Chemosphere
May 2022, Volume 295 Pages 133945 (12p.)
https://doi.org/10.1016/j.chemosphere.2022.133945
https://archimer.ifremer.fr/doc/00756/86823/

Use of groundwater and reclaimed water for agricultural irrigation: Farmers' practices and attitudes and related environmental and health risks

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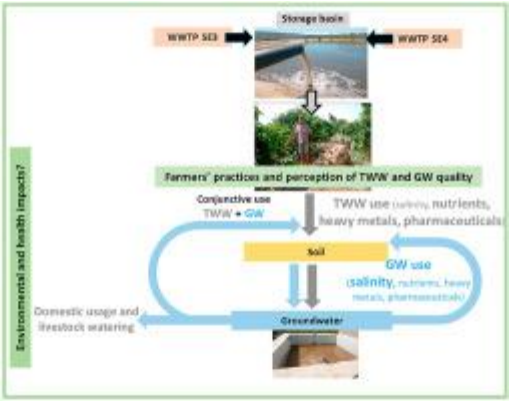
Abstract :

Agricultural reuse of treated wastewater (TWW) for irrigation is widely practiced. Its conjunctive use with freshwater is becoming more common to guarantee food security, while the rationale behind and its sustain ability are quite arguable. The objective of this study is to better understand the drivers of the conjunctive use of TWW and groundwater (GW) in Nabeul region, Tunisia, and the potential environmental and health impacts taking into account farmers' practices and attitudes toward reuse. TWW used for irrigation exhibited relatively high salinity and high microbiological load. GW has a very high salinity. TWW and GW showed low concentrations of heavy metals (Al, Cd, Co, Cu, Cr, Mn, Ni, Pb, and Zn). Concentrations of pharmaceutical compounds were between Limits of Quantification and 13 mu g/L. In GW, values were relatively high, especially for caffeine, carbamazepine, ofloxacin, and ketoprofen. Farmers have a low perception of the polluting load of TWW and GW and of their potential long-term impacts on agricultural environment, human health, and agricultural productivity. GW availability has facilitated its conjunctive use with TWW, either to augment water quantity and/or to improve its quality. Despite its low quality, GW timeliness for irrigation was the main driver to guarantee a better yield and quality of produces. Soil microbial community, bacterial biomass, denitrifying potential and carbon oxidation profiles were similar under TWW, GW and their conjunctive use. Though an effect of the sampling period was observed with a high abundance of denitrifying bacteria in the wet season and a low carbon oxidation activity at the end of the dry season. The conjunctive use of TWW and GW is very likely unsustainable from health and environmental perspectives. Balancing farmers' economic profit against the preservation of agricultural activity, linked to cultural and natural heritage, remains one of the challenges for decision-makers and regional stakeholders.

Graphical abstract



Highlights

► Conjunctive use of reclaimed water (TWW) and groundwater (GW) is an unplanned practice. ► Conjunctive use can improve irrigation quality or/and increase its availability. ► Farmers have low awareness of the "invisible" pollutants and their related risks. ► The presence of pharmaceuticals compounds in GW is due to agricultural practices. ► Soil microbial functioning is not affected by individual or conjunctive use of TWW or GW.

Keywords : Conjunctive use, Farmers' attitude, Soil microbial activity, Emerging contaminants, Sustainability

1. Introduction

Food security and economic welfare are inextricably related to agricultural production and water security as the global food production has to increase by 70% by 2050 to meet the population needs (FAO, 2009). Achieving food security implies accomplishing jointly the Sustainable Development Goals for the year 2030 (SDG) SDG2 (food security) and SDG6 (water and sanitation) (WWAP, 2015). However, the availability of freshwater resources is jeopardized by anthropogenic activities and climatic conditions. Human activities are putting high pressure on renewable resources through the release of pollutants in wastewater. In addition to salinity and heavy metals, contaminants of emerging concern, like pharmaceutical active compounds, are increasingly detected in the agricultural environment and their occurrence in water resources is still not well addressed under irrigated agriculture in developing countries (Madikizela et al., 2020;; Scheytt et al., 2006; Siler, 1999; Tahrani et al., 2016). Climate change is exacerbating water shortage and rain fed agriculture is suffering most because of the increasing drought frequency and duration. Irrigation is a way to make up rainfall deficit. However, 20% of the world's aquifers are being over-exploited leading to sea water intrusion (WWAP, 2015). In 2014, irrigated agriculture represented about 20% of the total cultivated land, contributing by 40% of the food production worldwide; these figures may vary over developing countries. Timeliness of the irrigation water, in time and place, is required to meet crops requirements and to guarantee acceptable yield, especially that most of the world irrigated agriculture is laying in developing and scarce water countries (Alexandratos and Bruinsma, 2012). Coping with water scarcity is constraining farmers either to reduce the irrigation demand, by reducing either the cultivated area or the distributed water amount (Zairi et al., 2003), or to rely on alternative water resources of lower quality, like reclaimed water or brackish water or a combination of both (Pereira et al., 2002). Augmentation of irrigation water supply by the reuse of urban treated wastewater (TWW) is recognized worldwide as a sustainable option to combat drought, and a way to shifting the demand away from the aquifer (Roumasset and Wada, 2010) provided it complies with minimum requirements for a safe reuse (WHO, 2006). High precaution is required in managing the chemical and biological polluting load to prevent harmful accumulation in soil and plants and/or infiltration to groundwater. Nutrients (nitrogen and phosphorus) present in TWW may indeed represent a threat to the environment when irrigation water is applied copiously (gravity/surface irrigation) and chemical fertilizers are applied to soil (El Ayni et al., 2011). Meanwhile, organic matter (suspended and dissolved moieties) present in TWW is reported to potentially improve soil fertility. Salts and other types of organic and inorganic chemical pollutants may also be brought to soil and, impact soil microbial biomass, diversity and activities such as those linked to the N- P- and C-cycles. For instance, the relative abundance of bacteria involved in the nitrification or denitrification pathway often increase – mainly in the top soil- after irrigation with TWW and their concentration can be significantly lowered by improving wastewater treatment strategy (Becerra-Castro et al., 2015; Ibekwe et al., 2018; Michel et al., 2014). In addition, irrigation using TWW can bring a microbial biomass including pathogens. The abundance of pathogens was shown to increase mainly in top soil (Malkawi and Muhammad, 2003) To tackle water shortage, the conjunctive use of water resources is an alternative to augment the water availability for irrigation. The most commonly addressed practice is the conjunctive use of surface water and groundwater (GW) (Templer, 2001). In Tunisia, conjunctive use of surface and groundwater is covering 15-20% of all private irrigated area (Hamdane, 2014). The conjunctive use of TWW and other water resources is not well studied (Al Khamisi et al., 2013; Ejaz and Peralta, 1995). The implementation of this "strategy" is strongly influenced by farmers' social, economic, cultural conditions and attitudes while environmental and health aspects are largely overlooked. This research work was motivated by the prevailing agricultural practices, up-to-date, in response to critical water scarcity. The objectives are three folds: 1) to better understand the motivations behind the use of TWW and GW for irrigation either conjunctively or separately, 2) to assess the polluting load of TWW and GW, especially in terms of pharmaceutical compounds, and 3) to evaluate the potential impacts on soil and GW quality while taking into consideration farmers' attitudes toward reuse.

2. Material and Methods

2.1 General context and historical background of reuse

The area of Oued Souhil, irrigated with TWW since the late 1980's, is located in the Nabeul Governorate (district), in the peninsula of Cap Bon Region, northeast of Tunisia (Figure 1, Supplementary Table 1). The economic activity is based on agriculture, industry and tourism. The region is well renowned for growing a large variety of citrus trees in addition to the production of orange and bitter orange flowers' extracts representing one of the region's heritages preciously preserved by the communities. Famous international brands of perfumers rely on neroli produced in Nabeul region which contributes by around 5 million Tunisian Dinar (around 1,700,000 US Dollar) to its economic benefit. Therefore, the preservation of the agricultural land against urbanization and the increasingly fragmentation is a paramount. The region is also the first touristic area of Tunisia with about 150 hotels having an approximate capacity of about 990,000 tourists per year (Nabeul Governorate, 2016). In order to preserve the agricultural activity especially citrus cultivation, the effluents of the wastewater treatment plant

(WWTP) SE4 were reused to replenish the aquifer and to protect it from sea water intrusion (Mahjoub et al., 2009). Currently, the area of Oued Souhil is under serious water shortage despite the availability of TWW which is in large proportion (80%) discharged into the sea because of the low storage capacity and the lack of transfer infrastructure to the irrigated areas.

Figure 1. Location of Tunisia and Nabeul city (A) and irrigated agricultural plots (1: GW+TWW, 2: GW, 3: TWW), treatment plants (WWTP-SE3 and WWTP-SE4), storage basin and wells (Well 1 and Well 2) (B)

2.1.1. Hydrogeological context and groundwater resources exploitation

Farmers used to rely on the aquifer Hammamet-Nabeul that stretches over 195 km^2 with 4 to 31 m depth. The vadose zone varies between 10 and 13 m (Kallali et al., 2007). The aquifer is composed of two geological units: the first is unconsolidated sand to gravel layers at 13.8 m depth, representing the porous media with a permeability of 10^{-4}-10^{-6} m/sec and the second is consolidated silt to clay beds layer below 13.8 m, as hydrogeological basement where the conductivity is less than 10^{-9} m/sec (Kallali and Yoshida, 2002). Hammamet-Nabeul aquifer has experienced an excessive withdrawal to grow garden crops and fruits trees. In the 1950's, the area was classified as "protected area" and GW abstraction from surface wells has been prohibited. However, exploitation of the shallow aquifer has been growing since the years 70's (Rekaya, 1986; UNDP/OPE 1987) and still continues.

2.1.2 Climatic conditions and agricultural activity

Nabeul region lays under semi-arid climate. Rainfall varies between less than 100 and 200 mm with a mean of about 445 mm/year. Yearly mean temperature is around 19°C, and rises up to 30°C in summer resulting in a high evaporation and severe water shortage (Mahjoub et al., 2009). The area of Oued Souhil used to cover about 430 ha. Currently, around 280 ha are irrigated, including fruit trees, olive trees, fodders, and tobacco (GDA Oued Souhil, 2014; UNDP/OPE, 1987).

