2019). The reuse of water for irrigation is widely applied in agriculture because of the benefits of nutrient recovery possibilities, socio-economic implications, reduction of fertilizer application, and effluent disposal (Candela *et al.*, 2007; Alsubih *et al.*, 2017; Ibekwe *et al.*, 2018). Even though the irrigation with treated wastewater (TWW) offers many advantages, its use can however affect the physicochemical properties of the soil and consequently crop production (Feigin *et al.*, 2012; Chen *et al.*, 2004). These effects depend on several parameters such as the quality and the quantity of irrigation water, soil type, duration of irrigation, and local climate (Tarchouna *et al.*, 2010).

However, the applications of TWW are several in different domains (industry, urban and recreational uses, aquaculture, and groundwater recharge). Indeed, the scarcity of conventional water resources constitutes a social, agricultural, and economic problem in most of the countries located in the southern Mediterranean basin (Laraus, 2004). Additionally, water shortage results from climatic conditions and population growth contributing to

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an increase in water needs (Vörösmarty *et al*., 2000). The search for an alternative source to be made available for agriculture and to replace the enormous need for water in this area has become a necessity (Angelakis *et al*., 1999). Many countries in the Mediterranean region such as Cyprus, Jordan, and Tunisia have proven that municipal wastewater reuse can be a realistic alternative for agriculture (Angelakis *et al*., 1999; Mahjoub *et al*., 2018; Elkiran *et al*., 2019; Abu Qdais *et al*., 2019). Meaningfully, Morocco is part of this region characterized by the scarcity of conventional water. The estimated volume of wastewater produced in Morocco is 640 million (m^3) in 2010 and the volume will increase to 1039 million (m^3) in 2030 (FAO, 2016).

Currently, the direct use of treated wastewater concerns the agricultural sector as well as watering golf courses and green spaces. Moreover, only 10% of wastewater was recycled in 2008 reaching 170 million (m^3) and 325 million (m^3) in 2020 and 2030, respectively (FAO, 2016). Generally, the water quality criteria applied for agricultural reuse have been mainly based on microbiological aspects, more specifically the presence of pathogenic potential (viruses, bacteria, and protozoa), which can cause health risks (WHO, 1989). Different legislative approaches are used in the world to determine the level of contamination of the wastewater that can be used in agriculture. The WHO guidelines (Blumenthal *et al*., 2000), using empirical epidemiological evidence, classified the reuse of TWW in three categories (unrestricted, restricted, and localized irrigations) according to the degree of fecal coliforms contamination.

In addition, wastewater is recognized to have direct effect on soil chemical properties. It affects macro and micronutrients, pH, dissolved particles, and salinity (Beltrán, 1999; Mohammad & Mazahreh, 2003; Bedbabis *et al*., 2014). The biological and chemical criteria should indicate all potential pathogens and chemical poisonings that may create a risk (Salgot *et al*., 2006; Ibekwe *et al*., 2018; Farhadkhani *et al*., 2018). Other studies have investigated the possible risk of pesticide leaching from golf courses (Candela *et al*., 2007). Otherwise, the long-term effect of wastewater reuse on the quality of soils and plants have been reported in many works (Cohen *et al*., 1999;. Candela *et al*., 2007; Dère *et al*., 2007; Rusan *et al*., 2007). Recently, some public health-related studies have focused on emerging contaminants (Christou *et al*., 2017; Diaz-Sosa *et al*., 2020).

Despite the socio-economic benefits of wastewater application in irrigation, it remains a practice that presents a significant risk to human and environmental health (Cirelli *et al*., 2012; Forslund *et al*., 2012; Szkup-Jablonska *et al*., 2012).

In this context, the objective of our study is to assess the irrigation impact driven by treated wastewater to two compartments (soil and turfgrass) in a golf course. The study is carried out in Anza-Taghazout zone located in the region of Agadir (southern Morocco). Agadir zone is considered a touristic and agricultural region characterized by an arid climate suffering from water scarcity and nutrient poor soils.

Along the study area, the wastewater treatment plant of Aourir provides the reused water treated by the activated sludge process, to Taghazout golf located in the touristic resort “*Taghazout bay*” (Abelouah *et al*., 2022). Overall, the current study aims to evaluate the short-term impact of irrigation on a Moroccan sandy soil irrigated with UV treated wastewater (UV-TW) during one year. The experiment led us to make a comparison between two different states of the same land irrigated differently, and to understand the microbial and physicochemical behavior of the soil and the turf towards the source of irrigation water. The main soil properties (pH, conductivity, exchangeable cations) as well as the macronutrients and micronutrients of the organic matter were analyzed. Total coliforms, fecal coliforms, *E. coli*, fecal enterococci were selected as indicators of fecal contamination, whereas *Salmonella* spp. was selected as pathogenic bacteria.

2. Materials and methods

2.1. Study site

The study area is located in the north of Agadir (Morocco) 30° 31′ 59″ N, 9° 42′ 00″ W (Figure 1). The Aourir treatment plant (WWTP) is near the ocean about 700 m south of the touristic resort Taghazout bay. This treatment plant uses an activated sludge system with UV disinfection as a tertiary treatment. The process is divided into three essential stages. The effluent treatment channels will include a water treatment system with pre-treatment, biological treatment and tertiary treatment, as well as sludge treatment and a stale air treatment line (deodorization). A conventional pretreatment constitutes a succession of screening, grit and oil removal. The biological treatment consists of two aeration basins and two clarification basins. The tertiary finalization treatment is a series of mechanical filtration (10 µm microfiltration) and UV disinfection operations. The total flow of wastewater to be treated at the Aourir wastewater treatment plant would be around 7,600 m^3/day at the saturation horizon of the project (2030), which corresponds to an hourly peak flow of 840 m^3/h. Tertiary treated wastewater UV-TW is pumped and stored before use in an open basin located at the golf course. The latter is located 5 km from the WWTP and is a part of the touristic resort, *Taghazout bay*. It was built in 2014 and was irrigated with drinking water FW until 2019 date from which the golf course began to be irrigated with UV-TW. In this study, the zero state of the golf course, chosen as a reference, corresponds to the period when it was irrigated by fresh water FW.

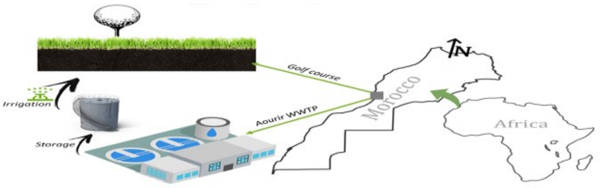

Figure 1. Geography location of Aourir's treatment plant and the studied golf course

2.2. Sampling strategy

Water irrigation (FW, UV-TW), turfgrass, and soil samples were collected from January 2018 to December 2019. Golf course was divided into three blocks, and a composite of turfgrass and soil samples was taken from each block. Soil samples were taken at depth of 20 cm. All samples were collected in sterile plastic bags for bacteriological analysis, while water samples in sterile glass bottles, and then stored at + 4 °C. The bacteriological analyses were carried out within 24 h from samples collection.

