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**URBAN WASTEWATER REUSE IN AGRICULTURE:
RECENT PERSPECTIVES FROM CASE STUDIES IN
SICILY**

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FOREWORD

Irrigated agriculture implies the largest consumptive water use and water withdrawal (Frenken and Gillet, 2012).

Water for irrigation and food productions constitutes one of the greatest pressures on freshwater resources. Agriculture accounts for about 70% of global freshwater withdrawals (up to 90% in some fast-growing economies). Irrigation is only a modest part of agricultural water consumption but it accounts for more than 40% of the world's production on less than 20% of the cultivated land.

Withdrawals for agriculture tend to decrease with increasing levels of development. In many countries, water availability for agriculture is already limited and uncertain, and is set to worsen. Agricultural water withdrawal accounts for 44% of total water withdrawal in OECD (Convention on the Organisation for Economic Co-operation and Development). In the BRIC countries (Brazil, Russian Federation, India and China), agriculture accounts for 74% of water withdrawals (this ranges from 20% in the Russian Federation to 87% in India). In the least developed countries (LDCs), more than 90% (FAO, 2011b) of the available water is used for irrigation. Globally, irrigated crop yields are ~2.7 times those

of rainfed farming, hence irrigation will continue to play an important role in food production. The area equipped for irrigation increased from 170 million ha in 1970 to 304 million ha in 2008.

The world population is expected to grow from 6.9 billion in 2010 to 8.3 billion in 2030 and to 9.1 billion in 2050 (UNDESA, 2009a). Although projections vary considerably based on different scenario assumptions and methodologies, FAO estimates an 11% increase in irrigation water consumption from 2008 to 2050. This will imply a ~5% increase of the actual water withdrawal for irrigation, which corresponds to about 2,740 km³. Although this seems a modest increase, much of it will occur in regions already suffering from water scarcity (FAO, 2011a).

Economic growth and individual wealth are shifting diets from predominantly starch-based to meat and dairy, which require more water. Producing 1 kg of rice, for example, requires ~3,500 L of water, 1 kg of beef ~15,000 L, and a cup of coffee ~140 L (Hoekstra and Chapagain, 2008). This dietary shift is the greatest to impact on water consumption over the past 30 years, and is likely to continue well into the middle of the twenty-first century (FAO, 2006).

As of 2010 the world's aggregated groundwater abstraction is estimated at $\sim 1,000 \text{ km}^3$ per year, $\sim 67\%$ of which is used for irrigation, 22% for domestic purposes and 11% for industrial purposes (EUROSTAT, 2011; FAO 2011b, IGRAC, 2010; Margat, 2008; Siebert et al., 2010). The rate has at least tripled over the past 50 years and continues to increase by $1\text{--}2\%$ per year. The estimates suggest that the abstraction of groundwater accounts for $\sim 26\%$ of total global water withdrawal and equals $\sim 8\%$ of mean global groundwater recharge (WWAP, 2009).

North Americans, the highest per capita water users in the world, consume 2.5 times what Europeans use. One reason for this is that water is relatively inexpensive compared to other industrialized countries (CEC, 2008). European and North American populations consume a considerable amount of virtual water embedded in imported food and products. Each person in North America and Europe (excluding former Soviet Union countries) consumes at least 3 m^3 per day of virtual water in imported food, compared to 1.4 m^3 per day in Asia and 1.1 m^3 per day in Africa (Zimmer and Renault, n.d.).

Irrigation with treated wastewater (TWW) is a common practice in many parts of the world, although its diffusion is

still a debatable point (Scott et al., 2004). A realistic estimation confirms that 20 million ha in the world are irrigated by raw, treated and/or partially diluted wastewater (Hamilton et al., 2006). TWWs are produced in large volumes, which if not re-used would be discharged into the environment and do not contribute to increase water availability for the agricultural context. Furthermore, it is well known that discharge of raw effluents into the environment, particularly natural water bodies such as lakes, rivers and the coastal marine environments, may cause severe degradation of these water bodies. The degradation is often related to the presence of organic and inorganic nutrients, which cause problems such as eutrophication and algal blooms. Reusing these discharged effluents can significantly reduce or completely remove the impact of these effluents on receiving bodies. In addition, the TWW reuse for agricultural irrigation reduces the amount of water that needs to be extracted from environmental water resources. TWW can often contain significant concentrations of organic and inorganic nutrients for example nitrogen and phosphate that may be used as a fertilizer source when the water is recycled for irrigation. Moreover, soil microorganisms have been

observed to increase their metabolic activity when sewage effluent is used for irrigation (Toze, 2006).

One of the most economically feasible agricultural uses of reclaimed TWW is the irrigation of high-value horticultural crops, which typically has high returns per volume of water invested in (Hamilton et al., 2006). But this practice has been approached with trepidation, owing primarily to concerns about risks to human health via contamination of food with pathogenic microorganisms (Lazarova, 2005, Hamilton et al., 2005, Toze, 2006). There have been a number of risk factors identified for using TWWs for agricultural irrigation purposes. Some risk factors are short term and vary in severity depending on the potential for human, animal or environmental contact (e.g. microbial pathogens), while others have longer term impacts which increase with continued use of recycled water (e.g. saline effects on soil). A pragmatic approach to allow and justify such concerns, which has been gaining favour in recent years, is the application of WHO 2006 guidelines (updated in 2008) based on a risk assessment and management procedure. In particular, the approach for microbial risks is (i) to define a tolerable maximum additional burden of disease, from which is possible (ii) to derive tolerable risks of disease and infection,

(iii) to set health-based targets for pathogens reductions, (iv) to determine how the required pathogen reductions can be achieved, and (v) to set up a system for verification monitoring.

Finally, the main purposes of this study were to assess the health risk associated with the use of wastewater in irrigation and to monitor the impact on different crops of drip and sub-drip irrigation with treated municipal WW. Faecal Coliform (FC), *Escherichia Coli* (E. Coli), *Enterococcus Faecalis* (EF) were selected as indicators of faecal contamination, whereas *Salmonella* was selected as pathogenic bacteria. In addition, effects of TWW reuse on irrigation system, main production features, hydrological soil behaviour and microbial soil contamination were investigated.