2.1.3 Wastewater treatment plants

Wastewater effluents treated in the WWTP-SE4 (treatment capacity of 9585 m^3/day) are collected from the cities of Nabeul, Béni Khiar, and Dar Châabane El Fehri. Effluents undergoing activated sludge treatment are mainly of domestic origin; industrial effluents are released, either pretreated or untreated. TWW used for irrigation in Oued Souhil is also supplied by WWTP-SE3 (3500 m^3/day) established in 1990's. Effluents deriving mainly from hotels and industrial units are treated using oxidation ditches process. Variable portions of both effluents are pumped into a 4500 m^3 capacity storage basin (SB) located in the upstream to supply the farmlands by gravity (Supplementary Figure 1).

2.2 Water sampling and determination of quality parameters

2.2.1. Treated wastewater

TWW was collected, almost monthly, as a grab sample, from June 2013 to May 2016 at the outlet of WWTP-SE4 (23 samples) and WWTP-SE3 (23 samples), and less frequently from SB (12 samples) as this depends on water availability in the basin. TWW was collected in polyethylene containers, transferred to the laboratory in cooling boxes, and preserved at 4°C until analysis that took place immediately or within 24h. The following parameters were determined: pH, electrical conductivity (EC) (dS/m), total suspended solids (TSS) (mg/L), biological oxygen demand after 5 days (BOD_5) (mgO_2/L), chemical oxygen demand (COD) (mgO_2/L), nitrogen as NO_2^--N, dissolved phosphorous (PO_4^{3-}-P), K^+, Na^+, HCO_3^-, SO_4^{2}, and Cl^-. Analyses were performed according to standards methods (Supplementary Table 2). Heavy metals (Al, Cd, Co, Cu, Zn, Mn, Total Cr, Ni, and Hg) were analyzed in water samples (4 to 8 per sampling point) collected during the irrigation period from March to October 2015. Samples were digested with aqua regia according to NF ISO 15587-1 (2009) and analyzed with ICP according to the standard method NF ISO 1185 (2007). For Hg analysis, samples' digestion took place in a closed system according to the same standard method cited above and analysis was performed with ICP with hydride generation-atomic absorption spectrometry. Microbiological parameters including Total Coliforms (TC) and *E. coli* were determined on grab samples collected into sterile containers and treated the same day according to the most probable number (MPN) method based on the standard method ISO 9308-2 (1990). Helminth eggs were determined in a 5 liters grab sample by the modified Bailenger method according to WHO (Ayers et al., 1996).

2.2.2. Groundwater

Samples were collected from farms wells, during the irrigation period, after activating the pump for several minutes. Two wells were selected based on their use: Well 1 whose GW is conjunctively used with TWW, and Well 2 whose GW is exclusively used for irrigation (Supplementary Table 2). The same water quality parameters as for TWW were determined, except BOD_5, COD, and TSS.

2.3 Determination of pharmaceutical active compounds

Treated wastewater samples were collected at the outlet of WWTP-SE4 and from SB, and from groundwater for the determination of 40 pharmaceutical substances encompassing parent compounds and metabolites (Supplementary Table 3). WWTP-SE3 was not included due to the limited volume transferred to SB and the frequent failures during the sampling period. Samples were collected in amber glass bottles rinsed with distilled water and methanol beforehand and were kept in a cooling box at 4°C until reaching the laboratory. All the samples were immediately frozen and then shipped to BRGM-France for analysis. After thawing, samples were analyzed using HPLC/MS-MS (Method reference, ANA-I10.MOA.68.B standard) by an external provider (LABOCEA, Brest, France).

2.4 Soil sampling and analysis

Soil samples were collected from three irrigated lands cultivated with citrus trees and characterized by different irrigation practices: i) Irrigation using conjunctively GW from Well 1 and TWW, ii) Irrigation exclusively using GW from Well 2, and iii) Irrigation exclusively using TWW from SB (Figure 1). As the irrigation season stretched usually over May-October, sampling was performed in May 2015 (i.e., before the irrigation period), July and August (i.e., during the irrigation period), and in November (i.e., after the irrigation period). For each plot, 5 soil samples were collected from the top layer (0-5 cm) after discarding leaves or small plants and mixed together to obtain one representative composite sample. Three replicates were used to check the reproducibility of the results. All the samples were stored at 4°C in a cooling box during transport to the laboratory.

Bacterial DNA was extracted from 0.5 g of soil samples using the FastDNA™ Spin Kit for Soil (MP Biomedicals, USA) according to manufacturer's recommendations. Abundance of the bacteria universal marker (16S rRNA gene, for microbial biomass quantification) and of nitrate-reducing bacteria (*narG* and *napA* genes, both involved in NO_3^- -> NO_2^- reactions) were assessed by real-time quantitative PCR (RT-qPCR) according to Bru et al.(2007). For samples collected in May, July and August, microbial potential towards the Carbon cycle was evaluated using Biolog EcoPlates that allows testing the ability of a microbial community to oxidize/metabolize a panel of 31 carbon sources that can be grouped together into six families: amino acids, carbohydrates, carboxylic acids, amines, phenolic compounds and polymers. Tests were done on microbial suspension obtained by mixing each soil with a sterile NaCl solution (0.85 %) at a ratio of 1 g soil for 10 mL NaCl 85 %, for 1 h at 50 rpm (Heidolph REAX 2) followed by a centrifugation step (10 min, 1000 rpm) to detach microorganism from soil particles. Biolog® EcoPlates were inoculated with 100 µL of microbial suspension in aerobic conditions, and incubated at 25°C for 5 days in the dark. Absorbance at 590 nm was measured twice a day using a UV/visible spectrometer for microplates (µQuant BIO-TEK INSTRUMENT) (De Lipthay et al., 2004; Janniche et al., 2012). Kinetics of oxidation (as Vmax) of the 31 carbon sources were measured as the Average Well Color Development (AWCD) along time.

2.5 Farmers' survey: health and environmental risks perception of water reuse

In order to better understand farmers' perception of water quality and their attitude with regard to the irrigation practices (i.e., the exclusive use of TWW or GW, or their conjunctive use), a survey covering 25% of the farmers' community (70 farmers) was carried out. Farmer's selection was based on the records of the Water Users' Association (GDA) Souhil considering mainly land size (due to land segmentation). Thus14 categories of land size were set as follows: 0.05 <<0.1 ha; 0.1 <<0.2 ha; 0.2<<0.3 ha; 0.3<<0.4 ha; 0.4<<0.5 ha; 0.5<<0.6 ha; 0.6<<0.7 ha; 0.7<<0.8 ha;

0.8<<0.9 ha; 0.9<<1 ha; 1<<1.5 ha; 1.5<<2 ha; 2<<3 ha; 3<<5 ha. Farmers were selected randomly within each of these categories based on random table number. The questionnaire encompassed open, semi-open, and closed questions covering several aspects of the reuse of TWW and includes mainly the following sections: (1) general information on the farm; (2) farmer's profile; (3) water resources quality and management and quality including TWW and GW; (4) soil quality perception; (5) agricultural practices; (6) health and hygiene; and (7) environmental and health risks perception. A first round of interviews confirmed the existence of three main categories of use of TWW and GW for irrigation within farmers' community as mentioned above: i) Farmers using exclusively TWW, ii) Farmers practicing the conjunctive use of TWW and GW either by blending or by alternating, and iii) Farmers using exclusively GW. The first two categories were of particular interest as they are both using TWW.

2.6. Statistical analysis

Data were processed using ExcelStat software using descriptive analysis for all the quality parameters and PCA for pharmaceutical compounds observed in TWW (WWTP-SE4 and SB) and GW (Well 1 and Well 2). For soil microbial analysis, the effect of sampling date or irrigation type (TWW, GW, TWW+GW) as well as the irrigation status (irrigated in the last days or not) was assessed using Kruskal Wallis analysis followed by Dunn as post-hoc test.

3. Results

3.1. Irrigation water quality

3.1.1. Physico-chemical parameters

pH was between 6.98-8.06, with the highest values registered for WWTP-SE4 (Table 1). Electrical conductivity was between 2.21 dS/m (WWTP- SE3) and 4.21 dS/m (WWTP-SE4). COD and BOD_5 were ranging from 75 to 484 mgO_2/L and from 8 to 75 mgO_2/L, respectively. TSS was highly fluctuating (2-198 mg/L). Nitrites were between 0.01 and 1.58 mg/L. PO_4 and K were between 0.0 (zero) and 7.23 mg/L and 11 and 53 mg/L, respectively. Sulfates were ranging from 212 to 624 mg/L. Globally, Na and Cl concentrations were very high with up to 600 mg/L. As for GW, mean pH was comparable for Well 1 (7.37) and Well 2 with (7.14) and EC was very high for Well 1 (6.54 dS/m) compared to Well 2 (3.41 dS/m). Phosphorous was at a mean of 0.040 mg/L in both wells. Overall, K mean concentrations in Well 1 were almost half those observed in Well 2 (28mg/L). Sulfates have shown high concentrations (883for Well 1 and 5363 mg/L for Well 2). Mean concentrations of Na and Cl were exceptionally high with 822 and 1320 in Well 1, and 590 mg/L and 439 mg/L for Well 2, respectively.

3.1.2. Microbiological quality

Total coliforms (TC) and *E. coli* counts in TWW ranged from <100 MPN/100 mL to > 1.1×10^5 MPN/100 mL (Table 1). Helminth eggs were only found in WWTP-SE4. TC and *E. coli* were detected at more than1.1×10^3 MPN/100 mL in both wells. Globally, TC were more frequently detected in Well 2 (80% of samples) while *E. coli* was more frequently found in Well 1 (60%). No helminth eggs were found in both wells.

Table 1. Physicochemical and microbiological parameters of TWW collected from WWTP-SE3, WWTP-SE4, SB, and GW collected from Well 1 and Well 2

3.1.3. Heavy metals

In TWW, the majority of heavy metals were below the limits of detection (LOD) except sporadically Mn and Zn. Al was quantified in all the samples with concentrations ranging from 0.018 to 0.502 mg/L (Table 2). Hg was also detected at concentrations up to 0.0056 mg/L. In GW, almost all the trace elements were below LOD except for Mn and Hg. The latter was registered at a maximum of 0.0096 mg/L.