2.3. Physicochemical and bacteriological analysis of irrigation water

2.3.1. Physicochemical analysis of irrigation water

Water samples were collected monthly throughout 2019 to characterize irrigation water quality. Samples were transported in a cooler with ice to the laboratory, then stored and processed. The overall physicochemical parameters (Temperature, pH, dissolved Oxygen, and electrical conductivity) were measured *in situ* using a "Conductivimeter" and a "pH meter" by THERMO Scientific electrode, and BANTE electrode instrument for dissolved oxygen measurement. The five-day biological oxygen demand (BOD_5), the chemical oxygen demand (COD), the suspended solid (SS) were measured on monthly composite samples of treated wastewater. For suspended solids (SS), the measurement method adopted is differential weighing by filtration on GFC filter and drying at 105 °C (AFNOR, T90-105). The five-day biological oxygen demand (BOD_5) was determined by the manometric method with a respirometer (type WTW), according to the AFNOR standard (AFNOR, T90-103). The chemical oxygen demand (COD) was determined by potassium dichromate oxidability (AFNOR, T90-101).

The concentrations of macronutrients (Ca, Mg, Na, K) were determined by ion chromatography (AFNOR, T90-048), and micronutrients (Fe, B, Mn, Cu, Zn) were determined according to the standard method (AFNOR, 14870) by flame atomic absorption spectrometry. Anions and chlorides by Mohr's method (AFNOR, 9297), nitrates with the method based on sodium salicylate (AFNOR T90-013), phosphates by spectrometric method (AFNOR T90-023), and sulfates by titrimetric method (AFNOR, T90-009).

2.3.2. Bacteriological analysis of irrigation water

Fecal enterococci, total and fecal coliforms and, *E. coli* were selected as indicators of fecal contamination. The membrane filtration method was used to enumerate these bacteriological indicators in treated wastewater (UV-TW) and freshwater samples (FW). 10 ml and 100 ml of water samples were filtered through 0.45 µm membrane filters (Millipore), with incubation on TTC-Tergitol-Agar for 24 h at 44 °C for fecal coliforms (ISO 9308-1, 2000), and 24 h at 37 °C for total coliforms. Besides, the TBX agar during 24 h at 44 °C was used for *E. coli* (ISO 9308-1, 2000) and The BEA Agar at 37 °C during 48 h for fecal enterococci (ISO 7899-2, 2004). To detect a possible presence of *Salmonella* spp, 5 l of water were filtered through a 0.45 µm cellulose acetate filter, and then the filters were placed in 225 ml of buffered peptone water and incubated at 37 °C for 18 to 24 h. A 0.1 ml enrichment of this pre-enrichment was transferred to 10 ml of RV10 Rappaport–Vassiliadis broth and incubated at 44 °C for 18 to 24 h. The isolation was done on a selective medium, and it consists of seeding the Hektoen and XLD medium from the enrichment broth and then incubating at 37 °C for 24-48 h (ISO 6759-1, 2017). At the end, typical colonies (red colony with black center) were selected and streaked onto nutrient agar at 37 °C for 24 h and identified biochemically by the API 20E gallery. The results are expressed in presence/absence by filtered volume.

2.3.3. Physicochemical analysis of the soil

The physicochemical analyses were performed on three replicates of dry soil samples. The soil had to be dried and sieved (<2 mm), then pH and electrical conductivity of the soil were measured in a soil/water ratio of 1:5, according to ISO 10390. Additionally, the total limestone is determined by the volumetric method and active limestone using ammonium oxalate. The organic matter continent is estimated according to NM 13.1.004. Furthermore, the analysis is conducted also on Kjeldahl nitrogen (NM EN 16169), macronutrients Na, Ca, Mg, and K (NM ISO 11260), as well as micronutrients Zn, Cu, Mn, Fe, and B (NM ISO 14870).

2.3.4. Bacteriological analysis of the soil and turfgrass

For soil and turf analysis, 10 grams of each sample was added to 90 ml of tryptone salt broth, and homogenized using a stomacher, then 0.1 ml of serial dilutions were plated onto plates containing BEA fecal enterococcus Agar and TBX for *E. coli* according to the procedure (ISO 9308-1, 2000). The same samples were analyzed for total coliforms (TC) and fecal coliforms (FC), by the incorporation of VRBL, with incubation at 30 °C and 44 °C, respectively (NF V08-060, 2009). All bacteriological tests were repeated in triplicate. For the detection of *Salmonella* spp., 25 g of soil and turf were placed in 225 ml of pre-enrichment medium (buffered peptone water), after that, the same procedure described previously for water is carried out in the soil and turfgrass analysis.

2.4. Data analysis

The monthly variation of the bacteriological parameters was presented in a heatmap with a color scale. The assumption of normality of the datasets was validated (Shapiro-Wilk test, $p < 0.05$) before statistical analysis. Data were analyzed by analysis of variance (ANOVA) followed by Tukey's post hoc test. Additionally, the box plot graphs were carried out between values of UV-treated wastewater and freshwater values by considering months of irrigation as repetitions. In addition, pH and electrical conductivity were presented by Double Y axis column line symbol graph of two years. The level of significance was set to 0.05. The statistical tests and graphs were conducted in GraphPad Prism, Excel, and OriginLab.

3. Results and discussion

3.1. Physicochemical and bacteriological quality of the irrigation water

The physicochemical characteristics of the treated wastewater used for irrigation varied over the year of application (Table 1). The treated wastewater was, on average, alkaline with a basic pH value of 7.67 and had a low suspended solids (SS) level of 15.6 mg/l. The electrical conductivity recorded an average value of 1291.9 ± 8.834 µS/cm, chlorides revealed a low concentration in the treated water with an average value of 11.62 ± 0.03 mg/l, dissolved oxygen reached an average value of 9 ± 9.098 mg/l, also, the biochemical and chemical oxygen demand recorded 21.3 ± 3.364 and 85.3 ± 3.236, respectively. Besides, nitrates registered 2.04 ± 1.36 mg/l and phosphorus were 5.59 ± 0.06 mg/l.

Table.1 Physicochemical and bacteriological quality of Aourir treated wastewater

Parameters	UV-TW		Standard of irrigation
Macroelements (ppm)	Na	9.02 ± 0.05	9
	K	23 ± 14	-
	Ca	92 ± 18	-
	Mg	43 ± 12	-
Microelements (ppm)	Fe	0.04 ± 0.03	5
	B	1.02 ± 0.04	3
	Mn	0.05 ± 0.02	0.2
Heavy metals (ppm)	Cu	0.03 ± 0.01	2
	Zn	0.09 ± 0.02	2
Anions (mg/l)	Nitrates	2.04 ± 1.36	30
	Phosphates	5.59 ± 0.06	-
	Chlorure	11.62 ± 0.03	15
	Sulphates	98 ± 16	250
Physicochemical parameters	pH	7.67 ± 0.098	6.5–8.4
	EC (µS/cm)	1291.9 ± 8.834	1200
	DO (mg/l)	9 ± 9.098	-
	SS (mg/l)	15.2 ± 2.053	100
	BOD_5 (mg/l)	21.3 ± 3.364	50
	COD (mg/l)	85.3 ± 3.236	100
Microbiological parameters	Total coliforms (log10)	2.13 ± 0.052	-
	Fecal coliforms (log10)	1.67 ± 0.060	< 3
	Fecal enterococci (log10)	1.45 ± 0.061	< 3
	E. coli (log10)	1.77 ± 0.086	< 3

On the other hand, the concentrations of micronutrients and heavy metals in the treated wastewater were relatively low with 0.04 ± 0.03 mg/l of Fe, 1.02 ± 0.04 of B, 0.05 ± 0.02 of Mn, 9.02 ± 0.05 of Na, 23 ± 14 of K, 92 ± 18 of Ca, 43 ± 12 of Mg, 0.03 ± 0.01 of Cu and 0.09 ± 0.02 of Zn. None of the parameters exceeded the limit values for wastewater reuse in irrigation except for electrical conductivity, which was close to the standard, confirming the need for monitoring due to possible nutritional imbalance (Figure 2).