OBJECTIVES OF THE THESIS

On the basis of the previously described context of water use for irrigation in agriculture, and due to the necessity of finding new and alternative water resources to guarantee the economy of the primary sector, my PhD thesis had the following main objectives:

- analyse the European legislation, and World Health Organization (WHO) guidelines regarding the real possibility of reusing wastewater in the agriculture context;
- evaluate the role of a natural tertiary treatment system (i.e. constructed wetland and lagoons) for supplying good quality water for irrigation purposes;
- analyse the effects of tertiary treatment wastewater reuse on vegetable crops production and quality characteristics;
- identify any potential effects of tertiary wastewater reuse on soil contamination and soil hydraulic properties modification;
- analyse the performance of micro-irrigation systems (i.e. drip and sub-drip irrigation) supplying tertiary treatment wastewater for irrigation;
- identify the presence of microbial biofilm growth within the irrigation pipelines supplying wastewater for irrigation;
- test bacteriostatic pipelines for preventing the biofilm growth in the irrigation pipelines.

To find reliable argumentations to the above cited research objectives, experimental campaigns were realized by the

Dipartimento di Agricoltura, Alimentazione e Ambiente of the University of Catania, within the area of S. Michele di Ganzaria (in province of Catania, Eastern Sicily). The trials were carried out in an experimental field close to the Constructed Wetland (CW) systems of the municipality (i.e. the CW system serves as tertiary wastewater treatment system), during the period 2013-2015.

1. ON THE USE OF URBAN TREATED WASTEWATER IN AGRICULTURE

1.1 EUROPEAN LEGISLATION AND WHO GUIDELINES

The Italian legislation (n. 152/2006) states that natural freshwater sources can be used as a priority for the municipal water supply, and that the recycling and reuse of water are viable alternatives for meeting industrial and agricultural needs. Putting these strategies into practice requires that concentration limits, best treatment practices and irrigation technology options are defined to support treated wastewater (TWW) reuse in agriculture. These specifications are still under discussion; currently, there are strict regulations for TWW reuse (Ministry Decree, D.M. n. 185/03), especially for levels of some chemical compounds and for microbial parameters. In many cases, the quality standards for reclaimed wastewater are the same as for drinking water (Cirelli et al., 2008).

These regulations have made it difficult to promote TWW reuse, especially when advanced treatments are needed to ensure compliance with very strict standards, making water

recycling uneconomical. Another obstacle is the excessive number of parameters (54) taken into account by the Italian legislation and their related monitoring protocols, which also have to comply with regional regulations. In addition, the legislation makes no distinction among different alternatives of TWW reuse, namely, urban non-potable, industrial and agricultural reuse. For agriculture in particular, the regulations do not distinguish between crops using restricted irrigation and those using unrestricted irrigation. Additionally, the regulations do not consider the impact of different irrigation options (i.e., subsurface drip irrigation versus spray irrigation) on reducing sanitation-related risks. In summary, current legislation (Table 1) is a major constraint to the development of municipal TWW reuse for irrigation in Italy.

Table 1 Chemical-physical and microbiological parameter reuse limits according to MD 185/2003.

PARAMETERS	STANDARDS	PARAMETERS	STANDARDS
pH	6.0 ÷ 9,5	Sulphites [mg SO ₃ /L]	0.5
Sodium Adsorption Rate	10.0	Sulphates [mg SO ₄ /L]	500
Coarse solids	absent	Chlorine residual [mg/L]	0.2
TSS [mg/L]	10.0	Chlorides [mg Cl/L]	250
BOD ₅ [mg/L]	20.0	Fluorides [mg F/L]	1.5
COD [mg/L]	100.0	Animal/vegetal oils & fats [mg/L]	10.0
Phosphorus [mg P/L] (total)	2.0	Mineral oils [mg/L]	0.05
Total Nitrogen [mg N/L]	15.0	Phenols [mg/L] (total)	0.1
Ammonia [mg NH ₄ /L]	2.0	Pentachlorophenol [mg/L]	0.003
EC _w [dS/m]	3.0	Aldehydes [mg/L] (total)	0.5
Aluminium [mg Al/L]	1.0	Tetra/tricloro-ethylene [mg/L]	0.01
Arsenic [mg As/L]	0.02	Chlorinated solvents [mg/L] (total)	0.04
Barium [mg Ba/L]	10.0	TTHM [mg/L]	0.03
Boron [mg B/L]	1.0	Aromatic solvents [mg/L] (total)	0.001
Cadmium [mg Cd/L]	0.005	Benzene [mg/L]	0.01
Cobalt [mg Co/L]	0.05	Benzo(a)pyrene [mg/L]	0.00001
Chromium [mg Cr/L] (total)	0.1	Org. nitr. solvents [mg/L] (tot.)	0.01
Chromium VI [mg Cr _{Vl} /L]	0.005	Surfactants [mg/L] (total)	0.5
Iron [mg Fe/L]	2.0	Chlorinated biocides [mg/L]	0.0001
Manganese [mg Mn/L]	0.2	Phosphorated pesticides [mg/L]	0.00001 [^]
Mercury [mg Hg/L]	0.001	Other pesticides [mg/L] (total)	0.05
Nickel [mg Ni/L]	0.2	Vanadium [mg V/L]	0.1
Lead [mg Pb/L]	0.1	Zinc [mg Zn/L]	0.5
Copper [mg Cu/L]	1.0	Cyanides [mg CN/L] (total)	0.05
Selenium [mg Se/L]	0.01	Sulphides [mg H ₂ S/L]	0.5
Tin [mg Sn/L]	3.0	<i>E. Coli</i> [UFC/100 mL]	10*
		(80% of samples)	
		CWs & Stabilisation ponds	50**
Thallium [mg Tl/L]	0.001	<i>Salmonellae</i> [UFC/100 mL]	absent

[^] for any single item;

* 100 CFU/100 mL will be allowed as a maximum for a single isolated sample and for the first three years of application of the new Act;

** 200 CFU/100 mL will be allowed as a maximum for a single isolated sample.

E.coli: *Escherichia coli*; * limit value for 80% of samples; a maximum of 200 CFU/100 mL is allowed in 20% of samples;
n.d.: not detected; ** 100% of samples.