Table 2. Heavy metals concentrations in TWW collected from WWTP-SE3, WWTP-SE4, SB, and GW collected from Well 1 and Well 2 (mg/L)

3.1.4 Pharmaceutical active compounds

Seventeen pharmaceutical compounds, out of the 40 investigated substances, were detected in TWW collected from WWTP-SE4 and SB (Table 3). The highest concentrations were measured for caffeine (CAF) (13.0 µg/L). Ofloxacin (OFL) was found at 1.11 µg/L in WWTP-SE4 and 1.16 µg/L in SB. Globally, the concentrations in WWTP-SE4 and SB were in the same range except for ketoprofen. For GW, seven and nine compounds were detected in Well 1 and Well 2, respectively. The highest values were observed for doxycycline (DOX) (ca. 0.1 µg/L) in both wells. As for metabolites, only 10,11-epoxy-carbamazepine (CBZ-EP) was detected at 0.058 µg/L in both WWTP-SE4 and SB and at 0.016 and 0.008 µg/L in Well 1 and Well 2, respectively. The rest of substances, including hormones and antibiotics, were below LOQ (Supplementary Table 4).

Table 3. Concentrations of pharmaceutical compounds quantified in at least one sample of effluents from WWTP-SE4, SB and GW collected from Well 1 and Well 2 (µg/L)

Principal component analysis (PCA) of pharmaceuticals compounds (CAF, CBZ, DIC, DOX, CBZ-EP, ERY, and HYD) detected in TWW and GW measured in Well 1, Well 2, WWTP-SE4, and SB showed two main factors explaining 99.52% of the total variability: F1 with an eigenvalue of 2.030 representing 50.76% and F2 with an eigenvalue of 1.951 explaining 48.77% (Supplementary Table 5). The correlation matrix (Supplementary Table 6) showed that WWTP-SE4 and SB as well as Well1 and Well 2 were significantly and positively correlated with coefficients of 0.9999 and 0.9613, respectively. Accordingly, F1 axis would correspond to the status of contamination thus separating contaminated and non-contaminated samples; the contaminated samples having positive values (Supplementary Figure 2). Meanwhile, F2 axis separates GW and TWW samples and would correspond to the levels of contamination in samples, farm wells being less contaminated than TWW. High correlations between TWW and F1 and between Wells and F2 were registered (Supplementary Table 7).

3.2. Soil microbial analysis

Biomass (as 16S RNA gene copy number) and *narG* functional gene abundance were in the same range in the 23 analyzed soils samples (from 9.5 to 10.4 log cp 16S/ g sol (dw), and from 6.6 to 7.7 log cp narG/ g sol (dw)). Function gene *napA* has been quantified in 9/23 samples (from 4.6 to 6.5 log cp napA/g sol (dw)). *narG* and *napA* proportion in the total biomass (16S RNA gene) were <0.3 % and <0.0003 %, respectively, showing that bacteria having *narG* genes were dominant in the soils. Date of sampling (during and after the dry season) and typical irrigation type (TWW, GW or both) in each irrigated plot had no significant effect on the bacterial biomass in the soil (16S copy, $p > 0.05$) (Figure 2). Abundance of *narG* gene was also similar in the three plots ($p > 0.05$).However the date of sampling significantly affected *narG* gene abundance, with a higher abundance of bacteria having the *narG* gene in November than in May and August suggesting a higher denitrifying activity during the wet season.

Figure 2. Bacterial biomass (measured as the copy number of 16 S rRNA gene per g of dry soil) and denitrification (measured as *narG* and *napA* gene abundance) in soil sampled from 3 plots irrigated with both TWW+GW, only TWW or only GW during May, July, August, and November 2015. Differences between sampling dates for 16S and *narG* genes quantification are shown only when they are significant (*: $p < 0.05$, Kruskal Wallis with Dunn test as post hoc)

The impact of irrigation practices on the ability of soil communities' function/activity linked to the carbon cycle was also evaluated (Table 4). All soil samples could utilize a similar and large panel of carbon sources (from 22 to 29 carbon sources over the 31 carbon sources available in the microplate) and were thus characterized by a high substrate utilization richness. During our study, the type of irrigation did not impact soil microbial properties linked to the carbon cycle. However, an increase of Vmax in July followed by a decrease in August was observed for all soils. This observation about soil microbial activity would need to be verified by a statistical approach with more data, though, it may suggest a weaker oxidation activity of carbon sources in August possibly due to the impact of a long period/exposition to drought and high temperatures.

Table 4. Community-level physiological profile in soils collected from the 3 plots irrigated either conjunctively with TWW and GW, or only TWW or only GW

3.3 Water quality perception, farming practices and attitudes, and environmental and health risks

Due to large number of questions (about 130) covered in the survey, only selected ones were reported in this paper. About 70% of the interviewees were more than 60 years old and almost 70% have received elementary educational level i.e., primary and Qur'anic School (Table 5). The majority (77%) are landowners and more than 75.7% have less than 1 ha. In majority, TWW reuse started in the 1980's and irrigation stretches from April to September and even to November. For 88%, water availability is the main motivation behind the reuse of TWW. However, only 19% did link the reuse to the presence of nutrients and their benefits on yield, and more than 90% acknowledged spreading various types of manure. Their evaluation of TWW quality is rather based on the presence of foam (91.4%), color (84.3%), and odor (85.8%). Regarding good practices and the use of protecting measures, about 96% acknowledge not wearing gloves and 44% not wearing boots and globally 91.4% were not taking any type of vaccination. Meanwhile, more than 77% and 40% think that TWW can carry microbes and harmful chemicals, respectively. Regarding the impacts of reuse on soil, more than 57% declared having no idea. In revenge, 70% stated that TWW has very good to average impact. About 90% of the interviewees have open wells used for different purposes like domestic usage, animal watering, and irrigation. For 47% of interviewees GW is not polluted whereas 29% have no idea on its quality parameters. Conjunctive use (blending or alternating TWW and GW) is practiced as 86% declared relying on GW to satisfy the crops water needs.

Table 5. Selected questions and results of the farmers' survey, expressed in percentage of interviewees (%)

4. Discussion

Even though the study was carried out during the period 2014-2016, its outcomes remain valid and up-to-date because TWW quality and availability did not improve.

4.1. Irrigation water quality and risk assessment

Variability of TWW quality parameters is due to aging infrastructures of the WWTP and operation over biological and hydraulic capacity on one hand. On the other hand, effluents from slaughterhouses, olive mills, flowers extraction units, tanneries, and textiles industries are released without pre-treatment causing fluctuation of TWW quality, especially salinity. pH and EC of all TWW samples were complying with the discharge standards (Order of 26 March 2018) and the Tunisian Standards of Reuse in Agriculture (NT 106.03, 1989). Compared to WWTP-SE3 and WWTP-SE4, SB was moderately saline (Ayers and Westcott, 1994). Bearing in mind that citrus trees can resist to up to 1.3 dS/m, which is far below the mean value of SB, the impact on soil quality and yield is highly forecasted. High Na and Cl can originate from the release of textile and tanneries effluents, intrinsic salinity of raw effluents, blending TWW of different salinities, and evaporation (Mahjoub et al., 2009). Though the maximum value allowed by the standard is not exceeded (2000 mg/L), Cl concentration can harm citrus species that can stand up to 16.6 meq/L (approximately 580 mg/L), which is exceeded by far. High Cl content can have adverse impacts on soil and plants (Belaid et al., 2010; Hashem and Qi, 2021; Hidri et al., 2014). Sulfates were also very high contributing to salinity load. Therefore, salinity is a crucial aspect to be considered under TWW reuse. For TSS and COD, WWTP-SE3 and WWTP-SE4 were exceeding the discharge and reuse limit values for agricultural irrigation whereas BOD_5 was within the limit of 30 mg/L. Nutrients' load has no thresholds values when TWW is reused meanwhile it has to comply with the standards of discharge when it is not meant for agricultural irrigation. Because 80% of TWW is released in water bodies, PO_4, NO_2, and K being above the limit concentrations may be of environmental concern. Despite this fertilizing load, farmers are heavily amending the soil, using organic and mineral fertilizers. Farmers need to be informed about the fertilizing potential of TWW and to be provided with friendly-use tools to make a rough estimation of the amounts of fertilizers they need to apply.

TWW is used for irrigation, either exclusively or conjunctively with GW. Both practices affect GW quality through return flow. EC was high in both wells but higher in Well 1 because of pronounced seawater intrusion. GW salinity is caused also by aquifer material weathering (Anane et al., 2014; Hamzaoui Azaza et al., 2012; Rekaya, 1986) which is in line with the impact of agricultural activity and farmers' practices, like the cropping system (Anane et al., 2014). The relatively better GW quality of Well 2, according to farmers' saying, is motivating the exclusive and intensive use as it allows diversification of crops (garden crops). Well 1 is very likely to harm orange trees due to salinity; blending GW and TWW is the alternative "strategy" to reduce it, though the environmental impacts of this practice have never been evaluated by scientists. Apart from irrigation, GW is used for domestic purposes and animals watering. Variation of water quality is likely to cause animals to drink less which may affect their health status. Salinity should be below 5 dS/m for all classes of livestock and poultry (Ayers and Westcott, 1994). The sum of nitrates and nitrites should not exceed 100 mg/L with nitrites <10 mg/L and sulfates should be lower than 250 mg/L for livestock (Pfost and Fulhage,2001), which is largely exceeded in both wells.

As for the microbiological quality, TWW showed high contamination in all samples except helminth eggs detected only in WWTP-SE4. Comparable concentrations were reported for the same WWTP showing low treatment efficiency (Trad Raïs and Marzougui, 2017). The order of discharge has set a limit of 2000 MPN/100 mL for fecal coliforms. However, no restriction is set for reuse in irrigation. WHO Guidelines (2006) recommended applying the multi-barriers approach to achieve pathogen reduction and mitigate health risk to a tolerable level. These guidelines are merely disrespected especially by elders (Zantout et al., 2014). For helminth eggs, both limits for discharge and for agricultural reuse are set to <1/1000 mL. Their presence in WWTP-SE4 would be due to the release of effluents from poultry and slaughterhouses (Mahjoub et al., 2016).