The bacteriological quality of the treated wastewater from the Aourir WWTP is assessed by the abundance of fecal contamination indicators at the outlet of the plant for the water intended for golf course irrigation. Figure 3 represents, respectively, the different variations of total coliforms, fecal coliforms, *E. coli,* and fecal enteroccoci throughout the year 2019 from January to December. The average values are reported in the Table 1. For total coliforms, the highest concentration is noted in March (2.56 log10 CFU/100ml), while the lowest concentration was detected in December (1.70 log10 CFU/100ml). For fecal coliforms, the highest concentration did not exceed 2.30 log10 CFU/100ml, and the lowest concentration was noted in April with a value not exceeding 0.99 log10 CFU/100ml. The concentration of *E. coli* ranged from 0.88 (April) to 2.34 log10 CFU/100ml (August). The concentration of fecal enterococci ranged from 0.48 log10 CFU/100ml in April (minimum) to 2.36 log10 CFU/100ml in August (maximum). The analysis of *Salmonella* showed negative results for all the samples of treated wastewater analyzed, except in April 2019, when the biochemical identification showed the presence of *Salmonella* spp. The absence of these germs would probably be related to the control of UV treatment also to the presence of antimicrobial substances (polyphenols, tannins and fatty acids) (El Addouli *et al.*, 2009).

However, a previous published study of treated wastewater of M'zar treatement plant in Agadir showed the presence of different *Salmonella* species even after the installation of tertiary UV treatment (El Boulani *et al.*, 2016). The concentration rates of the fecal contamination indicators never exceeded the limit values for direct and indirect discharge and were always in accordance with the Moroccan standards in force (< 3 log10 CFU/100ml). For drinking water samples, no contamination was detected. Several studies on wastewater irrigation have confirmed the presence of bacteriological contamination and organic matter in wastewater, thing that can have different effects on environment quality and human's health (Al-Shammiri *et al.*, 2005). A recent study by our team confirmed the efficiency of the treatment by Aourir plant. The study revealed strong variations in the quality of the treated water linked to several parameters (Hajji *et al.*, 2021). The climatic variation of the region as well as the inadequate management of the tertiary treatment plays an important role in the quality of the treated wastewater (Hajji *et al.*, 2021). The irrigation by this water source is directly linked to its quality after treatment.

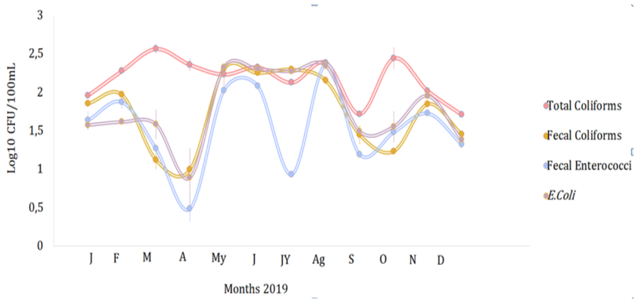

Figure 2. Monthly variation of fecal contamination in Treated wastewater of Aourir's treatment plant

4. Physicochemical characteristics of the soil

4.1. pH and electrical conductivity

The golf course soil is characterized by a sandy texture (Table 2). Figure 4 (a, b) show the values of the physicochemical parameters (pH and Electrical Conductivity) of soil irrigated by FW and UV-TW. The pH values of soil irrigated with FW varies between 7.76 and 8.50, and between 8.41 and 9.02 for soil irrigated with UV-TW. A significant difference <0.01 is recorded for the pH between the two irrigation periods (2018-2019) (Table 3). The increase in pH is most noticeable in the period when the soil is irrigated with UV-TW as compared to the period when the soil is irrigated with FW. This result is similar to those reported by Bihadassen *et al.*, (2020) who worked on two golf courses in Agadir, one irrigated with fresh water and the other irrigated with treated wastewater. They observed an increase in pH value following irrigation by treated wastewater. The increases in soil pH under irrigation with TW were previously reported by several researchers in other countries (Klay *et al.*, 2010; Kallel *et al.*, 2012; Shakir *et al.*, 2017; Vergine *et al.*, 2017). Tarchouna *et al.*, (2010) found that soil pH increased following several successive years of wastewater irrigation and attributed this increase to chemism and the high content of alkaline cations such as Na, Ca and Mg.

The highest electrical conductivity values are about 379.66 ± 8.02 µS/cm (April 2018) and 345.0 ± 1.02 µS/cm (May 2019) respectively for soil irrigated with FW, and soil irrigated with UV-TW. The low concentration is 194 ± 30.2 µS/cm (February 2018) for soil irrigated with FW and 220.33 ± 11.0 µS/cm (February 2019) for soil irrigated with UV-TW. These results showed that the irrigation of soil with treated wastewater led to an increase in its salinity level. A significant difference is recorded between the sampling months p<0.01, and a significant difference p<0.01 is recorded between the two irrigation periods (FW, UV-TW) (Table 3). Usually, this electrical conductivity growth comes from the salts present in TW as well as the resulting evaporation at the soil surface. Xu *et al.*, (2010) who worked on the impact of long-term irrigation of reclaimed wastewater on agricultural soils, found that higher conductivity values were found in the upper layer than in the lower layers. Several studies that have focused on long-term monitoring of treated wastewater irrigation on agricultural soils have concluded that changes in soil pH and conductivity are largely due to the displacement of cations or the addition of weak organic acids to soils (Rosabal *et al.*, 2007). Others have related this to excessive leaching of base cations (Gwenzi & Munondo, 2008). The suggested cause was the relatively high salinity of the TW (Farhadkhani *et al.*, 2018).

Table. 2 Main physical soil texture (mean values and standard deviation)

Granulometry (%) NM ISO/TS17892-4	
Clay X<2 µm	2.35% ± 0.0109
Silt 2 µm to 63 µm	10.94% ± 0.036
Sand 63 µm to 2 mm	86.71% ± 0.025
Textural Class	Sandy soil

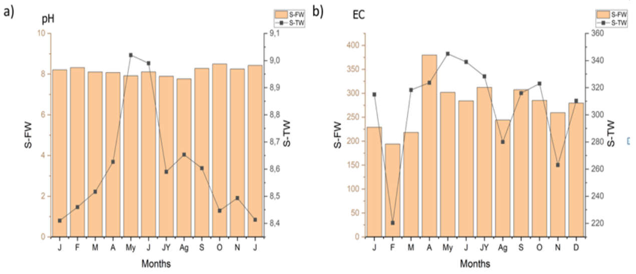

Figure 3. Monthly variations of pH (a) and Conductivity (b) in soil irrigated with freshwater (S-FW) 2018 and treated wastewater (S-TW) 2019

4.2. Soil organic matter (OM), macro- and micronutrients

The distribution of organic matter (OM) in examined soil was shown in Table 4. A small decrease in OM was identified in the UV-TW irrigation period (average of all samples 1.56% for S-FW and 1.39% for S-TW). However, no significant difference was recorded. Thisdecrease might be, probably, related to the intensification of microbial activity due to the labile C and N provided by treated wastewater. The comparison between FW and

UV-TW irrigation, recorded very small variations in the concentrations of N, P and K but no significant difference is recorded between the two irrigation periods (Table 4). The increase of N and P in the soil was small under UV-TW irrigation, which can be attributed to the fact that most of the N and P in the recycled water are in forms that can be easily used by the plants (Carrow *et al.*, 2008). The mean values of exchangeable Na^+, Mg^{2+}, Ca^{2+} and NH^{+4} given in Table 4, indicate a small increase and no significant difference is recorded which indicates that the UV-TW contribution to the medium was lower than the uptake by the plants. Soil micronutrients (Cu, Zn, Mn, Fe and B) in examined soil were shown in Table 5. A small increase in these parameters is recorded (Cu: 0.405 FW; 0.428 UV-TW), (Zn: 0.469 FW; 0.330 UV-TW), (Mn: 3.218 FW; 3.568 UV-TW), (Fe: 5.064 FW; 7.371 UV-TW), (B: 0.100 FW; 0.114 UV-TW). There were no significant differences between the two irrigation periods.