The World Health Organization (WHO) considers a different approach for evaluating any potential pathogenic risk associated with TWW reuse in agriculture. The approach is that of the: *the Disability-adjusted Life Years (DALYs)* (WHO, 2006). The DALYs index calculates the time lost because of disability from a disease compared with a long life free of disability in the absence of disease. Three index pathogens are selected from WHO: Rotavirus (a virus), *Campylobacter* (a bacterium) and *Cryptosporidium* (a protozoan). Results of the QMRA (Quantitative Microbial Risk Analysis, WHO 2006) are used to determine the total pathogen reductions targets to be achieved by a combination of wastewater treatment and a selection of post-treatment health protection control measures; these are based on a tolerable maximum additional burden of disease of one-million of a DALY loss per person per year (1×10^{-6} DALY loss pppy).

In the adopted approach, the model exposure scenario of “unrestricted irrigation”, based on the consumption of TWW-irrigated lettuce (Shuval et al., 1997), was considered within the “planned TWW use” definition. The selected scenario corresponds with the controlled use of TWW to grow crops that are normally eaten raw (WHO, 2006).

The QMRA procedure is illustrated in Table 2 for the consumption of TWW-irrigated lettuce.

As it can be seen, the risk of Rotavirus infection is higher than those of *Campylobacter* and *Cryptosporidium* and thus Rotavirus infection risk must be used to assess the safety of TWW irrigation practice. In particular, the calculation shows that, for the parameter value selected, the required rotavirus reduction from TWW to lettuce ingestion is 6 log units. This total reduction is achieved partially by wastewater treatment and partially by a selection of post-treatment health-protection control measures (i.e. low-cost drip irrigation techniques, pathogen die-off, produce washing and peeling, etc.).

Table 2. Unrestricted irrigation: median infection risks from the consumption of TWW-irrigated lettuce estimated by QMRA-Monte Carlo simulation (WHO, 2006)

TWW quality (<i>E.Coli</i> /100 ml)	Median infection risk pppy		
	Rotavirus	<i>Campylobacter</i>	<i>Cryptosporidium</i>
10^7 - 10^8	1	1	0.91
...			
10^3 - 10^4	0.3	1.1×10^{-2}	2.4×10^{-4}
100-1000	3.4×10^{-2}	1.1×10^{-3}	2.3×10^{-5}
10-100	3.5×10^{-3}	1.1×10^{-4}	2.3×10^{-6}
1-10	3.5×10^{-4}	1.1×10^{-5}	2.3×10^{-7}

In the study, the risk associated with the use of TWW to grow tomato crops, evaluated by the described DALY tool, was compared with the very restrictive approach adopted by the Italian legislation for TWW reuse (Ministry Decree, n. 185/03).

1.2 RECENT RESEARCHES

Conventional wastewater treatment consists of a combination of physical, chemical, and biological processes and operations to remove solids, organic matter and, sometimes, nutrients from wastewater. General terms used to describe different degrees of treatment, in order of increasing treatment level, are preliminary, primary, secondary, and tertiary and/or advanced wastewater treatment.

Natural treatment methods are mainly used for wastewater treatment from decentralized houses, small settlements, dwelling, hotels, recreational facilities, restaurants and summer camps, smaller municipalities or their parts, usually up to 2000 p.e. (population equivalent). According to the composition of wastewater, these methods are also applicable for treatment of industrial wastewater from the food processing industry, trade facilities (workshops) and selected small industrial plants, landfill leachate treatment, organically low-loaded agricultural runoff and wastewater agricultural facilities, polluted storm water runoff, erosion washes of polluted surface water. Natural technologies of wastewater treatment are especially represented by soil filters (SF), constructed treatment wetlands (CTW) and waste

stabilization ponds (WSP) that have been used in the last thirty years. Relatively considerable effort is devoted to the possibility of using aquatic plants systems in different arrangements.

The reuse of wastewater in agriculture is gaining wider acceptance in many parts of the world. It represents an agronomic option adopted, even more frequently, in regions with water scarcity, growing urban populations, and rising demand for irrigation water (Meli et al., 2002; FAO, 2011).

In this section, results of recent researches were briefly reported in order to give value to TWW reuse practice. In particular, even more researches are focusing on the evaluation of the effects of TWW reuse practice on row crops, mainly because these crops, due to their edible part, may be greatly affected by contamination. The research group of the Di3A of the University of Catania has conducted in these recent years several experiments on TWW reuse in agriculture, evaluating the effects on soil, irrigation system performance and on row crops productivity and quality characteristics (see Aiello et al., 2007; 2013; Barbagallo et al., 2012; Cirelli et al., 2012; Castorina et al., 2015.) These studies have confirmed the suitability of TWW reuse practice in agriculture; low effects on crops main features were

evidenced from these studies. In particular, following the WHO standards the cited researches provided fundamental results for standardized the reuse practice procedures.

Other researchers were in the same direction of those carried out by the University of Catania. For example, Gatta et al., (2014) have revealed interesting aspects of the TWW reuse: (i) yields of the irrigated tomato fruits were not significantly different from those obtained with fresh water; (ii) for all the TWW irrigation treatments, the most important morpho-qualitative parameters of the processing tomato fruit (i.e., dry matter content, pH, soluble solid content, colour parameters) were in agreement with those reported in the literature; and (iii) tomatoes microbial quality was very good for all the thesis.

As confirmed by studies (Lopez et al., 2006), Compared to olive trees grown in rainfed conditions, irrigation with the “simply” treated effluents caused a yield increase of 50%, improving fruit characteristics very important for marketing table-olives such as weight and flesh to pit ratio.

Other studies carried out by Lubello et al. (2004) confirm that treated wastewater can be reused on nurseries irrigation. In fact, The refinery treatment by filtration and disinfection with PAA (Peracetic Acid) and UV together were very effective in

bacteria removal. The value of 2 Total Coliforms MPN 100 ml⁻¹ set by law for unrestricted irrigation was constantly satisfied. The tertiary effluent can be considered as an important source of fertilizer for some container grown plants (e.g., Juniper, Myrtle and Cypress), with positive economic and environmental aspects related to reduction of synthetic fertilizers use (Lubello et al., 2004).