Bacteria can infiltrate to GW under reuse and in case of manure application and during grazing livestock, even though die-off may occur because of temperature, humidity, pH, and organic carbon in soil. The impact of this practice on *E. coli* or fecal concentrations are still unexplored (Petersen and Hubbart, 2020). Higher TC in Well 2 was probably resulting from the intensive and extended application use of manure for growing garden crops compared to trees where it is locally applied. Given the high soil hydraulic conductivity (Mahjoub et al., 2009), irrigation can remove 1 $\log_{10}$ to several $\log_{10}$ (Donn et al., 2020). Considering the maximum microbiological load of TWW, a removal is definitely taking place during infiltration, while die-off may continue in groundwater (Donn et al., 2020). Consequently, the presence of *E. coli,* at higher concentration in Well 1 could derive from both the reuse of TWW and the use of manure. TC includes fecal coliform bacteria such as *E. coli* and other types of coliforms naturally occurring in soil. Hence, the presence of TC does not necessarily indicate a recent water contamination by fecal waste, while fecal coliform does. TC in GW can be brought in agricultural runoff, sewage discharges and infiltration of domestic or wild animal fecal matter. TC was found in GW of the agricultural area of Korba region, located close to a managed aquifer recharge site, at concentration of up to > 5000 per 100 mL due to the use of TWW with a microbiological load of $9.3x10^2$ to $240x10^3$ per 100 mL (Cherif et al., 2013), which corroborates our findings. Microbiological parameters cannot be considered separately from physicochemical parameters which would greatly improve the accuracy of predictions regarding *E. coli* inactivation and survival in water resources. Recently, turbidity, pH, TDS, and EC were used in modelling to predict *E. coli* contamination of GW (Khan et al., 2021). In view of the predominant farmers' attitudes, the use of GW for irrigation would be of similar health concern, probably as high as using TWW because the presence of coliforms in GW could be a trigger to the presence of viruses as well. As for animals' health, water supplies for livestock should contain < 200 MPN/100 mL for TC and < 1 MPN/mL for *E. coli*. which is not the case of Well1 and Well 2 and requiring further investigation. As for parasites, GW was free of helminth eggs because they can settle with sludge during storage.

Heavy metals in TWW were often within the limit values of discharge and of reuse for irrigation. In near-neutral pH values, Al concentration may range from 0.001 to 0.05 mg/L and may reach up to 0.5-1 mg/L in water rich in organic matter (WHO, 2010), which is in line with our case study. This could corroborate the first quantitative risk assessment study carried out in Tunisia in 2015 in which high concentrations of Al in TWW and irrigated crops resulted in a high estimated health risk (ANCSEP, 2021). For Hg, the maximum concentrations were exceeding the limits of discharge (0.005 mg/L) and those reuse for irrigation (0.001 mg/L). Energy and industrial sectors, waste incineration, iron and steel production, cement production are among the potential sources, and combustion of fossil fuels, particularly coal, remains as the main anthropogenic source (Pacyna et al., 2006). In GW, Hg maximum concentration exceeded even the standards of reuse for agricultural irrigation (0.001 mg/L). Organic carbon and nutrients in TWW facilitate the mobilization of naturally occurring Hg. Mercury can derive from fertilizers like calcium superphosphate (5.1 mg/kg) and 15-5-5 NPK (1.2 mg/kg) (Zhao and Wang, 2010). Hg maximum acceptable value in drinking water for livestock is 0.01 mg/L (Ayers and Westcot, 1994). According to WHO guidelines, GW would be harmful to animals and unsuitable for irrigation as well, requiring moderate to severe restriction. Based on prevailing qualities of GW and TWW, their separate or conjunctive use would harm soil quality, irrigated crops, and even the agricultural environment and health of end-users. Impacts and potential risks related to blending or alternating these two water resources are still unknown up-to-date and tedious to estimate in the absence of extensive epidemiological studies.

Caffeine had the highest concentrations among the investigated contaminants in WWTP-SE4 and SB. It is the main ingredient of beverages, including coffee, and cosmetics. In a previous research work, it was detected at 165 µg/L in effluents of the WWTP-SE4 (Fries et al., 2016). The difference with our study lies in effluents initial composition related to consumption of products-containing caffeine (mainly deriving from households and hotels) especially that CAF mainly derive from consumption and excretion by humans (Ternes et al., 2001) and because of incomplete degradation under activated sludge process. CAF was even found in the Antarctic environment at 71.33 µg/L indicating the high human activities and the expansion of tourism (Gonzalez-Alonso et al., 2017). From environmental impact perspective. The European Food Safety Authority concluded that CAF is harmless to the environment after evidencing low Monitoring Trigger Levels (Drewes et al., 2018). Yet, some studies recommend its complete removal from TWW and surveillance due to toxicity on wildlife and the ecosystem (Korekar et al., 2020). Detection of CAF in GW indicates the infiltration with TWW during irrigation (Siler, 1999) especially that Hammamet-Nabeul is shallow aquifer (water table around 15 m-20 m depth). CAF concentration in Well 1 was twice as much in Well 2 possibly due to large amount of TWW poured during irrigation and as Well 2 is located in the upstream of the area. CAF was detected more recently in 4 wells in Oued Souhil area (unpublished results) at mean concentrations ranging between 0.319 and 10.441 µg/L. This is comparable with the concentrations detected in GW in Europe ranging between 0.02 and 23.97 µg/L (Gao et al., 2019). The use of GW for irrigation either exclusively or conjunctively with TWW is, in both cases, a source of contaminants to soil and potentially to irrigated crops. The usage of GW containing CAF for animals watering is suspected to be harmful by enhancing the effect of escape and avoidance of aversive stimuli (Kennedy et al., 2015). The presence of CBZ is also an indicator of contamination of GW with wastewater (Fenet et al., 2012; Ternes, 1998) and wastewater infiltration can have an immediate impact on CAF and CBZ concentrations in the aquifer (Dvory et al., 2018).

Ofloxacin is a fluoroquinolones antibiotic exclusively used for human's curative purposes. Concentrations observed in effluents of WWTP-SE4 and in SB are very high compared to those detected in previous studies. OFL was found in secondary TWW of 7 treatment plants in Tunisia at 190-648 ng/L (Moslah et al., 2017). Fluoroquinolones, including OFL, can degrade partially with a low to medium average removal efficiency of 40 to 60-70% (Gros et al., 2010). OFL has a high K_d (70-5000 L/kg) showing its high affinity with solids matrices (Schauss et al., 2009) and low biodegradability. Though, it can undergo photolysis (Andreozzi et al., 2003). In GW, DOX showed the highest concentration in both wells with concentration in Well 2 comparable to those in observed for SB. Doxycycline is used for veterinary purpose and is generally used for pet's and as growth promoter to enhance animals' production (meat and protein content), and to treat pathologies in the intensive animal farming (Cerbo et al., 2019). DOX is rather excreted in feces, is frequently detected in manure even after storage (Berendsen et al., 2018), and in manure-amended soils (78.5 mg/kg dry weight in broiler manure) (Ho et al., 2014). Hence, broiler, sheep, and/or bovine manures are significant potential sources of antibiotics to soil and GW (Buta et al., 2021; Gros et al., 2021). Depending on agricultural practices in Oued Souhil area, cattle, broiler or ovine manure are spread on soil on annual basis. The irrigated plot using Well 2 is heavily amended with broiler manure for growing garden crops because "it has a better impact on crops and it is more affordable, compared to bovine manure" farmer said. Consequently, infiltration of DOX is very likely to occur under sandy soil if not degraded under field conditions, mainly by photodegradation (Xu et al., 2021). DOX can cause enrichment of antibiotic resistance bacteria and mobile genetic elements in rhizosphere and bulk soil thus causing changes in bacterial community composition, especially in sandy soils (Blau et al., 2017). As for metabolites, CBZ-EP is the product of epoxidation of CBZ in the human body then it is converted to 10,11-dihydro-10,11-trans-dihydroxycarbamazepine. In Tunisia, this metabolite was detected for the very first time in the aquifer Hammamet-Nabeul at 2-6 ng/L while TWW contained 111-293 ng/L. Higher mobility in soil compared to the parent compound was evidenced (Fenet et al., 2012).

PCA performed for pharmaceutical compounds in TWW and GW supports the hypothesis that WWTP-SE4 and SB had very similar contamination profile and that the composition of TWW almost was not altered during the transfer to the storage basin by degradation/transformation processes. Pharmaceutical compounds in TWW are worth further investigation during the irrigation season to evaluate the polluting load of WWTP-SE3 effluents (that was not considered in the present study), and to detect any variation (improvement or degradation) of irrigation water quality. Regarding farm wells, the profile of contamination is consistent with the hydrological flow of the aquifer and would support the process of infiltration of substances deriving either from TWW or from other sources, like manure.

Risk is defined as the matching between "Impact" levels and "Likelihood" levels (Risk = Impact × Likelihood). The qualitative risk assessment requires the determination of these levels for both for the considered contaminant under the prevailing irrigation conditions using TWW. Four levels were considered for both Impact (1: Very Low, 2: Low- and Medium: 3: High) and Likelihood (1: Rare to 4: Likely). Consequently, the risk values are as follows: 1-3: low risks, 8-16: high risks (Australia 2004a, b). Accordingly, the risk associated to the occurrence of the pharmaceutical active compounds detected in GW of Oued Souhil is estimated to be the lowest with a score of 2 ; the highest score is related to salinity and nitrogen (Elgallal et al., 2016). Considering the levels of concentrations of the different entities, this result would be highly questionable and provide evidence that farmers' practices have to be seriously studied and taken into consideration, under GW or TWW or their conjunctive use when aiming at evaluating health and environmental impacts. Moreover, risk assessment is rarely conducted under real field conditions, integrating/simulating the actual behavior/attitudes of farmers which is very trivial.

4.2. Farming practices and environmental and health risks

The age and the educational level of farmers may inform on their strong ties to land meanwhile they largely influence their knowledge about water quality parameters and its perception. This is especially true for chemicals (except salinity) like heavy metals and emerging contaminants, and microbiological parameters as "invisible" pollutants. In Oued Souhil, the duration of the irrigation period depends strongly on rainfall and may stretch over November increasing the need to GW when TWW is not timely supplied. As per the conjunctive use of TWW and GW, it is driven firstly by the insufficient quantity of water and secondly by the low quality of TWW. It does not obey to a clear pattern and depends chiefly on farmers' perception of water quality/quantity and seasonal habits. For instance, when the irrigation schedule is unexpectedly modified and resulting in untimely supply, farmers may alternate TWW and GW. We can distinguish two types of conjunctive use that can definitely influence soil physical, chemical, and microbiological properties, crops yield, human health, and the agricultural environment:

- Blending TWW and GW is applied when TWW is available and visibly of low quality and farmers are planning to irrigate more crops. This requires a decision taken enough time beforehand based on farmers' objective, availability of equipment for pumping, etc.
- Alternating TWW and GW takes place when TWW supply is suddenly interrupted during a critical period of the irrigation season or when other types of crops are meant to be irrigated. It is also applied to avoid buildup of salinity brought by GW or decay of suspended solids on soil surface brought by TWW.