Table. 3 The mean values of soil pH and electric conductivity and their statistical description

Physicochemical parameters		FW	UV-TW	Significance
Soil electric conductivity µS/cm 25°C	Min	194 ± 30.26	220.3 ± 11.01	s*
	Max	379.6 ± 8.02	345 ± 1.00	
	Average	274.5 ±8.69	306.8 ± 5.10	
pH	Min	7.76 ± 0.09	8.41 ± 0.03	s*
	Max	8.5 ± 0.05	9.02 ± 0.04	
	Average	8.16 ± 0.05	8.6 ± 0.049	

Table. 4 The mean values of the soil OM and macronutrients parameters and their statistical description

Parameters		S-FW	S-TW	Significance
OM (%)	Min	1.21	1.08	ns
	Max	2.07	2.08	
	Average	**1.56**	**1.39**	
	SD	0.044	0.045	
Azote kjeldahl (%)	Min	0.063	0.103	ns
	Max	0.100	0.14	
	Average	**0.089**	**0.125**	
	SD	0.019	0.026	
$CaCO_3$ Total (%)	Min	19.623	22.173	ns
	Max	26.723	28.567	
	Average	**23.886**	**25.352**	
	SD	0.470	0.686	
$CaCO_3$ active (%)	Min	2.507	3.16	ns
	Max	3.957	3.803	
	Average	**3.058**	**3.454**	
	SD	0.108	0.121	
K (g/Kg)	Min	0.203	0.28	ns
	Max	0.518	0.397	
	Average	**0.326**	**0.332**	
	SD	0.020	0.021	
Mg (g/Kg)	Min	0.186	0.19	ns
	Max	0.32	0.347	
	Average	**0.245**	**0.275**	
	SD	0.031	0.019	
Ca (g/Kg)	Min	1.537	2.180	ns
	Max	2.622	2.763	
	Average	**2.099**	**2.345**	
	SD	0.022	0.044	
NH_4^+ (ppm)	Min	3.463	3.230	ns
	Max	3.633	3.670	
	Average	**3.523**	**3.551**	
	SD	0.020	0.021	
P (mg/Kg)	Min	59.204	43.680	ns
	Max	105.871	79.980	
	Average	**76.038**	**65.083**	
	SD	3.345	1.636	

*ns not significant, s significant to 0.05, ** significant to 0.01; *** *** significant to 0.001*

S-FW: soil irrigated with fresh water; S-TW: soil irrigated with treated wastewater

Table. 5 The mean values of the soil micronutrients parameters and their statistical description

Parameters		S-FW	S-TW	Significance
Cu (ppm)	Min	0.213	0.310	ns
	Max	0.536	0.727	
	Average	0.405	0.428	
	SD	0.015	0.020	
Zn (ppm)	Min	0.299	0.280	ns
	Max	0.782	0.403	
	Average	0.469	0.330	
	SD	0.029	0.023	
Mn (ppm)	Min	1.489	2.083	ns
	Max	4.302	4.333	
	Average	3.218	3.568	
	SD	0.033	0.029	
Fe (ppm)	Min	3.398	4.243	ns
	Max	6.619	10.643	
	Average	5.064	7.371	
	SD	0.021	0.036	
B (ppm)	Min	0.026	0.036	ns
	Max	0.212	0.213	
	Average	0.100	0.114	
	SD	0.004	0.007	

*ns: not significant, s significant, **p ≤ 0.01; ***p ≤ 0.001*

S-FW: soil irrigated with fresh water; S-TW: soil irrigated with treated wastewater

The accumulation of OM in effluent-irrigated soils could probably increase soil fertility. Soil organic matter can improve soil structural properties, acts as a nutrient reserve and counteracts the effects of salinity (Rattan *et al*., 2005; Ganjegunte *et al*., 2017). Previous studies of soils irrigated long-term with untreated and treated wastewater have reported an increase in soil C and N content (Friedel *et al*., 2000; Tarchouna *et al*., 2010; Bedbabis *et al*., 2014; Quemada *et al*., 2016). An increase in cation exchange capacity has been detected by several studies which they believe that can be attributed to the high content of organic compounds in the wastewater used (Angin *et al*., 2005). However, such increase was not observed when the wastewater was treated before its use (Qian, 2005). Other researchers reported accumulation of P in the soil with the application of wastewater and treated wastewater, which was attributed to the original content of these nutrients in the applied wastewater. The long duration of irrigation can also contribute to accumulation (Mohammad & Mazahreh., 2003). The upper part of the soil is most likely to receive an increase in micronutrient levels and then a decrease with depth (Ofori *et al*., 2021). In a long-term wastewater study, Ganjegunte *et al*., (2017) observed an improvement in the soil nutrient content after a six-year irrigation period.

There is no doubt that long-term application of treated wastewater will lead to a significant increase in OM and nutrients in soils. This can improve soil quality and good plant growth (Tarchouna *et al*., 2010; Adrover *et al*., 2012; Becerra-Castro *et al*., 2015; Farhadkhani *et al*., 2018).

In general, irrigation with wastewater treated by an activated sludge system and disinfected by UV has not recorded any negative effect on the soil or turf, as most of the nutrients supplied can be used directly by the plants or degraded in the soil.

5. Bacteriological quality of the soil and turfgrass

Bacterial contamination varied significantly in the two irrigation periods (FW and UV-TW irrigation). The concentrations of total coliforms, fecal coliforms, *E. coli* and enterococci in soil irrigated with FW and Soil irrigated with UV-TW are shown in Figure 4. Total coliforms in soil irrigated with FW, ranged from 2.11 log10 CFU/g (March 2018) to 5.25 log10 CFU/g (September 2018). Contamination by fecal coliforms in soil recorded values between 2.21 log10 CFU/g (March 2018) to 5.36 log10 CFU/g (June 2018). The concentration of *E. coli* varied between 0 log10 CFU/g (August 2018) and 1.55 log10 CFU/g (March 2018). Enterococci contamination ranged from 0 log10 CFU/g (October 2018) to 5.22 log10 CFU/g (April 2018).

The soil irrigated with UV-TW revealed the presence of total coliforms ranging from 2.19 log10 CFU/g (January 2019) to 5.41 log10 CFU/g (October 2019). The fecal coliform concentrations vary from 3.4 log10 CFU/g (March 2019) to 6.09 log10 CFU/g (August 2019). *E. coli* is present with concentrations ranging between 0 (January 2019) and 1.83 log10 CFU/g (August 2019). Enterococci indicated concentrations between 0 log10 CFU/g (July) and 3.82 log10 CFU/g (April).