A recent research conducted by A. Christou (2014) has evaluated the effects of tomato crop irrigation with treated WW effluent, as compared to groundwater irrigation, on soil geochemical properties, fruit safety and crop productivity. Irrigation using either effluent did not significantly affect soil pH, organic C or heavy metal content. Also, no impacts of WW irrigation on crop productivity, as compared to control irrigation, were registered. Furthermore, the heavy metal content of tomato fruits and leaves in all irrigation treatments was below the MPLs set for fruit safety and the critical tissue concentration for phyto-toxicity. Moreover, no microbiological contamination (TC, FC, *E. coli*, *Salmonella* spp., *Listeria* spp.) of tomato fruits was found, in any treatment.

1.3 WASTEWATER REUSE AS SUSTAINABLE PRACTICE IN AGRICULTURE

The use of treated wastewater (TWW) for crop irrigation is an alternative to the scarcity of quality water suffered in many countries of the Mediterranean basin. TWW can provide an important saving of fertilizers (i.e. nitrogen and phosphorus) as well as benefits for the environment, by avoiding the discharge of contaminated water into public waterways (Martínez et al., 2013) and providing consistent available water throughout the year. It is estimated, that the reuse of wastewater could reach 15% of the world's water consumption (Asano, 1998). Possible uses of reclaimed wastewater include irrigation of food or non-food crops, irrigation of green or leisure zones (with or without direct contact), aquaculture, industry (water for refrigeration, cleaning), municipal use and aquifer recharge, among others (Cirelli et al., 2009). The use of reclaimed wastewater for crop irrigation has been a common practice for some years now. This agricultural use has been tested in crops such as forage (Bole and Bell, 1978), alfalfa and radish (Rosas et al., 1984), wheat and maize (Al-Jaloud et al., 1993), trees (Tznakis et al., 2003) and vegetable crops (Rosas et al., 1984;

Keraita et al., 2007; Aiello et al., 2007; Cirelli et al., 2012). Moreover, different practices involving wastewater reuse, in terms of irrigation techniques (surface and subsurface drip irrigation) (Cirelli et al., 2012), cultivation systems (Pedrero and Alarc'on, 2009) and treatments technologies, (Pedrero et al., 2010, Barbagallo et al., 2003; Consoli et al., 2011; Barbagallo et al., 2012) have been tested.

The use of wastewater for crop irrigation is perhaps one of the main sources of pathogenic microorganism contamination (Assadian et al., 2005; Aiello et al., 2013). The risk associated with this type of water depends on the presence of pathogenic microorganisms and chemical substances, as well as on environmental conditions, safety measures, treatment types, irrigation methods and type of grown crops. Among the variety of existing crops, vegetables are the most vulnerable to contamination (Armon and Shelef, 1991), especially when they are consumed fresh, thus demanding high-quality water for irrigation.

1.3.1 Effects on soil characteristics

In recent years, a number of Mediterranean countries have experienced severe water supply and demand imbalances, with more frequent and longer lasting periods of drought. In particular, several regions in Italy have suffered successive droughts over the last 10 years (Coppola et al., 2004). Due to water scarcity, agricultural activities (using more than 50% of the total water resource extracted) are penalized, while higher priority demands (domestic and industrial) are satisfied.

Benefits apart, treated wastewater can be used for irrigation under controlled conditions to minimise hazards from pathogenic and toxic contaminants of agricultural products, soils, and ground water (Al-Nakshabandi et al., 1997). Furthermore, hydrological soil properties are specially sensitive to wastewater compounds. Indeed, numerous studies (Pescod, 1992; Bresler, 1981; Tarchitzky et al., 1984; Vinten et al., 1991; Aiello et al., 2007; Cirelli et al., 2012) have highlighted hydraulic conductivity reduction in wastewater irrigated soil, ascribing it to a partial biological clogging of soil pores due to increased biomass and suspended solids. Recycled wastewater applications generally result in a disturbed layer at a soil depth of 0.3 m, exhibiting

changes in physical properties (increase in bulk density). The investigated layer shows reduced soil porosity, translation of pore size distribution towards narrower pores and a consequent decrease in permeability. Near the surface level, soil samples (collected around the emitter) show a certain growth in microbial content (*E. coli* and FS) (Aiello et al., 2007).

Impact from wastewater on agricultural soil, is mainly due to the presence of high nutrient contents (Nitrogen and Phosphorus), high total dissolved solids and other constituents such as heavy metals, which are added to the soil over time. Wastewater can also contain salts that may accumulate in the root zone with possible harmful impacts on soil health and crop yields. The leaching of these salts below the root zone may cause soil and groundwater pollution (Bond 1999).

1.3.2 Effects on crop production and quality

The main health risks are associated with contamination of crops or groundwater by wastewater due to its chemical composition being somewhat different from most natural waters used in irrigation (Pereira et al., 2002). Urban

wastewater generally contains high concentrations of suspended and dissolved solids (chloride, sodium, boron and heavy metals) and little of any added salt is removed during conventional (secondary and tertiary) treatments. Pathogenic organisms (Helminths, Enteric bacteria, Enteric viruses) constitute one of the main health concerns in the use of wastewater for irrigation.

The safety of reclaimed water (e.g., municipal effluent from the Wastewater Treatment Plant (WWTP) is source of concern; toxic chemicals are one of dominant hazardous components in wastewater (Asano et al., 2007 and Xu et al., 2014). These residual toxic chemicals in WWTP effluents would accumulate and cause long term potential risks on human health (Asano et al., 2007). Considering the non-biodegradability and the persistent nature of heavy metals, the control of their levels in reclaimed water intended for irrigation is of vital concern. Indeed, such trace-elements may enter the human body via consumption of food crops and can cause various toxic effects (Khan et al., 2013, Li et al., 2012 and Liu et al., 2011).