The reuse in Oued Souhil was promoted as a valuable water and nutrients source. However, few farmers were aware of nutrients load. Similarly, in Iran, farmers of Marvdasht County were adding nitrogen fertilizers while reusing TWW in order to increase soil fertility and improve growth (Khanpae et al., 2020) and in Jordan, they applied excessive quantities of fertilizers to guarantee high productivity and good yield (Carr et al., 2011) despite their awareness of TWW nutrients content. Farmers of low educational level are in lack of simple and practical tools to estimate optimal doses of both nutrients and water, which have to be provided by practitioners of the agricultural sector. They cannot do without spreading manure because citrus trees are "positively sensitive" to organic fertilizers. Regarding the safe use of TWW, good practices are not widely applied as the majority of farmers do not wear protecting tools due to their low perception of health risks and polluting load. Some declared having acquired a kind of "immunity" by being frequently exposed to contaminants. From their perspective it is like "taking a vaccination against all kind of substances and microorganisms". Protective equipment usually causes inconveniences (allergies, itching, and blush) pushing farmers to irrigate barefoot in some cases. Farmers recognize that seasonal exposure to TWW deriving from hot spots polluting sources, like flowers distillation, olive mills, slaughterhouses, may result in relatively serious health problems. However, mixed opinions were noticed about the impact of reuse on soil and GW and farmers were not able to identify/describe it. They think that irrigation has no major impact on GW and it can still be used for irrigation but not for drinking purposes because of what they expressed as “bitterness” and salinity. Consequently, they can keep withdrawing GW. The current perception and attitudes toward reuse are expected to result in negative impacts on environment and health.

4.3. Soil microbial properties

Recently, Gallego et al. (2021) reported that under controlled conditions, the irrigation with wastewater containing a mixture of fourteen chemicals at 10 µg/L each, including pharmaceutical, biocide, and pesticide active substances, caused a drift in the composition of soil bacterial community. A hundred operational taxonomic units were identified as responsible for changes between treated and spiked wastewater irrigation treatments. This would suggest that under real field conditions, continuous irrigation with wastewater could result in changes in bacterial communities with unknown functional consequences. In our study we focused on microbial potential activities linked to the N and C cycle that are important parameters for soil health, in particular as they support plant growth. Our results did not reveal negative impacts of irrigation with GW and/or TWW on soil microbial activities as no great changes were observed over several months. The main modifications in soil microbial activities seemed to be linked to climate/season. Indeed, our results suggest that the wet season (November) could tend to increase bacterial biomass involved in the denitrification pathway, whereas the dry season (August) could tend to decrease activities linked to the C-cycle. Water amount might have thus been more determinant than water origin for soil microbial activity. During the study period, soil microbial functioning was not substantially affected by the use of TWW, TWW+GW or GW. The excessive use of organic fertilizers would have hindered the observation of differences in soil microbial activities and biomass between soils irrigated with TWW and GW. Higher soil microbial activities were observed after irrigation with TWW, as it brings nutrients and organic matter. In our case, the fertilizing load of TWW can be considered insignificant and has thus only little impact on microbial biomass and activities. However, it induced changes in soil microbial diversity. As underlined by Becerra-Castro et al. (2015), these changes can potentially be positive to soil as TWW can bring microorganisms involved in the N cycle. However, it is still not well established whether they can survive under soil conditions, and their interaction with the microbial community of the receiving soil has still to be determined.

5. Conclusions

In Oued Souhil, irrigated agriculture used to rely exclusively on GW resources. After serious depletion of the aquifer, TWW was the unique water resource available to safeguard the agricultural activity. Despite the availability of TWW, the transfer and distribution to the irrigated area is hindered by several factors. GW withdrawn from shallow farm wells was considered as a solution to meet the water crops demand, thus it was conjunctively used with TWW for augmenting the quantity and/or improving its quality. This practice is estimated to be sustainable from farmers' perspective. However, the use of GW from farm wells located in areas irrigated with TWW is banned by law. TWW and GW, as shown in this study, are both non-conventional water resources and their conjunctive use for irrigation is expected to be detrimental and environmentally unsound affecting the sustainability of such practice. In order to promote the safe reuse of TWW in the region while maintaining the pollution of GW at an acceptable level, water planners should guarantee a systematic reliable and timely supply of TWW with a better quality. Farmers' high acceptance of TWW and buy-in of reuse projects to preserve their lands should be considered as an asset to introduce innovative solutions that can mitigate the contaminants load introduced either by TWW/GW or their conjunctive use. Considering the gap of knowledge about reuse and water quality parameters, farmers' awareness should be raised about the environmental and health concerns of their practices through regular awareness campaigns and capacity building by the extension services. Risk assessment studies are recommended to be conducted at local and larger scale to promote evidence-based actions of policy and decision-makers in the whole country and environmental and health externalities need to be weighed against the economic viability of the conjunctive use of TWW and GW.

6. Acknowledgements

This research work was funded by the United States Agency for International Development (USAID) under the Further Advancing the Blue Revolution Initiative (FABRI) within the Research Project “Mitigating Environmental Risks of Wastewater Reuse for Agriculture” (2013-2017) (Grant No. PR&D 03-03). It has partially received funds from BRGM (Sorpantinano Project) for the analysis of emerging contaminants and microbiological soil quality. Are acknowledged all persons who contributed to this research study especially the Water Users’ Association (GDA) Souhil, farmers in Oued Souhil area, CRDA Nabeul and Mr. Sayed Selmi and Mr. Ammar Nefati for contributing to conducting the survey.

7. References

- Al Khamisi, S. A., Prathapar, S. A., Ahmed, M. 2013. Conjunctive use of reclaimed water and groundwater in crop rotations. Agric. Water Manag. 116, 228-234. https://doi.org/10.1016/j.agwat.2012.07.013

- Alexandratos, N. and Bruinsma, J. 2012. World agriculture towards 2030/2050: the 2012 revision. ESA Working paper No. 12-03. FAO. Rome.

- Anane, M., Selmi, Y., Limam, A., Jedidi, N., Jellali, S. 2014. Does irrigation with reclaimed water significantly pollute shallow aquifer with nitrate and salinity? An assay in a perurban area in North Tunisia. Environ. Monitoring and Assessment. 186, 4367-4390. https://doi.org/10.1007/s10661-014-3705-x

- ANCSEP. 2021. Etude d'évaluation des risques sanitaires liés à la présence des éléments traces métalliques dans les eaux usées traitées réutilisées à des fins agricoles. 175 pages.

- Andreozzi, R., Raffaele, M., Nicklas, P. 2003. Pharmaceuticals in STP effluents and their solar photodegradation in aquatic environment. Chemosphere. 50, 1319-1330. https://doi.org/10.1016/s0045-6535(02)00769-5

- Arrêté du ministre des affaires locales et de l’environnement et du ministre de l’industrie et des petites et moyennes entreprises du 26 mars 2018, fixant les valeurs limites des rejets d'effluents dans le milieu récepteur.

- Australia, S. 2004a. Risk Management Guidelines: Companion to AS/NZS4360: 2004. Homebush, NSW.

- Australia, S. 2004b. Risk Management, 3rd ed. Standards Australia. Homebush, NSW.

- Ayres, R.M., Mara, D. D., World Health Organization. 1996. Analysis of wastewater for use in agriculture: a laboratory manual of parasitological and bacteriological techniques/Rachel M. Ayres and D. Duncan Mara. World Health Organization. https://apps.who.int/iris/handle/10665/41832

- Ayers, R.S. and Westcot, D.W. 1994. Water Quality for Agriculture. FAO Irrigation and Drainage Paper 29, Revision 1, FAO, Rome, 174 p.

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

- Belaid, N., Neel, C., Kallel, M., Ayoub, T., Ayadi, A., Baudu, M. 2010. Effects of treated wastewater irrigation on soil salinity and sodicity in Sfax (Tunisia): A case study. Revue des sciences de l'eau. 23, 133-146. https://doi.org/10.7202/039905ar

- Berendsen, B.J.A., Lahr, J., Nibbeling, C., Jansen, L.J.M., Bongers, I.E.A., Wipfler, E.L., van de Schans, M.G.M. 2018. The persistence of a broad range of antibiotics during calve, pig and broiler manure storage. Chemosphere, 204, 267-276. https://doi.org/10.1016/j.chemosphere.2018.04.042

- Blau, K., Casadevall, L., Wolters, B., Meersche, V. D.T., Kreuzig, R., Smalla, K., Jechalke, S. 2017. Soil texture-depending effects of doxycycline and streptomycin applied with manure on the bacterial community composition and resistome. FEMS Microbiol. Ecol. 94, https://doi.org/10.1093/femsec/fix145

- Bru, D., Sarr, A., Philippot, L. 2007. Relative abundances of proteobacterial membrane-bound and periplasmic nitrate reductases in selected environments. Applied and Environ. Microbiol. 73, 5971-5974. https://doi.org/10.1128/AEM.00643-07

- Buta, M., Korzeniewska, E., Harnisz, M., Hubeny, J., Zieliński, W., Rolbiecki, D., Bajkacz, S., Felis, E., Kokoszka, K. 2021. Microbial and chemical pollutants on the manure-crops pathway in the perspective of "One Health" holistic approach. Sci. Total Environ. 785, 147411. https://doi.org/10.1016/j.scitotenv.2021.147411

- Carr, G., Potter, B. R., Nortcliff, S. 2011. Water reuse for irrigation in Jordan: Perceptions of water quality among farmers. Agric. Water Manage. 98, 847-854. https://doi.org/10.1016/j.agwat.2010.12.011

- Cerbo, D. A., Pezzuto, F., Guidetti, G., Canello, S., Corsi, L. 2019. Tetracyclines: Insights and updates of their use in human and animal pathology and their potential toxicity. The Open Biochem. J. 13, 1-12ca. https://doi.org/10.2174/1874091X01913010001

- Cherif, S., El Ayni, F., Jrad, A., Trabelsi-Ayadi, M. 2013. Aquifer Recharge by Treated Wastewaters: Korba case study (Tunisia). Sustainable Sanitation Practice. Issue 14/2013, 41-48.