The variation of fecal contamination in golf turfgrass is shown in Figure 5 for the two irrigation periods (FW, UV-TW). For the contamination by total coliforms of the turfgrass irrigated by FW recorded are maximum in June 2018 by 6.77 log10 CFU/g and minimum in March 2018 by 4.16 log10 CFU/g. Fecal coliforms recorded a concentration in the turfgrass varied between 2.19 log10

CFU/g in October 2018 to 5.17 log10 CFU/g in July 2018. *E. coli* contamination ranged from 0 recorded in March, July, October, and November 2018, to 3.27 log10 CFU/g recorded in April 2018. The maximum of enterococci in the turfgrass irrigated by FW is recorded in June 2018 by a value of 4.87 log10 CFU/g. The lowest concentration is recorded in July 2018 by 0 log10 CFU/g. For Turfgrass irrigated with UV-TW demonstrated concentrations of total coliforms between 2.34 log10 CFU/g (January 2019) to 6.53 log10 CFU/g (August 2019), 2.45 log10 CFU/g (April 2019) is the lowest concentration of fecal coliforms in turfgrass (UV-TW). 6.39 log10 CFU/g (July 2019) is the highest concentration. *E. coli* varied between 0 log10 CFU/g (April, September, November and December 2019) and 2.20 log10 CFU/g (May 2019). Fecal enterococci recorded on turfgrass with a concentration between 0 log10 CFU/g (January 2019) and 5.19 log10 CFU/g (August 2019). A complete absence of *Salmonella* spp in the soil and turfgrass of the golf course in the two years of irrigation by FW and UV-TW was noted.

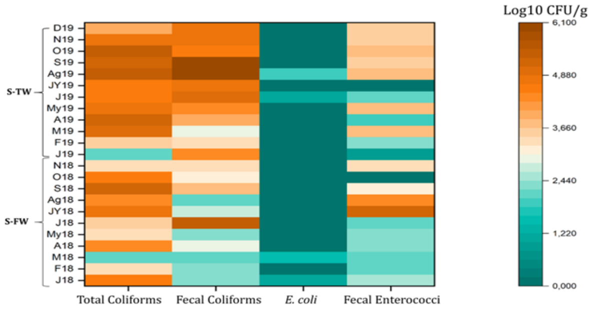

Figure 4. Heat map shows monthly variation of fecal contamination in soil irrigated with freshwater (S-FW) 2018 and treated wastewater (S-TW) 2019

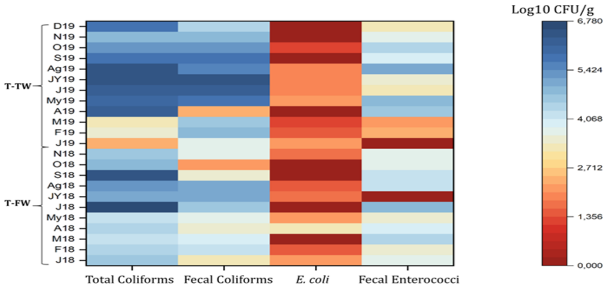

Figure 5. Heat map shows monthly variation of fecal contamination in turfgrass irrigated with freshwater (T-FW) 2018 and treated wastewater (T-TW) 2019

Statistical analysis between the two types of irrigation applied to soil and golf turfgrass indicated no significant difference between the concentrations of soil contamination and turfgrass by total coliforms, *E. coli* and fecal enterococci. Whereas fecal coliforms showed a high significant difference between FW and UV-TW ($p<0.001$) for soil (Figure 6), and a significant difference for turfgrass ($p<0.01$) (Figure 7).

As expected, the soil of the plots irrigated with the UV-TW were heavily contaminated by fecal coliforms, a similar result has been reported by Palese *et al*., (2009), using an irrigation water with coliform contamination. In addition, Vivaldi *et al*., (2013) found that the concentration of fecal coliforms contamination of soil irrigated by TW was statistically low compared to soil irrigated by secondary water, while freshwater FW remains very low than TW ($p<0.001$). Otherwise, Chevremont *et al*. (2012) found that watering with UV-LED WW does not increase the number of fecal coliforms and their diversity. On the other hand, their results agree with ours in the fact that the concentration of total coliforms, fecal enterococci, and *E. coli* does not mark any significant difference between watering with UV-LED WW and drinking water.

The reduction of fecal contamination in the soil depends on several parameters which are related to the method of irrigation and the type of soil. Cools *et al*., (2001) demonstrated that sandy soil allowed the best survival of fecal bacteria, while loamy sand and loamy soils with low organic content represented the worst conditions for exogenous bacterial survival. In our case, the method of irrigation by sprinkling on sandy soil can probably guarantee the necessary conditions for the survival of these bacteria and probably a large dispersion in space.

For an appropriate comparison, it must be noted that also with freshwater irrigation we observed a significant contamination of soil and turf. Benami *et al*., (2013); Forslund *et al*., (2012); Gatta *et al*., (2016) reported in their work that this may be due to occasional contamination by several factors, such as wild animals, birds and runoff. Especially in our case where the golf course is open to the surrounding natural area.

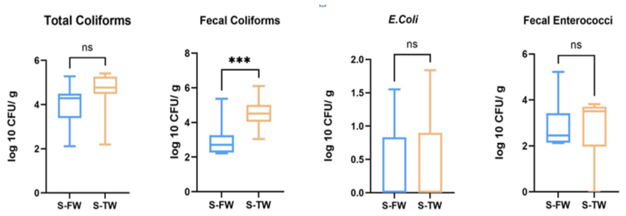

Figure 6. Boxplot graphs displaying the fecal contamination in soil, irrigated with freshwater (FW) and treated wastewater (TW).

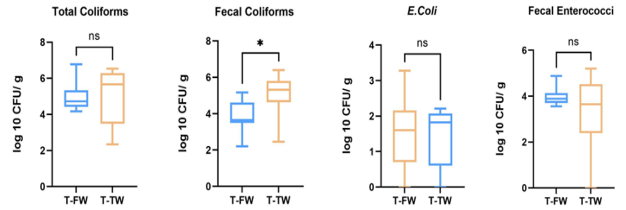

Figure 7. Boxplot graphs displaying the fecal contamination in turfgrass, irrigated with freshwater (FW) and treated wastewater (TW)

6. Safety of irrigating a golf course with treated wastewater

Golf courses occupy relatively large areas that are often havens of nature near or in the heart of urban areas, just like parks, gardens, and other green spaces. They are therefore potential host sites for original flora and fauna. Besides, they have an important role to play in ecological networks. For that, the measures taken in a golf project to preserve the environment are inseparable from the choices made in the organization of the space, the treatment of water resources, respect for biodiversity, and the control of pollution.

In the current study, the golf course is a space open to the surrounding natural environment and is integrated into the ecosystem of which it is home to various animal and plant species. This area is occupied by Argan forests (*Argania spinosa*), which currently extend only into the arid and semi-arid zones of southwest Morocco. Therefore, the preservation of this ecosystem is a national responsibility (Fahmi *et al*., 2013).

First, metal pollution is one of the main risks that can affect an area irrigated with wastewater that is not adequately treated. This is not the current case of the golf course studied because we have not experienced any significance increase. However, if heavy metals are present at high levels, they have toxic effects on humans (Bharti & Sharma, 2021). Explicitly, heavy metals enter the human body mainly through ingestion or inhalation through various ways such as living near or having direct contact with a site where these metals are improperly disposed. Otherwise, metals maintain growth and metabolism for several types of plants. Or, when metals are present in concentrations above the plants' needs, they cause toxic effects (Bharti & Sharma, 2021). Also, Gbaruko & Friday. (2007) reported the accumulation of metals among fauna and flora. Another study revealed the effect of irrigation by wastewater on soil macrofauna using the metal pollution as a criterion (Kanwal & Rana, 2020). Microplastics (MPs) are another group of emerging contaminants that can be found in treated wastewater. These contaminants pose a health risk to both humans and animals through the food chain. In addition, MPs together with their adsorbed contaminants could be leached out of the soil into groundwater, representing a potential exposure pathway to humans (He *et al*., 2018; Hurley & Nizzetto, 2018). Otherwise, we investigated microplastics occurrence in the UV-TW of Aourir wastewater treatment plant in a parallel study. Currently, the data is not published yet and reported high microplastic density in the samples destinated to the golf irrigation.