Study carried out by Aiello et al. (2007) revealed in microbial crop analysis a slight accumulation of *E. coli* on tomato fruits irrigated with wastewater, with a concentration of about 40

MPN/100 for 80% of the samples. Results also showed that tomato yield increased when irrigated with wastewater. Notwithstanding the combination of tertiary treated wastewater and irrigation technologies (DI and SDI), a not negligible level of contamination on tomato fruit and irrigation soil surface was determined; these results confirm that CW effluents cannot be used for irrigation without additional disinfection. (Aiello et al.,2007).

Wastewater is a rich source of plant food nutrients. Empirical studies, presented in an earlier section of this paper, show that the impact of wastewater irrigation on yield, varies from crop to crop. If the crops are under-supplied with essential plant food nutrients, wastewater irrigation will act as a supplemental source of fertilizer thus increasing crop yields. Alternatively, if plant food nutrients delivered through wastewater irrigation result in over supply of nutrients, yields may negatively be affected.

1.3.3 Effects on irrigation systems and biofilm growth

Modern irrigation (surface drip irrigation, and subsurface drip irrigation) has the possibility of adequately applying water

and chemicals both in respect of amount and in location throughout a field soil.

Selecting the most appropriate irrigation technique can reduce the environmental and health risks. Drip irrigation is the safest irrigation technique for wastewater reuse (Capra and Scicolone 2007) applying the reclaimed water onto the soil surface close to the plants. Thus, compared to other irrigation techniques, drip irrigation has the potential to optimize water application and minimize health and crop contamination risks. Nevertheless, clogging is a common problem when using drip irrigation (Ravina et al. 1997). It is generally observed on different components of irrigation systems (filters, pipes and emitters) and consequently can have significant economic impacts for farms. Indeed, irrigation systems become dysfunctional as the uniformity of distribution progressively decreases. This leads to serious yield losses, may generate local leaching and induce additional costs to either clean or replace the damaged irrigation devices. Clogging causes are categorized into physical, biological and chemical (Dosoretz et al. 2011). Physical deposits when using reclaimed water are mainly composed of organic matter, clay and silt-sized aggregates. Chemical precipitation may occur depending on water quality

parameters such as temperature, pH and the concentration of salt. These parameters may induce the precipitation and sedimentation of mineral elements to generate clogging. Treated effluents contain microorganisms and some nutrients that tend to develop a self-protection biofilm. Independently of other causes, the intensity of microbial slime development is usually related to water flow properties.

Biomass growth and disruption are conditioned by flow velocity (Beyenal and Lewandowski 2002; Stoodley et al. 1999; Mahfoud et al. 2008). Under high velocities, biofilm thickness is reduced due to high shear forces at its interface with flow. In addition, cohesion and density increases near to the attachment areas of biofilms as resistance to shear stress (Delron et al. 2007).

Biological clogging is often coupled to physical and chemical deposits (Gilbert et al. 1981; Adin and Sacks 1991) when suspended materials adhere to the biofilm (Figure 1).

Numerous studies have been carried out looking at the effect of wastewater quality on clogging of irrigation system components when using wastewater effluents (Bucks et al. 1979; Capra and Scicolone 2004, 2005; Dehghanisanij et al. 2004; Puig-Bargue`s et al. 2005; Liu and Huang 2009;

Duran-Ros et al. 2009; Bo et al.,2013), without analyzing the physical phenomena involved.

The complex chemistry of wastewater supports the rapid growth of several species of algae and bacteria resulting in slime-like gelatinous deposits of amorphous shape. Particles of definable shape were found in the matrix of the gelatinous substance (biofilms) which formed sediment in the emitters, and caused serious emitter blockage (Adin and Sacks, 1991) (Figure 1).

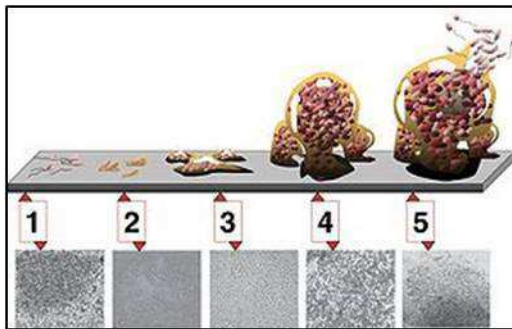


Figure 1. Stages of biofilm development: (1) Initial attachment, (2) Irreversible attachment, (3) Maturation I, (4) Maturation II, and (5) Dispersion.

Adapted from: <https://en.wikipedia.org/wiki/Biofilm>

More than 90% of the clogging composition included biological species and the clogging process was usually

initiated by bacterial biofilms (Gilbert *et al.*, 1981; Taylor *et al.*, 1995). Although the clogging materials are mainly suspended particles, some of the biofilm bacterial species may also cause emitter clogging through the precipitation of iron, manganese and sulfur minerals dissolved in reclaimed wastewater (James, 1988; Pitts *et al.*, 1990; Ahin *et al.*, 2005). Microbial cells that grow in association with surfaces often form biofilms, microorganisms immobilized and embedded within an organic polymer matrix (Liu *et al.*, 1994; Kristina *et al.*, 2007; Strathmann *et al.*, 2002). Microorganisms including various bacteria, fungi, protozoa, and metazoa with large quantities, multiple types, and fast growth rates in natural water play an important role in the ecosystem. In fact, over 90 % of microbes adhere to the substrate surface and exist in the form of biofilms (Dong *et al.* 2002; Kang *et al.* 2006; White *et al.* 1998). Biofilm is a 3D heterogeneous and functional aggregation containing a microbial community (bacteria, protozoa and fungi, etc.), solid particulates, extracellular polymers (EPS) secreted by microorganisms and other substances (Capra and Scicolone 2004, 2005; Liu and Huang 2009; Ravina *et al.* 1992, 1997). Although the water quality of reclaimed water reaches basic standards of irrigation, it still contains a certain amount of

pollutants. The nutrients, organic substances, and trace elements in the reclaimed water after secondary or even tertiary treatment can sustain the microorganisms' growth. Microorganisms and nutrients can pass through the filter and enter the drip irrigation system and then form biofilms following the “attachment-growth-detachment-decay” process (Li et al. 2010; Nakayama and Bucks 1991). The biological species in the biofilm excrete a protective mass of extracellular polysaccharides (EPS), a wide group of polymers that can be highly attached to the cell surface or released as extracellular slime in the surroundings of the cell (Knoshaug *et al.*, 2000).

