Urban raw or treated wastewater drip-irrigation for lettuce and leek crops: chemical and microbiological properties of soil and plants

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Abstract:

The aim of this study is to evaluate chemical and microbiological properties of soil, as well as crop yields, when lettuces and leeks are irrigated by raw wastewater (RW), treated wastewater (TW) and drinking water (DW) while in a greenhouse. After 2 lettuce and 1 leek growth cycles, the soil analyses showed an increase of electrical conductivity (EC), Cl, Na and nitrate-nitrogen concentration (NO_3-N) after irrigation with TW and particularly with RW compared to DW. The fresh weight of both crop yields was significantly higher with TW compared to DW and RW irrigation. The decay of fecal indicators (*E. coli*, *Enterococcus* sp., bacteriophages) in soils was slow, as shown by cultivation-based techniques and qPCR. Fecal indicators were found in plants irrigated with raw wastewater.

Keywords: soil proprieties; drip irrigation; crop yields; fecal indicator bacteria

Introduction

Several studies related to the use of treated wastewater in agriculture have been developed for different crops including some eaten raw, such as lettuce (Urbano et al. 2017), eggplant and tomato (Cirelli et al. 2012). There are many benefits to using treated wastewater for irrigation such as increased soil nutrients, increased crop yield and reduction in fertilizer quantity. However, there are also numerous disadvantages e.g. soil salinization, damage of sensitive crops, loss of soil infiltration capacity and contamination by pathogens (Nogueira et al, 2013). These different studies focus mainly on high quality wastewater treatment cases and rarely assess the impact of low-treatment or raw wastewater, which covers the majority of water reuse in the world (Theboa et al. 2017).The aim of study is to analyze the effects of using three different types of wastewater: treated wastewater (TW), raw wastewater (RW) and drinking water (DW) to irrigate lettuces and leeks. We take into account various factors: chemical and microbiological properties of soil, crop yields and possible transfer of microbial contaminants by measuring the concentration of fecal indicators in the roots and in the edible parts of the vegetables.

Material and Methods

The experiment conducted in a greenhouse at a wastewater treatment plant (treatment process with 3 waste stabilization ponds) in Murviel-Lès-Montpellier, France. The mean temperature and relative humidity during all test cycles were 27.2 ± 7.2 °C and 54 ± 21.7 % respectively. Global radiation varied between 463 MJ $month^{-1}$ (in June) and 696 MJ $month^{-1}$ (in July). In order to avoid field contamination, large bins (surface equal to 1 m^2) filled with a loamy clay soil (24% of clay, 25.6% of silt, 19.5% of very fine sand, 16.4 % of fine sand and 14.4 % of sand) were used to cultivate 16 Batavia lettuces (*Lactuca sativa*; four lettuces per bin) and 64 leek plants (*Allium porrum*; 16 leeks per bin). Lettuces and leeks were irrigated by 3 different water qualities: drinking water (DW), treated wastewater (TW) and raw wastewater (RW) which was pumped from the entrance of an anaerobic pond. To keep plants watered, humidity was controlled using time domain reflectometry sensors. A surface drip irrigation was installed at each lettuce and leek plant and a nominal flow rate equal to 2 $L.h^{-1}$ (at P=1bar) was used. Between June and September 2018, lettuce crops were cultivated for two cycles of 6 weeks and leeks for one cycle of 14 weeks. The total water irrigation for lettuce cycle 1 and cycle 2 was 52 mm and 58 mm respectively. For leeks, total irrigation was 198 mm.

Irrigation water analyses

Physico-chemical analyses of water quality were carried out weekly on each of the three water types. Total Suspended Solids (TSS), electrical conductivity (EC) and pH, nitrate and ammoniac nitrogen (NO_3-N, NH_4-N) and phosphorus (P) concentrations were measured. Macronutrient concentrations (Ca, Mg, Na, K) were analyzed once per crop cycle by an external lab (AUREA laboratory).Chemical properties analyzed for three types of water are given in Table 1. Electrical conductivity is higher for RW and TW compared to DW. This is probably related to the higher concentration of Ca, Mg, K, Na, Cl, and P in RW and TW.

Microbiological analyses using culturing techniques were performed by an external accredited laboratory (Eurofins). The concentration of *E. coli*, fecal streptococci, RNA-bacteriophages and spores of sulfite-reducing anaerobes were determined following NF EN ISO 9308-3, NF EN ISO 7899-1, NF EN ISO 10705-1 and NF EN 26461-1 norms respectively.