- De Lipthay, J. R., Johnsen, K., Albrechtsen, H. J., Rosenberg, P., Aamand, J. 2004. Bacterial diversity and community structure of a sub-surface aquifer exposed to realistic low herbicide concentrations. FEMS Microbiol. Ecol. 49, 59-69. https://doi.org/10.1016/j.femsec.2004.02.007

- Donn, M., Reed, D., Vanderzalm, J., Page, D. 2020. Assessment of E. coli attenuation during infiltration of treated wastewater: a pathway to future managed aquifer recharge. Water. 12, 173. https://doi.org/10.3390/w12010173

- Drewes, J. E., Anderson, P., Denslow, N., Jakubowski, W., Olivieri, A., Schlenk, D., Snyder, S. 2018. Monitoring Strategies for Constituents of Emerging Concern (CECs) in Recycled Water Recommendations of a Science Advisory Panel Convened by the State Water Resources Control Board. SCCWRP Technical Report. California State Water Resource Control Boards.

- Dvory, N. Z., Livshitz, Y., Kuznetsov, M., Adar, E., Gasser, G., Pankratov, I., Lev, O., Yakirevich, A. 2018. Caffeine vs. carbamazepine as indicators of wastewater pollution in a karst aquifer. Hydrol. Earth Syst. Sci., 22, 6371-6381. https://doi.org/10.5194/hess-22-6371-2018

- Ejaz, M. S. and Peralta, R. C. 1995. Maximizing conjunctive use of surface and groundwater under surface water quality constraints. Adv. Water Resources. 18, 67-75. 7–75. https://doi.org/10.1016/0309-1708(95)00004-3.

- El Ayni, F., Cherif, S., Jrad, A. 2011. Impact of Treated Wastewater Reuse on Agriculture and Aquifer Recharge in a Coastal Area: Korba Case Study. Water Res. Manag. 25, 2251-2265. https://doi.org/10.1007/s11269-011-9805-2

- Elgallal, M., Fletcher, L., Evans, B. 2016. Assessment of potential risks associated with chemicals in wastewater used for irrigation in arid and semiarid zones: A review. Agric. Water Manage. 177, 419-431. https://doi.org/10.1016/j.agwat.2016.08.027

- FAO. 2009. How to Feed the World in 2050. High Level Expert Forum. FAO. Rome.

- Fenet, H., Mathieu, O., Mahjoub, O., Zhi, L., Hillaire-Buys, D., Casellas, C., Gomez, E. 2012. Carbamazepine, carbamazepine epoxide and dihydroxycarbamazepine sorption to soil and occurrence in a wastewater reuse site in Tunisia. Chemosphere. 88, 49-54. https://doi.org/10.1016/j.chemosphere.2012.02.050

- Fries, E., Mahjoub, O., Mahjoub, B., Berrehouc, A., Lions, J., Bahadir, A. M. 2016. Occurrence of contaminants of emerging concern (CEC) in conventional and nonconventional water resources in Tunisia. Fresenius Environ. Bull. 25, 3317-3339
- Gallego, S., Brienza, M., Béguet, J., Chiron, S., Martin-Laurent, F., 2021. Impact of repeated irrigation of lettuce cultures with municipal wastewater on soil bacterial community diversity and composition. Environ. Sci. Pollut. Res. 2021. https://doi.org/10.1007/s11356-021-14734-4
- Gao, Q., Blum, K. M., Gago-Ferrero, P. 2019. Impact of on-site wastewater infiltration systems on organic contaminants in groundwater and recipient waters. Sci. Total Environ. 651, 1670-1679. https://doi.org/10.1016/j.scitotenv.2018.10.016
- GDA Oued Souhil. 2014. Activities Report. GDA Oued Souhil. Tunis.
- González-Alonso, S, Merino, L. M., Esteban, S., López de Alda, M., Barceló, D., Durán, J. J., López-Martínez, J., Aceña, J., Pérez, S., Mastroianni, N., Silva, A., Catalá, M., Valcárcel, Y. 2017. Occurrence of pharmaceutical, recreational and psychotropic drug residues in surface water on the northern Antarctic Peninsula region. Environ. Pollut. 229, 241-254. https://doi.org/10.1016/j.envpol.2017.05.060
- Gros, M., Petrović, M., Ginebreda, A., Barceló, D. 2010. Removal of pharmaceuticals during wastewater treatment and environmental risk assessment using hazard indexes. Environ. Int. 36, 15-26. https://doi.org/10.1016/j.envint.2009.09.002
- Gros, M., Catalan, N., Mas-Pla, J., Petrovic, M., Farre, M. 2021. Groundwater antibiotic pollution and its relationship with dissolved organic matter: Identification and environmental implications. Environ. Pollut. 289, 117927. https://doi.org/10.1016/j.envpol.2021.117927

- Hamdane, A. 2014. La gestion des ressources en eau souterraines (nappes et aquifères) comme biens communs : Cas de la Tunisie. SCET, Tunisie, Version provisoire. Janvier 2014.

- Hamzaoui Azaza, F., Bouhlila, R., Gueddari, M. 2012. Characterization of the mineralization of the Triassic water table (South-East Tunisia) by geochemical and statistical methods. Geo-Eco-Trop. 36, 49-62.

- Hashem, M. S. and Qi, X. 2021. Treated Wastewater Irrigation-A Review. Water 13, 1527. https://doi.org/10.3390/w13111527

- Hidri, Y., Fourti, O., Eturki, S., Jedidi, N., Charef, A., Hassen, A. 2014. Effects of 15-year application of municipal wastewater on microbial biomass, fecal pollution indicators, and heavy metals in a Tunisian calcareous soil. J. Soils and Sediments, 14, 155-163. https://doi.org/10.1007/s11368-013-0801-4

- Ho, Y. B., Zakaria, M. P., Latif, P. A., Saari, N. 2014. Occurrence of veterinary antibiotics and progesterone in broiler manure and agricultural soil in Malaysia. Sci Total Environ. 488-489: 261-267. https://doi: 10.1016/j.scitotenv.2014.04.109

- Ibekwe, A.M., Gonzalez-Rubio, A., Suarez, D.L. 2018. Impact of treated wastewater for irrigation on soil microbial communities. Sci. Total Environ. 622-623, 1603-1610 https://doi.org/10.1016/j.scitotenv.2017.10.039

- Institut National de la Normalisation et de la Propriété Industrielle. 1989. Protection de l'environnement - Utilisation des eaux usées traitées en agriculture - Spécifications physiques, chimiques et biologiques, Norme Tunisienne NT 106.03 (1989).

- ISO 11885: 2007. Water quality - Determination of selected elements by inductively coupled plasma optical emission spectrometry (ICP-OES).

- ISO 15587-1: 2002. Water quality - Digestion for the determination of selected elements in water - Part 1: Aqua regia digestion.

- Janniche, G. S., Spliid, H., Albrechtsen, H.-J. 2012. Microbial Community-Level Physiological Profiles (CLPP) and herbicide mineralization potential in groundwater affected by agricultural land use. J. Contam. Hydrol., 140–141, 45-55. https://doi.org/10.1016/j.jconhyd.2012.08.008

- Kallali, H. and Yoshida, M. 2002. Soil and subsoil characterization of Souhil Wadi (Nabeul) pilot plant for wastewater infiltration-percolation. Proceedings of InternationalSymposium on Environmental Pollution Control and Waste Management (EPCOWM'2002). 7th-10th January, Tunis. Part II: Sessions 3 and 4, p. 831-837.

- Kallali, H., Anane, M., Jellali, S., Tarhouni, J. 2007. GIS-based multicriteria analysis for potential wastewater aquifer rehcarge sites. Desalination 215, 111-119. https://doi.org/10.1016/j.desal.2006.11.016

- Kennedy, A. E., Grimes, L. M., Labaton, R. S., Hine, J. F., Warzak, W. J. 2015. A methodological discussion of caffeine research and animal avoidance behavior. J. Caffeine Res. 5, 176-186. https://doi.org/10.1089/jcr.2015.0010

- Khan, F. M., Gupta, R., Sekhri, S. 2021. Superposition learning-based model for prediction of E. coli in groundwater using physico-chemical water quality parameters. Groundw. Sustain. Dev. 13, 100580. https://doi.org/10.1016/J.GSD.2021.100580

- Khanpae, M., Karami, E., Maleksaeidi, H., Keshavarz, M. 2020. Farmers' attitude towards using treated wastewater for irrigation: The question of sustainability. J. Cleaner Produc. 243, 118541. https://doi.org/10.1016/j.jclepro.2019.118541

- Korekar, G. G., Kumar, A. Ugale, C. 2020. Occurrence, fate, persistence and remediation of caffeine: a review. Environ. Sci. Pollut. Res. 27, 34715-34733. https://doi.org/10.1007/s11356-019-06998-8

- Madikizela, L. M., Ncube, S., Chimuka, L. 2020. Analysis, occurrence and removal of pharmaceuticals in African water resources: A current status. J. Environ. Manage. 253, 109741. https://doi.org/10.1016/j.jenvman.2019.109741

- Mahjoub, O., Leclercq, M., Bachelot, M., Casellas, C., Escande, A., Balaguer, P., Bahri, A., Gomez, E., Fenet, H. 2009. Estrogen, Aryl Hydrocarbon and Pregnane X Receptors Activities in Reclaimed Water and Irrigated Soils in Oued Souhil Area (Nabeul, Tunisia). Desalination. 246, 425-434. https://doi.org/10.1016/j.desal.2008.03.064

- Mahjoub, O., Mekada, M., Gharbi, N. 2016. Good Irrigation Practices in the Wastewater Irrigated Area of Ouardanine, Tunisia. In: Hettiarachchi, H. and Ardakanian, R. (ed). Safe Use of Wastewater in Agriculture: Good Practice Examples. UNU-FLORES, 101-120, ISBN 978-3-944863-31-3.