2. CASE STUDIES IN SICILY

2.1 WASTEWATER AS ALTERNATIVE RESOURCE FOR IRRIGATION IN SICILY

The economical sustainability of the agricultural sector in Sicily (southern Italy) has to cope with the availability and management of water resources for irrigation.

Crop water requirements are, generally, unfulfilled for relevant percentages and the need to use alternative water sources, like urban TWW, is urgent. Moreover, reusing these discharged effluents can significantly reduce or completely remove the impact of these effluents from receiving environments. In addition, TWW reuse for agricultural irrigation reduces the amount of water that needs to be extracted from environmental water resources. TWW can often contain significant concentrations of nutrients, for example nitrogen and phosphate, which might be used as a fertilizer source when the water is recycled for irrigation.

In this section, a recent study conducted by Barbagallo et al., 2012 was taken as reference. In the study, the potential of TWW reuse in Sicily was analyzed and irrigated areas, eligible to receive TWW from wastewater treatment plants

were identified. In particular, among those analyzed, 24 out of 37 irrigation areas were eligible to receive TWW from 59 WWTPs (Figure 2). Overall, TWW potentially reusable was about $87 \times 106 \text{ m}^3 \text{ y}^{-1}$, while water deficit was $65 \times 106 \text{ m}^3 \text{ y}^{-1}$. Obviously, by considering each district, deficit or surplus would still exist. The irrigation districts with a resource surplus may extend their irrigated area or save high quality water for other uses or avoid aquifer overexploitation. TWW volumes coming from the excluded 247 WWTPs (about $117 \times 106 \text{ m}^3 \text{ y}^{-1}$) could be used to irrigate extra areas currently not irrigated, or served by private water supplies. This would represent a fundamental opportunity to improve the agricultural sector, to save fresh water, to preserve the environment and to develop the internal marginal areas of Sicily. Of course, this approach would imply a more accurate study to allocate in a correct way the nonconventional resources. TWW may be successfully used, under specific experimental conditions, to irrigate and grow crops that are normally eaten raw. In particular, the risk assessment analysis highlighted that, by applying post-treatment health-protection control measures (such as product washing, disinfection, peeling and/or the natural pathogen die-off after last irrigation), the acceptable rotavirus infection risk was

generally preserved, although *E. coli* content of TWW was often over the limits set by the Italian legislation (Barbagallo et al., 2012).

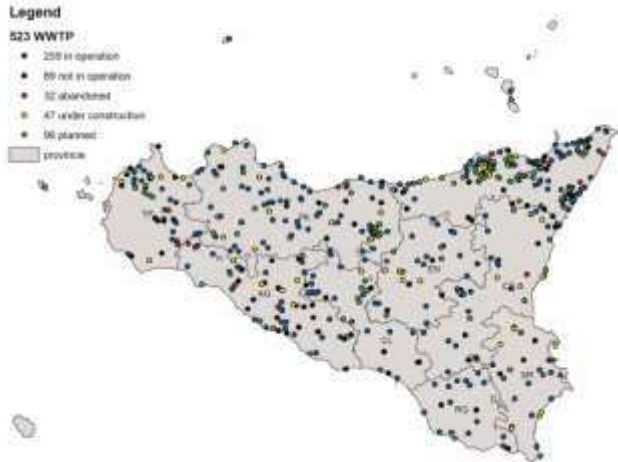


Figure 2. Potential TWW reuse in Sicily

2.2 CASE STUDY DESCRIPTION

The experimental activity of this PhD thesis was carried out in an open field near the constructed wetland (CW) system of S. Michele di Ganzaria ($37^{\circ}17'29.27''\text{N}$ $14^{\circ}25'16.76''\text{E}$) in the east part of Sicily. The study was conducted during 3 different irrigation seasons in 2013, 2014 and during the first months of 2015 on different vegetable crops. The constructed wetland unit treats secondary urban effluents from the

conventional wastewater treatment plant of the municipality (approximately 5,000 inhabitant) (Figure 3). The area has a Mediterranean-semiarid climate, with an average of 500 mm/year of rain.

The experimental plants is equipped with 4 constructed wetland (Horizontal Sub Surface Flux) reed beds (Figure 4) (i.e. surface area of about 2,000 m³) followed by three storage reservoirs (Figure 5), for the tertiary treatment of wastewater coming from the conventional treatment plant. The reed beds 1, 2 and 3 are vegetated with *Phragmites australis*, the bed 4 is vegetated with *Typha latifolia*. During the year 2013, TWW were collected from the outlet of HSSF-2, in 2014 from the outlet of HSSF-3. The flow rate of the systems is about 240 m³/day, and the hydraulic loading rate is about 0.11 m³/day. The filter bed depth is of 0.6 m, filled with gravel size of 5-15 mm, with porosity of 0.4.

The Hydraulic Retention Time (HRT) of TWW in each CW system is about 2 days.