Second, the irrigation of the golf course with treated wastewater has revealed the presence of significant bacteriological and pollution in the soil and turf. This contamination poses a risk to golfers and golf employers. Direct contact with the soil, turf or instruments used, exposes humans to possible microbiological infection. Although this contamination causes a wide range of infections, such as diarrhea, dysentery, skin and tissue infections, etc., other forms of pathogens can cause disease, such as typhoid, dysentery, and other intestinal disorders (Carter, 2005; Khalid *et al*., 2018; Okoh *et al*., 2010).

Otherwise, due to the proximity of the golf course to the beach, we noticed the presence of migratory birds in our study field. This may indicate other indirect contamination problems related to transportation. Several steams of pathogenic micro-organisms to homoeothermic vertebrates, including humans, have been associated with free-living migratory birds. Various species of migratory birds may play an important role in the ecology and circulation of some arboviruses (Hubálek, 2004; Figuerola *et al*., 2009; Battisti *et al*., 2020).

7. Conclusion and recommendations

The current study gives us an overview about the effect of irrigation with treated wastewater from an activated sludge system and UV disinfection as tertiary treatment. The results reported several advantages of irrigation with treated wastewater not only for soil and turf but also for the management of water scarcity in the region. In this context, we concluded that several parameters, playing an important role, must be taken in consideration to assure the success of this practice. Considering the health risk associated with irrigation, the possibility of implementing control measures has become a necessity. Meaningfully, the treatment plant must determine a sampling frequency that allows adequate control of the treatment efficiency of the equipment as well as the bacteriological and physico-chemical contamination. In addition to climatological variations, this sampling frequency must take into account the possibility of variations in treatment efficiency (flows during heavy rainfall, turbidity, etc.). Otherwise, the UV disinfection system needs to be closely monitored, including technologies to detect faults or malfunctions. For this, the turbidity should not compromise the effectiveness of UV disinfection and thus ensure microbial quality below the recommended thresholds.

In order to limit the exposure of people using the golf course, sprinklers should not be used when the public and employees are likely to be in the irrigated areas. In addition, the equipment used to irrigate wastewater should be clearly identified to prevent accidental use of this water source. The wastewater distribution system should be monitored regularly to ensure that it is being used properly. In addition, the personnel involved in the use of treated wastewater for irrigation should be adequately trained and made aware of the risks associated with the use of treated wastewater. To protect public health, golf course users should be informed that treated wastewater is used for irrigation so that they do not expose themselves to it. Similarly, at any location where wastewater is used, it should be posted to indicate that it is non-potable water and to specify restrictions on its use. However, it is necessary to limit the exposure of the population living near irrigated land by minimizing the risk of direct or indirect contact with irrigation water. To be successful, it is recommended that windbreaks (e.g. trees) be used at the edge of residential properties and other frequented areas. Sprinklers should not be used during periods of high winds or when blowing towards aerosol-sensitive areas. By complying with these guidelines, we consider that the risk of microbial infection and physico-chemical pollution, although very low, remains possible.

Availability of data and materials

The datasets supporting the conclusions of this article are included within the article.

Competing interests

The authors declare no competing interests

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References

Abelouah M.R., Ben-Haddad M., Rangel-Buitrago N., Hajji S., El Alem N. and Alla A.A. (2022). Microplastics pollution along the central Atlantic coastline of Morocco. *Marine pollution bulletin*, **174**, 113190. https://doi.org/10.1016/j.marpolbul.2021.113190

Adrover M., Farrús E., Moyà G. and Vadell J. (2012). Chemical properties and biological activity in soils of Mallorca following twenty years of treated wastewater irrigation. *Journal of environmental management*, **95**, S188–S192. https://doi.org/10.1016/j.jenvman.2010.08.017

Al-Shammiri M., Al-Saffar A., Bohamad S. and Ahmed M. (2005). Wastewater quality and reuse in irrigation in Kuwait using microfiltration technology in treatment. *Desalination*, **185**(1–3), 213–225. https://doi.org/10.1016/j.desal.2005.02.078

Alsubih M., Arthur S., Wright G. and Allen D. (2017). Experimental study on the hydrological performance of a permeable pavement. *Urban Water Journal*, **14**(4), 427–434. https://doi.org/10.1080/1573062X.2016.1176221

Angelakis A., Do Monte M.M., Bontoux L. and Asano T. (1999). The status of wastewater reuse practice in the Mediterranean basin: Need for guidelines. *Water research*, **33**(10), 2201–2217. https://doi.org/10.1016/S0043–1354(98)00465–5

Angin I., Yaganoglu A. and Turan M. (2005). Effects of Long-Term Wastewater Irrigation on Soil Properties. *Journal of Sustainable Agriculture*, **26**, 31–42. https://doi.org/10.1300/J064v26n03_05

Asano T. (2006). Water reuse via groundwater recharge. *International Review for Environmental Strategies*, **6**(2), 205–216.

Battisti E., Urach K., Hodžić A., Fusani L., Hufnagl P., Felsberger G., Ferroglio E. and Duscher G.G. (2020). Zoonotic pathogens in ticks from migratory birds, Italy. *Emerging Infectious Diseases*, **26**(12), 2986. https://doi.org/ 10.3201/eid2612.181686

Becerra-Castro C., Lopes A.R., Vaz-Moreira I., Silva E.F., Manaia C.M. and Nunes O. C. (2015). Wastewater reuse in irrigation: A microbiological perspective on implications in soil fertility and human and environmental health. *Environment international*,**75**, 117-135.https://doi.org/10.1016/j.envint.2014.11.001

Bedbabis S., Rouina B.B., Boukhris M. and Ferrara G. (2014). Effect of irrigation with treated wastewater on soil chemical properties and infiltration rate. *Journal of environmental management*, **133**, 45–50.

Beltrán J.M. (1999). Irrigation with saline water: Benefits and environmental impact. *Agricultural water management*, **40**(2–3), 183–194. https://doi.org/10.1016/S0378–3774(98)00120–6

Benami M., Gross A., Herzberg M., Orlofsky E., Vonshak A. and Gillor O. (2013). Assessment of pathogenic bacteria in treated graywater and irrigated soils. *Science of the total environment*, **458**, 298–302. https://doi.org/10.1016/j.scitotenv.2013.04.023

Bharti R. and Sharma R. (2021). Effect of heavy metals: An overview. *Materials Today: Proceedings*. https://doi.org/10.1016/j.matpr.2021.06.278

Bihadassen B., Hassi M., Hamadi F., Aitalla A., Bourouache M., Boulani A.E. and Mimouni R. (2020). Irrigation of a golf course with UV-treated wastewater: Effects on soil and turfgrass bacteriological quality. *Applied Water Science*, **10**(1), 7. https://doi.org/10.1007/s13201–019–1095–5

Blumenthal U.J., Mara D.D., Peasey A., Ruiz-Palacios G. and Stott R. (2000). Guidelines for the microbiological quality of treated wastewater used in agriculture: Recommendations for revising WHO guidelines. *Bulletin of the World Health Organization*, **78**, 1104–1116.

Candela L., Fabregat S., Josa A., Suriol J., Vigués N. and Mas J. (2007). Assessment of soil and groundwater impacts by treated urban wastewater reuse. A case study: Application in a golf course (Girona, Spain). *Science of the total environment*, **374**(1), 26–35.