- Malkawi, H. I. and Muhammad, M. J. 2003. Survival and accumulation of microorganisms in soils irrigated with secondary treated wastewater J. Basic Microbiol. 43, 47-55. https://doi.org/10.1002/jobm.200390004

- Michel, C., Joulian, C., Ollivier, P., Audrey Nyteij, Cote, R., Surdyk, N., Hellal, J., Casanova, J., Besnard, K., Rampnoux, N., Garrido, F. 2014. Multi-bioindicators to assess soil microbial activity in the context of an artificial groundwater recharge with treated wastewater: A large-scale pilot experiment. J. Microbiol. Biotechnol. 24, 843-53. https://doi.org/10.4014/jmb.1312.12010

- Moslah, B., Hapeshi, E., Jrad, A., Fatta-Kassinos, D., Hedhili, A. 2017. Pharmaceuticals and illicit drugs in wastewater samples in north-eastern Tunisia. Environ. Sci. Pollut. Res. 25, 18226-18241. https://doi.org/10.1007/s11356-017-8902-z

- Nabeul governorate. http://www.nabeul.gov.tn/fr/evennements/eau-de-fleurs-dorangers-le-plaisir-du-palais-la-joie-du-nez/ Accessed on 06 December 2021.

- Pacyna, E. G., Pacyna, J. M., Fudala, J., Strzelecka-Jastrzab, E., Hlawiczka, S., Panasiuk, D. 2006. Mercury emissions to the atmosphere from anthropogenic sources in Europe in 2000 and their scenarios until 2020. Sci. Total Environ. 370, 147-156. https://doi.org/10.1016/j.scitotenv.2006.06.023
- Pereira, L. S., Oweis, T., Zairi, A. 2002. Irrigation management under water scarcity. Agric. Water Manage. 57, 175-2006. https://doi.org/10.1016/S0378-3774(02)00075-6
- Petersen, F. and Hubbart, J. A. 2020. Physical factors impacting the survival and occurrence of Escherichia coli in secondary habitats. Water 12, 1-15. https://doi.org/10.3390/w12061796
- Pfost, D. L. and Fulhage, C. D. 2001. Water Quality for Livestock Drinking. Environmental quality, MU Guide. MU Extension. University of Missouri-Columbia. https://extension.missouri.edu/publications/eq381 Accessed on 06 December 2021.
- Rekaya, M. 1986. Expérimentation pilote de recharge artificielle à partir des eaux usées traitées. Cas de la nappe de l'Oued Souhil (Nabeul). DGRE. Tunis.
- Roumasset, J. and Wada, C. 2010. Optimal Conjunctive Use of Groundwater and Recycled Wastewater. University of Hawaii at Manoa, Department of Economics. https://www.economics.hawaii.edu/research/workingpapers/WP_10-13.pdf
- Schauss, K., Focks, A., Heuer, H., Kotzerke, A., Schmitt, H., Thiele-Bruhn, S., Smalla, K. 2009. Analysis, fate and effects of the antibiotic sulfadiazine in soil ecosystems. Trends Anal. Chem. 28, https://doi.org/10.1016/j.trac.2009.02.009
- Scheytt, J. T. Mersmann, P., Rejman-Rasinski, E., These, A. 2006. Tracing pharmaceuticals in the unsaturated Zone. J. Soils Sediments. 7, 75-84. https://doi.org/10.1065/jss2006.12.200

- Siler, R. L. 1999. Caffeine and pharmaceuticals as indicators of wastewater contamination in wells. Ground Water. 37, 405-410. https://doi.org/10.1111/J.1745-6584.1999.TB01118.x

- Tahrani, L., Loco, J. V., Mansour, H. B., Reyns, T. 2016. Occurrence of antibiotics in pharmaceutical industrial wastewater, wastewater treatment plant and sea waters in Tunisia. J. Water and Health. 14.2, 208-2013. https://doi.org/10.2166/wh.2015.224

- Templer, O. W. 2001. Municipal conjunctive water use on the Texas High Plains. The Soc. Sci. J. 38, 597-604. https://doi.org/10.1016/S0362-3319(01)00153-7

- Ternes, T. A. 1998. Occurrence of drugs in German sewage treatment plants and rivers. Water Res. 32, 3245-3260. https://doi.org/10.1016/S0043-1354(98)00099-2

- Ternes, T., Bonerz, M., Schmidt, T. 2001. Determination of neutral pharmaceuticals in wastewater and rivers by liquid chromatography-electrospray tandem mass spectrometry. J. Chromatogr. A. 14,175-85. https://doi:10.1016/s0021-9673(01)01205-5.

- Trad Raïs, M. and Marzougui, N. 2017. Réduction des bactéries à intérêt sanitaire par les traitements appliqués aux éaux usées urbaines dans les stations d'épuration tunisiennes. Eur. J. Sci. Res.148, 78-92.

- UNDP/OPE. 1987. Reclaimed water reuse in agriculture, Groundwater recharge, Technical Report (in French), Project RAB/80/011, Water Resources in the North African Countries, Water Resources Directorate (Tunisia) - United Nations Development Program. Tunisie.

- WHO. 2006. WHO Guidelines for the safe use of wastewater, excreta and greywater. Volume II: Wastewater in agriculture. WHO-UNEP-FAO.

- WHO. 2010. Aluminum in drinking-water. Background document for development of WHO Guidelines for Drinking-water Quality. WHO/HSE/WSH/10.01/13.

- WWAP. 2015. The United Nations World Water Development Report 2015: Water for a Sustainable World. UNESCO. Paris.

- Xu, X., Ma, W., An, B., Zhou, K., Mi, K., Huo, M., Liu, H., Wang, H., Liu, Z., Cheng, G., Huang, L. 2021. Adsorption/desorption and degradation of doxycycline in three agricultural soils. Ecotoxicol. Environ. Saf. 224: 112675. https://doi.org/10.1016/j.ecoenv.2021.112675.

- Zairi, A., El Amami, H., Slatni, A., Pereira, L.S., Rodrigues, P.N., Machado, T. 2003. Coping with drought: deficit irrigation strategies for cereals and field horticultural crops in Central Tunisia. In: Rossi, G., Cancelliere, A., Pereira, L.S., Oweis, T., Shatanawi, M., Zairi, A. (Eds.), Tools for Drought Mitigation in Mediterranean Regions. Kluwer, Dordrecht, pp. 181-201.

- Zantout, R.S., Ullman, J. L. Russo, S. L., Mahjoub, O. 2014. Analysis of Human Health Risks Linked to Irrigation with Treated Wastewater in Oued Souhil, Tunisia. 30th Annual AIAEE Conference Integrated Agricultural Systems for Environmental Sustainability and Production: Educating and Feeding Seven Billion People. 28-30 April 2014, Miami, Florida. Supplement of the J. Int. Agric. Ext. Educ., 21 https://doi.org/10.5191/jiaee.2014.21207

- Zhao, X. and Wang, D. 2010. Mercury in some chemical fertilizers and the effect of calcium superphosphates on mercury uptake by corn seedlings (Zea mays. L). J. Environ. Sci. 22, 1184-1188. https://doi.org/10.1016/s1001-0742(09)60236-9

Figures (to be printed in color)

Figure 1. Location of Tunisia and Nabeul city (A) and irrigated agricultural plots (1: GW+TWW, 2: GW, 3: TWW), treatment plants (WWTP-SE3 and WWTP-SE4), storage basin and wells (Well 1 and Well 2) (B)

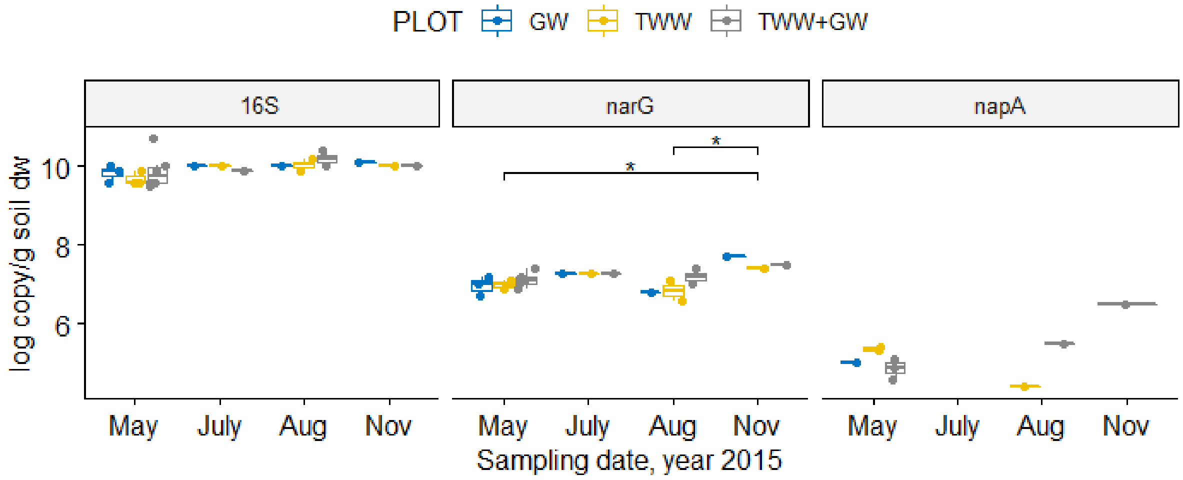


Figure 2. Bacterial biomass (measured as the copy number of 16 S rRNA gene per g of dry soil) and denitrification (measured as *narG* and *napA* gene abundance) in soil sampled from 3 plots irrigated with both TWW+GW, only TWW or only GW during May, July, August, and November 2015. Differences between sampling dates for 16S and *narG* genes quantification are shown only when they are significant (*: $p < 0.05$, Kruskal Wallis with Dunn test as post hoc).