Figure 3. Experimental area for TWW reuse in San Michele di Ganzaria

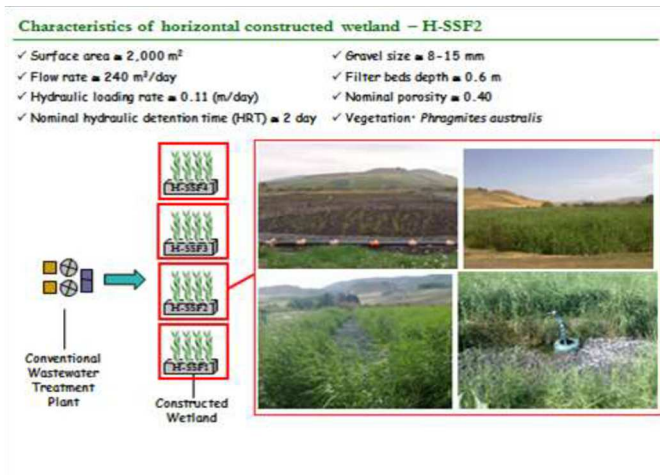


Figure 4. Horizontal CW systems

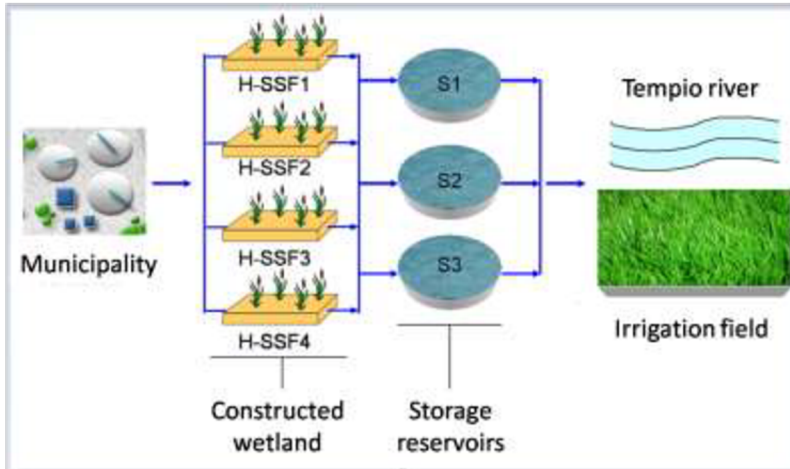


Figure 5: Scheme of constructed wetland – San Michele di Ganzaria (CT) - (Barbagallo et al., 2009)

2.3 METHODOLOGY

The study was carried out during 3 consecutive irrigation seasons in the years 2013, 2014 and during the first months of the year 2015.

During the years of trial, different vegetable crops were transplanted to the field, in order to verify their adaptability to irrigation with TWW. Lettuce crops (*Lactuca sativa* var. *Canasta*), zucchini (*Cucurbita pepo* var. *President*) and eggplant of two different variety (*Solanum melongena* var. *Dalia* and var. *Birga*) were analysed during the year 2013,

while industrial cultivar of tomato *Solanum lycopersicum* (var. *Missouri*) and eggplant of two different variety (*Solanum melongena* var. *Tasca* and var. *Gloria*) were tested in 2014.

During the last cycle, autumn-winter 2015, lettuce of two variety (*Lactuca sativa* var. *Canasta* and var. *Romana*) and cabbage of two variety (*Brassica oleracea gongylodes* var. red *Acitano* and var. white *Luna*) were analysed as test crops.

2.3.1 Set up of the experimental tertiary treatment plant

During 2013, tertiary treated effluents coming from the horizontal sub-surface flow constructed wetland system (HSSF- 2), after filtration, were reused to irrigate vegetable crops.

Before to be supplied to the experimental irrigation field, CW wastewater were stabilized within a reservoir (i.e. lagoon), that has a capacity of about 50 m³, hydraulic loading rate of 2.5 L/day and a nominal hydraulic retention time of 20 days. Wastewater passed in a storage tank (earth made), with a capacity of 100 m³ and then filtered by sand filter (model Irritec EGV 2” diam. 600 mm, max flow rate 28 m³/h, with

150 Kg of sand media inside, with automatic backwash) (Figure 6).

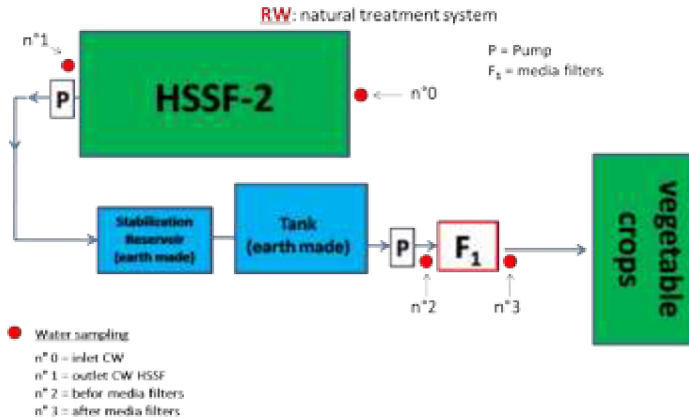


Figure 6: Lay-out out of the experimental treatment system adopted for the year 2013

The experimental field was also supplied by fresh water for comparison. Fresh water (FW) were collected by municipal pipe, stored within a P.E. tank and then disk filtered (model Irritec EUV rotofilter monotesta, 120 mesh and max flow rate 30 m³/h with automatic backwash) (Figure 7).

In the year 2013, the experimental field was equipped with two plots, supplying:

- Reclaimed wastewater (RW)
- Fresh water (FW).

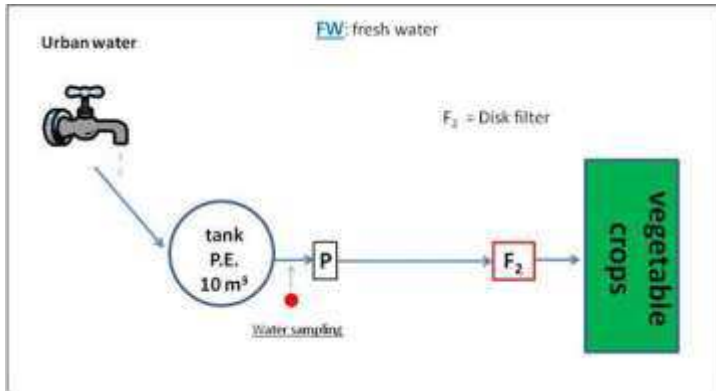


Figure 7: Lay-out out of the FW supplying system

Each plot was equipped with 4 blocks (Figure 8), consisting of 2 surface drip irrigation laterals (DI), and 2 sub-surface drip irrigation laterals (SDI), this latter buried at 0.05 m of depth. To avoid evaporation increasing due to bordering conditions (which can be significant, especially under windy conditions) the two outermost laterals (BL) of each plot were planted and irrigated, but not sampled during the experiment. The total length of the irrigated plots was 30 m. All the plots (supplied by RW and FW) were equipped with polyethylene laterals with 16 mm external diameter. All the laterals were

supplied by in-line labyrinth drippers (model i-siplast Tape) with a theoretical discharge rate of 0.8 L h^{-1} at a pressure of 55 kPa.