Carrow R., Duncan R.R. and Huck M.T. (2008). *Turfgrass and landscape irrigation water quality: Assessment and management*. CRC Press. https://doi.org/10.1201/9781420081947

Carter M. (2005). Enterically infecting viruses: Pathogenicity, transmission and significance for food and waterborne infection. *Journal of applied microbiology*, **98**(6), 1354–1380.

Chen Y., Wang C., Wang Z. and Huang S. (2004). Assessment of the contamination and genotoxicity of soil irrigated with wastewater. *Plant and Soil*, **261**(1), 189–196. https://doi.org/10.1023/B:PLSO.0000035565.65775.3c

Chevremont A.C., Boudenne J.L., Coulomb B. and Farnet A.M. (2013). Impact of watering with UV-LED-treated wastewater on microbial and physico-chemical parameters of soil. *Water research*, **47**(6), 1971–1982. https://doi.org/10.1016/j.watres.2013.01.006

Christou A., Agüera A., Bayona J.M., Cytryn E., Fotopoulos V., Lambropoulou D., Manaia C.M., Michael C., Revitt M. and Schröder P. (2017). The potential implications of reclaimed wastewater reuse for irrigation on the agricultural environment: The knowns and unknowns of the fate of antibiotics and antibiotic resistant bacteria and resistance genes–a review. *Water research*, **123**, 448–467. https://doi.org/10.1016/j.watres.2017.07.004

Cirelli G. L., Consoli S., Licciardello F., Aiello R., Giuffrida F. and Leonardi C. (2012). Treated municipal wastewater reuse in vegetable production. *Agricultural Water Management*, **104**, 163–170. https://doi.org/10.1016/j.agwat.2011.12.011.

Cohen S., Svrjcek A., Durborow T. and Barnes N.L. (1999). Water quality impacts by golf courses. *Journal of environmental quality*, **28**(3), 798–809. https://doi.org/10.2134/jeq1999.00472425002800030010x

Cools D., Merckx R., Vlassak K. and Verhaegen J. (2001). Survival of *E. coli* and Enterococcus spp. Derived from pig slurry in soils of different texture. *Applied Soil Ecology*, **17**(1), 53–62. https://doi.org/10.1016/S0929–1393(00)00133–5

Dère C., Lamy I., Jaulin A. and Cornu S. (2007). Long-term fate of exogenous metals in a sandy Luvisol subjected to intensive irrigation with raw wastewater. *Environmental Pollution*, **145**(1), 31–40. https://doi.org/10.1016/j.envpol.2006.04.002

Diaz-Sosa V.R., Tapia-Salazar M., Wanner J. and Cardenas-Chavez D.L. (2020). Monitoring and ecotoxicity assessment of emerging contaminants in wastewater discharge in the City of Prague (Czech Republic). *Water*, **12**(4), 1079. https://doi.org/10.3390/w12041079

El Addouli J., Chahlaoui A., Berrahou A., Chafi A., Ennabili A. and Karrouch L. (2009). Influence de rejets d'eaux usées sur les qualités physicochimique et bactériologique d'eaux utilisées en irrigation. *Rev Francoph Ecol Ind*, **4**, 23–28.

El Boulani A., Mimouni R., Chaouqy N., Hamadi F., Azelmad K. and \Hello L.S. (2016). Characterization and antibiotic susceptibility of Salmonella strains isolated from wastewater treated by infiltration percolation process. *Moroccan Journal of biology*, **13**, 2351–8456.

Fahmi F., Tahrouch S. and Hatimi A. (2013). Geoclimatic influences on flavonoids contents of the leaves of the argan tree Influences géoclimatiques sur la composition en flavonoides des feuilles de l'arganier Argania spinosa. *J. Mater. Environ. Sci*, **4**(6), 881–886.

FAO. (2016). *Food and Agriculture Organization of the United Nations (FAO). AQUASTAT*.

Farhadkhani M., Nikaeen M., Yadegarfar G., Hatamzadeh M., Pourmohammadbagher H., Sahbaei Z. and Rahmani H.R. (2018). Effects of irrigation with secondary treated wastewater on physicochemical and microbial properties of soil and produce safety in a semi-arid area. *Water research*, **144**, 356–364. https://doi.org/10.1016/j.watres.2018.07.047

Feigin A., Ravina I. and Shalhevet J. (2012). *Irrigation with treated sewage effluent: Management for environmental protection* (17). Springer Science & Business Media.

Figuerola J., Baouab R. E., Soriguer R., Fassi-Fihri O., Llorente F. and Jímenez-Clavero M.A. (2009). West Nile virus antibodies in wild birds, Morocco, 2008. *Emerging Infectious Diseases*, **15**(10), 1651. 10.3201/eid1510.090340

Forslund A., Ensink J., Markussen B., Battilani A., Psarras G., Gola S., Sandei L., Fletcher T. and Dalsgaard A. (2012). Escherichia coli contamination and health aspects of soil and tomatoes (Solanum lycopersicum L.) subsurface drip irrigated with on-site treated domestic wastewater. *Water research*, **46**(18), 5917–5934. ttps://doi.org/10.1016/j.watres.2012.08.011

Friedel J.K., Langer T., Siebe C. and Stahr K. (2000). Effects of long-term wastewater irrigation on soil organic matter, soil microbial biomass and its activities in central Mexico. *Biology and Fertility of Soils*, **31**(5), 414–421. https://doi.org/10.1007/s003749900188

Ganjegunte G., Ulery A., Niu G. and Wu Y. (2017). Effects of treated municipal wastewater irrigation on soil properties, switchgrass biomass production and quality under arid climate. *Industrial crops and products*, **99**, 60–69. https://doi.org/10.1016/j.indcrop.2017.01.038

Gatta G., Libutti A., Beneduce L., Gagliardi A., Disciglio G., Lonigro A. and Tarantino E. (2016). Reuse of treated municipal wastewater for globe artichoke irrigation: Assessment of effects on morpho-quantitative parameters and microbial safety of yield. *Scientia horticulturae*, **213**, 55–65. https://doi.org/10.1016/j.scienta.2016.10.011

Gbaruko B. and Friday O. (2007). Bioaccumulation of heavy metals in some fauna and flora. *International Journal of Environmental Science & Technology*, **4**(2), 197–202.

Gwenzi W. and Munondo R. (2008). Long-term impacts of pasture irrigation with treated sewage effluent on nutrient status of a sandy soil in Zimbabwe. *Nutrient Cycling in Agroecosystems*, **82**(2), 197–207.

Hajji S., Alla A.A., Noureddine S., Haddad M.B. and Moukrim A. (2021). Study of physicochemical and bacteriological quality of treated wastewater by the new Aourir plant (Southwestern of Morocco) using activated sludge technology in a semi-arid region. *Journal of Ecological Engineering*, **22**(6). https://doi.org/10.12911/22998993/137360

He D., Luo Y., Lu S., Liu M., Song Y. and Lei L. (2018). Microplastics in soils: Analytical methods, pollution characteristics and ecological risks. *TrAC Trends in Analytical Chemistry*, **109**, 163–172. https://doi.org/10.1016/j.trac.2018.10.006

Hubálek Z. (2004). An annotated checklist of pathogenic microorganisms associated with migratory birds. *Journal of wildlife diseases*, **40**(4), 639–659. https://doi.org/10.7589/0090–3558-40.4.639

Hurley R.R. and Nizzetto L. (2018). Fate and occurrence of micro (nano) plastics in soils: Knowledge gaps and possible risks. *Current Opinion in Environmental Science & Health*, **1**, 6–11. https://doi.org/10.1016/j.coesh.2017.10.006

Ibekwe A., Rubio G.A. and Suarez D. (2018). Impact of treated wastewater for irrigation on soil microbial communities. *Science of the Total Environment*, **622**, 1603–1610. https://doi.org/10.1016/j.scitotenv.2017.10.039

ISO 7899–2. (2004). *Water Quality-Detection and enumeration of intestinal Enterococci-Part 2: Membrane filtration method*.