Tables

Table 1. Physico-chemical and microbiological parameters of TWW collected from WWTP-SE3, WWTP-SE4, SB, and GW collected from Well 1 and Well 2

		pH	EC (dS/m)	TSS (mS/cm)	COD (mg/L)	BOD$_5$ (mg/L)	PO$_4$ (mg/L)	NO$_2$ (mg/L)	K (mg/L)	Na (mg/L)	Cl (mg/L)	SO$_4$ (mg/L)	TC	*E. coli*	H. E
WWTP SE3	n	23	22	19	19	13	21	22	18	19	22	23	6	6	6
	Min	6.98	2.21	2	30	5	0.01	0.01	11	267	351	212	<100	<100	Absence
	Max	7.96	3.55	155	343	48	7.23	0.20	34	495	755	591	>110000	>110000	Absence
	Mean	7.39	2.82	50	160	26	2.33	0.06	21	384	525	371	-	-	-
	SD	0.29	0.31	37	95	14	2.42	0.05	7	66	101	118	-	-	-
WWTP SE4	n	23	24	22	22	16	23	23	20	20	24	23	7	7	7
	Min	7.41	2.45	6	75	8	0.00	0.01	11	315	367	254	12000	<100	Absence
	Max	8.06	4.21	198	484	75	4.14	1.58	53	669	939	545	>110000	>110000	Presence
	Mean	7.70	3.32	92	217	38	0.93	0.13	28	452	618	413	-	-	-
	SD	0.16	0.52	60	112	21	1.11	0.32	13	99	149	73	-	-	-
SB	n	12	12	10	12	12	12	12	10	10	12	13	4	4	4
	Min	7.18	2.67	2	71	10	0.25	0.01	16	277	434	235	<100	<100	Absence
	Max	7.65	3.84	85	200	48	4.12	0.11	37	632	778	624	>110000	>110000	Absence
	Mean	7.47	3.18	42	113	21	2.25	0.05	24	406	570	408	-	-	-
	SD	0.17	0.44	24	38	10	1.38	0.03	6.0	105	118	119	-	-	-
Well 1	n	23	23	-	-	-	21	-	18	20	24	24	5	5	5
	Min	7.10	4.97	nd	nd	nd	0.001	nd	8	642	963	516	0	0	Absence
	Max	7.82	7.63	nd	nd	nd	0.12	nd	25	1031	1690	1239	>1100	>1100	Absence
	Mean	7.37	6.58	nd	nd	nd	0.04	nd	14	822	1320	883	-	-	-
	SD	0.21	0.77	nd	nd	nd	0.03	nd	4.1	100	197	208	-	-	-
Well 2	n	8	8	-	-	-	8	-	7	8	9	8	5	5	5
	Min	7.06	3.05	nd	nd	nd	0.02	nd	15	327	482	462	0	0	Absence
	Max	7.24	3.96	nd	nd	nd	0.06	nd	45	588	811	650	>1100	>1100	Absence
	Mean	7.14	3.41	nd	nd	nd	0.04	nd	28	439	590	536	-	-	-
	SD	0.07	0.35	nd	nd	nd	0.02	nd	9	96	114	60	-	-	-
Discharge standards*		**6.5-8.5**	**5**	**30**	**125**	**30**	**2**	**0.5**	**50**	**700**	**700**	**600**	**2000**	**-**	**< 1/1000 mL**
Agricultural Reuse standards**		**6.5-8.5**	**7**	**30**	**90**	**30**	**-**	**-**	**-**	**-**	**2000**	**-**	**-**	**-**	**< 1/1000 mL**
FAO Guidelines for GW***		**6.5 - 8.4**	**< 0.7 - > 3.0**	**-**	**-**	**-**	**-**	**-**	**-**	**< 3 – > 9**	**< 4 - > 10**	**-**	**-**	**-**	**-**

* Order 2018-315 related to the discharge of effluents in the receiving environment; ** Tunisian Standards for wastewater reuse for agricultural irrigation (NT 106.03, 1989) currently under revision; nd: not determined; *** 3 classes of restrictions based on EC, Na expressed as SAR, Cl, B, and NO_3-N contents and type of irrigation; TC: total coliforms; H.E: helminth eggs.

Table 2. Heavy metals concentrations in TWW collected from WWTP-SE3, WWTP-SE4, SB, and GW collected from Well 1 and Well 2 (mg/L)

		Al	Cd	Co	Cu	Total Cr	Mn	Ni	Pb	Zn	Hg
WWTP-SE3	n	6	6	6	6	6	6	6	6	6	6
	Min	0.004	<0.0027	<0.0070	<0.0097	<0.0071	<0.014	<0.010	<0.010	<0.0059	<0.044
	Max	0.188	0.004	<0.0070	<0.0097	<0.0071	0.016	<0.010	<0.010	0.0480	0.0027
WWTP-SE4	n	8	8	8	8	8	8	8	8	8	8
	Min	0.038	<0.0027	<0.0070	<0.0097	<0.0071	<0.014	<0.010	<0.010	<0.0059	0.0066
	Max	0.060	0.004	<0.0070	<0.0097	<0.0071	0.026	<0.010	0.012	0.0500	0.0056
SB	n	4	4	4	4	4	4	4	4	4	4
	Min	0.018	<0.0027	<0.007	<0.0097	<0.0071	<0.014	<0.010	<0.010	<0.0059	<0.044
	Max	0.502	<0.0027	<0.007	<0.0097	<0.0071	0.030	<0.010	0.032	0.0200	0.0042
Well 1	n	5	5	5	5	5	5	5	5	5	5
	Min	<0.0028	<0.0027	<0.007	<0.0097	<0.0071	<0.014	<0.010	<0.010	<0.0059	<0.044
	Max	<0.0028	<0.0027	<0.007	<0.0097	<0.0071	0.016	<0.010	<0.010	<0.0059	0.0096
Well 2	n	5	5	5	5	5	5	5	5	5	5
	Min	<0.0028	<0.0027	<0.0070	<0.0097	<0.0071	<0.014	<0.010	<0.010	<0.0059	<0.044
	Max	<0.0028	<0.0027	<0.0070	<0.0097	<0.0071	<0.014	<0.010	<0.010	<0.0059	0.0015
Discharge decree*		**5** **(Al+Fe)**	**0.01**	**0.5**	**2**	**0.005/0.5**	**1**	**0.2**	**0.1**	**5**	**0.005**
Reuse standards**		**-**	**0.01**	**0.1**	**0.5**	**0.1**	**0.5**	**0.01**	**1**	**5**	**0.001**
FAO Guidelines***		**5**	**0.01**	**0.05**	**0.20**	**0.10**	**0.20**	**0.20**	**5**	**2**	**-**

* Decree 2018-315 related to the discharge of effluents in the receiving environment; ** Tunisian Standards of treated wastewater reuse for agricultural irrigation NT 106.03 (1989) (currently under revision); figures underlined are above the standards of discharge or reuse; GW (Well 1 and Well 2) cannot be compared with the standards of reuse even if they are exceeding the threshold values. *** Ayers and Westcott (1994).

Table 3. Concentrations of pharmaceutical compounds quantified in at least one sample of effluents from WWTP-SE4, SB and GW collected from Well 1 and Well 2 (µg/L)

Compound	WWTP SE4	SB	Well 1	Well 2	LOQ*
			(µg/L)		
Caffeine	13.0	12.7	0.037	0.018	0.001
Carbamazepine	0.444	0.609	0.019	0.011	0.001
Diclofenac	0.073	0.106	<0.001	<0.001	0.001
Doxycycline hyclate	0.210	0.135	0.097	0.114	0.020
10,11-epoxy-carbamazepine	0.058	0.058	0.016	0.008	0.001
Erythromycin A dehydrate	0.098	0.115	<0.002	<0.002	0.002
Ketoprofen	0.417	0.179	<0.01	0.010	0.010
Lincomycin hydrochloride monohydrate	0.081	0.073	<0.001	<0.001	0.001
Losartan potassium	0.576	0.739	<0.006	0.006	0.006
Metoprolol tartrate	0.013	0.022	<0.001	<0.001	0.001
Naftidrofuryl (Nafronyl oxalate salt)	0.015	0.016	<0.001	<0.001	0.001
Ofloxacin	1.11	1.16	0.023	0.016	0.001
Oxazepam	0.016	0.025	<0.001	<0.001	0.001
Tetracycline	0.026	<0.005	0.009	0.013	0.005
Sulfamethoxazole	0.072	0.108	0.013	0.008	0.001
Sulfamethazine	0.010	0.009	<0.001	<0.001	0.001
Propranolol hydrochloride	0.069	0.061	<0.001	0.005	0.001

* LOQ: limit of quantification

Table 4. Community-level physiological profile in soils collected from the 3 plots irrigated either conjunctively with TWW and GW, or only TWW or only GW

Irrigation type	GW			TWW			TWW+ GW		
Month	May	July	August	May	July	August	May	July	August
AWCD Max (T=4 days)	1.33	1.26	0.74	1.49	1.38	1.22	1.17	1.45	1.17
Vmax (AWCD/h)	0.023	0.027	0.014	0.031	0.037	0.027	0.018	0.032	0.018
Richness (T= 4 days)	27	26	22	28	29	26	26	28	26

AWCD: Average Well Color Development

Table 5. Selected answers of the farmers' survey, expressed in percentage of interviewees (%)

Questions	Results (%)				
Farmers' age	20-40	40-60	>60		
	5.7	25.7	68.6		
Educational level	Quranic School	Primary school	High shcool	No answer	
	21.4	51.4	12.9	14.3	
Land tenure	Heritage	Purchase	Renting	Sharecropping	
	77.1	21.4	12.9	1.4	
Land size	<1 ha	1 ha - 2 ha	> 3 ha		
	75.7	12.9	11.4		
Irrigation start	March	April	May	June	August
	4.3	20.0	54.3	24.3	1.4
Irrigation ceasing	August	September	October	November	December
	10.0	27.1	35.7	30.0	2.9
Reuse activity TWW	Before 1980's	After 1980's			
	95.7	4.3			
Reason behind reuse of TWW	Availability	Gain of fertilizer	Availability + gain of fertilizer	Availability + water scarcity	Other
	71.4	5.7	12.9	4.3	5.7
Use of manure	Yes	No			
	91.4	8.6			
Parameters used to evaluate TWW quality*	Odor	Color	Foam	Turbidity	Other
	85.7	84.3	91.4	67.1	1.4
Wearing gloves during irrigation	Yes	No			
	4.3	95.7			
Wearing boots during irrigation	Yes	No	No answer		
	55.7	44.3	2.9		
Vaccination of the farmer (or person in charge of irrigation)	Yes	No			
	8.6%	91.4%			
Contaminants that farmers think are present in TWW*	Microbes	Chemicals	No answer		
	77.1	40.0	17.1		

Impact of TWW reuse on soil according to farmers	Crusts on surface	Soil surface affected	Water stagnation	Unknown	
	4.3	35.7	2.9	57.1	
Evaluation of the impact of reclaimed water globally according to farmers	Very good	Good	Average	Bad	Very bad
	10	22.9	37.1	18.6	12.9

* Multiple answers are accepted

Graphical abstract