Table 1: Chemical characteristics of drinking water (DW), treated wastewater (TW) and raw wastewater (RW).

Parameters	Units	DW	TW	RW
EC at 20°C	mS cm^{-1}	0.56 ± 0.16	1.28 ± 0.15	1.39 ± 0.10
$N-NO_3$	mg L^{-1}	0.4 ± 0.49	0.59 ± 0.17	0.98 ± 0.12
P	mg L^{-1}	0.57 ± 0.09	6.97 ± 1.49	7.80 ± 3.08
Cl	mg L^{-1}	41.68	120.1	168.84
$N-NH_4$	mg L^{-1}	0.39 ± 0.16	28.40 ± 0.17	33.4 ± 7.5
K	mg L^{-1}	1.03	20.21	24.79
Mg	mg L^{-1}	7.18	13.15	14.51
Ca	mg L^{-1}	51.74	93.26	82.49
Na	mg L^{-1}	18.95	83.68	125.34
SAR	meq L^{-1}	2.55	8.37	13.23

SAR: Sodium adsorption ratio

Soil analysis and crop yield

Various chemical parameters of soil (between 0-30cm depth) were evaluated by external lab (AUREA laboratory), before and after each growing cycles: pH, Organic matter (OM), EC, Ca, K, Mg, Na, P, NO_3-N, NH_4-N, total nitrogen (N), Cl, carbonate (CO_3), bicarbonate (HCO_3). Taking into account the water and initial soil analyses, results show the NPK concentration was not enough for plant demands. An inorganic fertilizer (to avoid bacterial contamination) of ammo nitrate 33.5% and phosphorus potassium 25% was placed in each bin before planting (Müller-Schärer (1996)) leeks and lettuces. The two crop yields were compared by measuring the fresh and dry weight of lettuces and leeks. Diameter of lettuces was measured weekly.

Microbiological analyses on soil samples

Samples of 50 g of topsoil (first 10 cm) were sent to an external accredited laboratory (eurofins) for analyses of fecal indicators by culturing techniques, according to the NF EN ISO 7899-1 norm for fecal streptococci, NPP method for E. coli and NF EN ISO 10705-1 for RNA-bacteriophages. For molecular analyses, samples of the topsoil of each bin were collected and immediately processed: a subsample of 0.3-0.4g was taken and frozen in dry ice before being stored at -20°C in the laboratory. Genomic

DNA of soil samples was extracted using the FastDNA SPIN kit for soil from MP Biomedicals. The number of *E. coli* was quantified by qPCR using a TaqMan system targeting the *uid A* gene that codes for β-glucuronidase (Frahm and Obst, 2003). Illumina sequencing for analysis of the bacterial community in soil was performed as reported in Lequette et al. 2019.

Results and Discussion

Irrigation water quality impacts on soil

An increase in EC and cumulative concentrations of Na and Cl was observed in the soil after irrigation (Tab. 2) for both lettuces and leeks, with TW and especially RW.

Table 2: Chemical characteristics of soil before planting and after the end of irrigation cycles by (DW), (TW) and (RW).

			Lettuces (After 2 Cycles)			Leeks (After 1 Cycle)		
Parameters	Units	Before planting	DW	TW	RW	DW	TW	RW
EC	mS/cm	0.13 ± 0,01	0.21	0.37	0.4	0.2	0.26	0.41
NO_3-N	mg/kg	7.20 ± 1,90	29.2	64.9	96.6	2.2	45	44.1
NH_4-N	mg/kg	0.43 ± 0,23	2.9	4.7	4.1	3.7	1.6	4.7
Na	mg/Kg	13.23 ± 1,93	40	172.8	150.5	46.5	232.3	249
Cl	mg/kg	44.67 ± 2,31	135	283	320	151	253	313