ISO 9308–1. (2000). *Water quality—Detection and enumeration of Escherichia coli and coliform bacteria—Part 1: Membrane filtration method.*

Kallel M., Belaid N., Ayoub T., Ayadi A. and Ksibi M. (2012). Effects of treated wastewater irrigation on soil salinity and sodicity at El Hajeb region (Sfax-Tunisia). *Journal of Arid Land Studies*, **22**(1), 65–68. https://doi: 10.21162/pakjas/20.9485

Kanwal S. and Rana N. (2020). Use of sewage wastewater in agriculture and its effects on soil macrofauna. *Pakistan Journal of Agricultural Sciences*, **57**(3). https://doi: 10.21162/pakjas/20.9485

Khalid S., Shahid M., Bibi I., Sarwar T., Shah A.H. and Niazi N.K. (2018). A review of environmental contamination and health risk assessment of wastewater use for crop irrigation with a focus on low and high-income countries. *International journal of environmental research and public health*, **15**(5), 895. https://doi.org/10.3390/ijerph15050895

Klay S., Charef A., Ayed L., Houman B. and Rezgui F. (2010). Effect of irrigation with treated wastewater on geochemical properties (saltiness, C, N and heavy metals) of isohumic soils (Zaouit Sousse perimeter, Oriental Tunisia). *Desalination*, **253**(1–3), 180-187. https://doi.org/10.1016/j.desal.2009.10.019

Laraus J. (2004). The problems of sustainable water use in the Mediterranean and research requirements for agriculture.

Annals of Applied Biology, **144**(3), 259–272. https://doi.org/10.1111/j.1744–7348.2004.tb00342.x

Mohammad M.J. and Mazahreh N. (2003). Changes in soil fertility parameters in response to irrigation of forage crops with secondary treated wastewater. *Communications in soil science and plant analysis*, **34**(9–10), 1281–1294. https://doi.org/10.1081/CSS–120020444

Mujeriego R. and Sala L. (1991). Golf course irrigation with reclaimed wastewater. *Water Science and Technology*, **24**(9), 161–171.

Ofori S., Puškáčová A., Růžičková I. and Wanner J. (2021). Treated wastewater reuse for irrigation: Pros and cons. *Science of The Total Environment*, **760**, 144026.

Okoh A.I., Sibanda T. and Gusha S.S. (2010). Inadequately treated wastewater as a source of human enteric viruses in the environment. *International journal of environmental research and public health*, **7**(6), 2620–2637. https://doi.org/10.3390/ijerph7062620

Palese A.M., Pasquale V., Celano G., Figliuolo G., Masi S. and Xiloyannis C. (2009). Irrigation of olive groves in Southern Italy with treated municipal wastewater: Effects on microbiological quality of soil and fruits. *Agriculture, ecosystems & environment*, **129**(1–3), 43–51. https://doi.org/10.1016/j.agee.2008.07.003

Qian Y. (2005). Long-Term Effects of Recycled Wastewater Irrigation on Soil Chemical Properties on Golf Course Fairways. *Agronomy Journal–AGRON J*, **97**. https://doi.org/10.2134/agronj2004.0140

Quemada M., Delgado A., Mateos L. and Villalobos F.J. (2016). Nitrogen fertilization I: The nitrogen balance. In *Principles of agronomy for sustainable agriculture* (341–368). Springer.

Rattan R., Datta S., Chhonkar P., Suribabu K. and Singh A. (2005). Long-term impact of irrigation with sewage effluents on heavy metal content in soils, crops and groundwater–A case study. *Agriculture, ecosystems & environment*, **109**(3–4), 310–322. https://doi.org/10.1016/j.agee.2005.02.025

Rosabal A., González M.E., López U.T., Porras M.C., Justo Á. and Herencia J.F. (2007). *Long-term impacts of wastewater irrigation on Cuban soils*.

Rusan M.J.M., Hinnawi S. and Rousan L. (2007). Long term effect of wastewater irrigation of forage crops on soil and plant quality parameters. *Desalination*, **215**(1–3), 143-152. https://doi.org/10.1016/j.desal.2006.10.032

Salgot M., Huertas E., Weber S., Dott W. and Hollender J. (2006). Wastewater reuse and risk: Definition of key objectives. *Desalination*, **187**(1–3), 29-40. https://doi.org/10.1016/j.desal.2005.04.065

Shakir E., Zahraw Z. and Al-Obaidy A.H.M. (2017). Environmental and health risks associated with reuse of wastewater for irrigation. *Egyptian Journal of Petroleum*, **26**(1), 95–102.

Szkup-Jablonska M., Karakiewicz B., Grochans E., Jurczak A., Nowak-Staz G., Rotter I. and Prokopowicz A. (2012). Effects of blood lead and cadmium levels on the functioning of children with behaviour disorders in the family environment. *Annals of Agricultural and Environmental Medicine*, **19**(2).

Tarchouna L.G., Merdy P., Raynaud M., Pfeifer H.R. and Lucas Y. (2010). Effects of long-term irrigation with treated wastewater. Part I: Evolution of soil physico-chemical properties. *Applied Geochemistry*, **25**(11), 1703–1710. https://doi.org/10.1016/j.apgeochem.2010.08.018

Ventura D., Consoli S., Barbagallo S., Marzo A., Vanella D., Licciardello F. and Cirelli G. L. (2019). How to overcome barriers for wastewater agricultural reuse in Sicily (Italy)? *Water*, **11**(2), 335. https://doi.org/10.3390/w11020335

Vergine P., Lonigro A., Salerno C., Rubino P., Berardi G. and Pollice A. (2017). Nutrient recovery and crop yield enhancement in irrigation with reclaimed wastewater: A case study. *Urban Water Journal*, **14**(3), 325–330. https://doi.org/10.1080/1573062X.2016.1141224

Vivaldi G.A., Camposeo S., Rubino P. and Lonigro A. (2013). Microbial impact of different types of municipal wastewaters used to irrigate nectarines in Southern Italy. *Agriculture, ecosystems & environment*, **181**, 50–57. https://doi.org/10.1016/j.agee.2013.09.006

Vörösmarty C.J., Green P., Salisbury J. and Lammers R.B. (2000). Global water resources: Vulnerability from climate change and population growth. *science*, **289**(5477), 284–288.

WHO. (1989). *WHO (1989). Wastewater use in agriculture and aquaculture: A health recommendation. World Health Organization, Geneva*.

Xu J., Wu L., Chang A.C. and Zhang Y. (2010). Impact of long-term reclaimed wastewater irrigation on agricultural soils: A preliminary assessment. *Journal of hazardous materials*, **183**(1–3), 780–786. 780–786. https://doi.org/10.1016/j.jhazmat.2010.07.094

Yuan J., Van Dyke M.I. and Huck P.M. (2016). Water reuse through managed aquifer recharge (MAR) : Assessment of regulations/guidelines and case studies. *Water Quality Research Journal of Canada*, **51**(4), 357–376. https://doi:10.2166/wqrjc.2016.022.