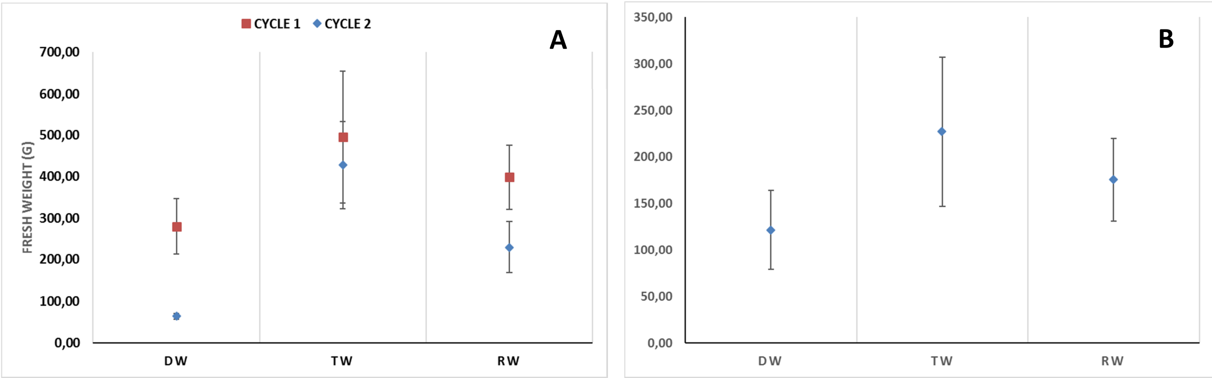
EC: electrical conductivity;

Soil nitrogen concentration is higher for TW and RW compared to DW in both crops but with higher values for the lettuce, probably due to lower nitrate requirements. Moreover, the increased in NO_3-N concentration in the soil is more pronounced than in NH_4-N, which is likely caused by the nitrification process. This accumulation can be explained by the soil proprieties; loamy clay soil used here has low hydraulic conductivity, which promotes element accumulation in the top (0-30 cm) soil layer (Musazura et al. 2019).

Wastewater irrigation effect on crops

Concerning crop yields after two lettuce cycles, Fig.1 shows the fresh weight of the lettuces and leeks after harvest. DW irrigation has less of an effect on fresh weight compared to TW and RW treatments. Leeks irrigated with DW have a smaller fresh mass compared to TW and RW. This can be explained by higher ammonium nitrogen (NH_4-N) concentration in wastewaters. During the RW irrigation we also observed damage on those lettuce leaves in contact with the soil. This damage was probably caused by the high Na and Cl concentrations in raw wastewater.

Figure 1: Comparison of the fresh weight after lettuce (A) and leek (B) harvest.



Monitoring of fecal indicators in water, soil and plants

Fecal indicator bacteria and bacteriophages were monitored only in bins irrigated with RW. In July, RW contained in average 10^6 *E. coli*/100 mL and 10^5 fecal streptococci/100 mL (MPN).

Table 3: Concentration of E. coli and fecal streptococci sp. in soil (1h and 4h after an irrigation event by RW)

		E. coli (NPP/g)	*Fecal streptococci (NPP/g)*
bin1, replicate 1	1h	$5,8x10^2$	$1,9\ x10^2$
	4h	$2,6\ x10^2$	$2,5\ x10^2$
bin1, replicate 2	1h	$1,2\ x10^3$	$1,8\ x10^2$
	4h	$2,6\ x10^3$	$5,0\ x10^2$
bin2, replicate 1	1h	$5,8\ x10^1$	$5,8\ x10^1$
	4h	detected, $<5,6x10^1$	detected, $<5,6x10^1$
bin2, replicate 2	1h	$1,8\ x10^2$	$5,6\ x10^1$
	4h	$1,8\ x10^2$	$1,2\ x10^2$

Analyses of soils samples collected 1h and 4h after irrigation showed that concentration of *E. coli* and *Enterococcus* sp. in soil remain stable, indicating that an extended time period (>4h) is necessary to inactivate the fecal bacteria (Table 4). Vergine et al. (2015) also showed that in the first two days after a fecal contamination event (under dry conditions) the reduction observed in the topsoil was less than half an order of magnitude. *E. coli* was found to contaminate roots of lettuces (at concentration of 10-30 CFU/g fresh material) and roots of leeks (10^2-10^3 CFU/g) but fecal streptococci were below quantification limits in the same samples. Fecal streptococci were detected in one sample of lettuce leaves at low concentration, which may be linked to internalization, although this hypothesis needs to be further investigated.

Conclusion

The rate of die-off of fecal bacteria was slow in topsoils irrigated with RW under experimental conditions. Although preliminary, contamination by fecal indicators of plants irrigated by RW was observed. Irrigation with TW and RW induced an increase of nitrogen in the soil. However, this concentration was not enough to meet the nutritive demands of plants, so fertilizer must be added. Lettuces and leeks irrigated with RW had a better yield in comparison with DW and those irrigated with TW even more so.

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