Pipelines thickness was 8 mil and emitters were spaced at 0.1 m; distance between rows was 1.2 m. Each plot was equipped by flow meters and manometers.

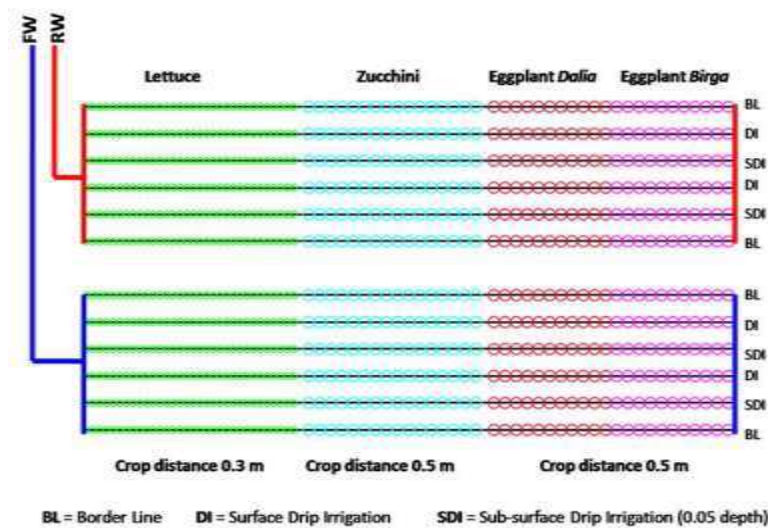


Figure 8: Experimental irrigation field for vegetable crops irrigation realized during summer 2013



Figure 9: Lettuce, zucchini and eggplants growth in 2013

The rows were covered by a black/white plastic mulching in order to reduce soil evaporation, water infiltration and control weeds. Buffer zone with laterals was 1.2 m; the middle corridor between plots was 2.0 m (Figure 9).

Lettuce (*Lactuca sativa* L., Cv *Canasta*) crops were transplanted at a density of 2.8 plants m^{-2} , zucchini (*Cucurbita pepo*. L. Cv *President*) and eggplant (*Solanum melongena* L.) of two cultivars *Dalia* – Da and *Birga* – Bi, at density of 1.7 plants m^{-2} ; four replicates of 11 eggplants, 32 lettuce and 16 zucchini were used for the all the determinations and comparisons.

During the year 2014, tertiary treated effluents coming from the horizontal sub-surface flow constructed wetland system

(HSSF- 3), after a filtration, were reused to irrigate vegetable crop. Differently from the previous year of trial, in 2014, HSSF-3 outlets were treated by two different lines, named ARU1 and ARU2 (see Figures 10 and 11).

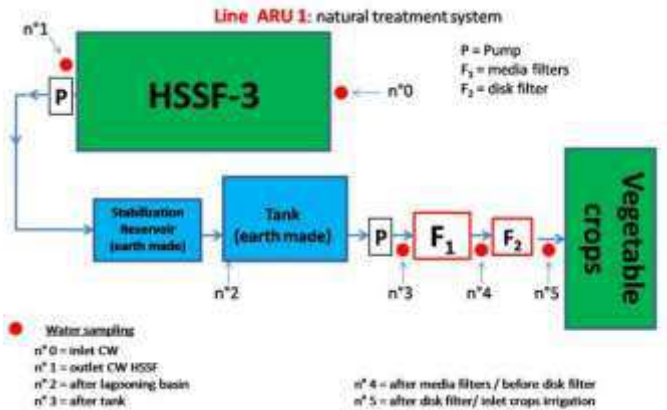


Figure 9: ARU1 treatment system

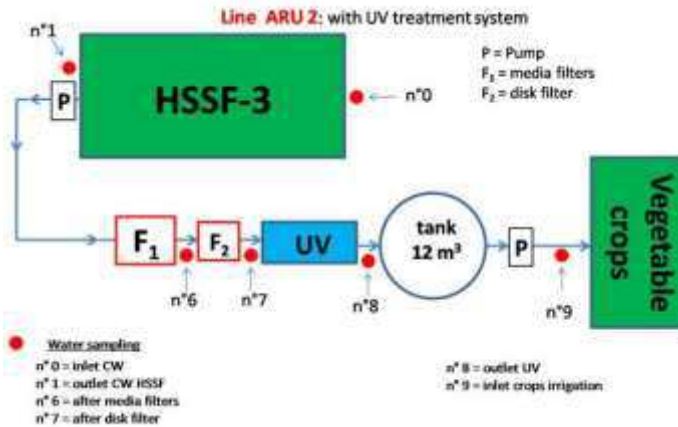


Figure 10: ARU2 treatment system

The treatment system ARU 1 includes in series: horizontal sub-surface constructed wetland (CW), lagoon basin (of about 50 m³), storage reservoir (of about 100 m³), and sandy filters (model Irritec EGV 2”), while the ARU 2 includes in series: horizontal sub-surface CW, sandy filters, ring filters (Irritec EUV rotofilter with automatic backwash) and ultraviolet (UV) system (WEDECO AG, LBX 10).

The UV unit was equipped with a three low-pressure mercury lamps (wavelength (λ) = 254 nm) that provide for a maximum flow rate of 6m³ h⁻¹, max pressure 16 bar and a minimum dose of 700 J cm⁻² to the water (minimum value for a efficiency water disinfection treatment) with 70% transmittance (Figure 11).

The ARU1 system may represent a valid treatment option for those countries where the irrigation reuse practice is not limited by binding standards, like countries of middle east, or where the WHO guidelines are adopted.

The ARU2 treatment option guarantees the complete removal of the microbiological component of TWW, thus implying high cost for disinfection. The treatment strategy is most suitable in those countries, like Italy, where the high quality standards of the reuse regulation have to be meet.



Figure 11: UV system, mod. WEDECO AG, LBX 10 used in ARU2 treatment system

In 2014, the scheme of the irrigation plant is that of Figure 12.

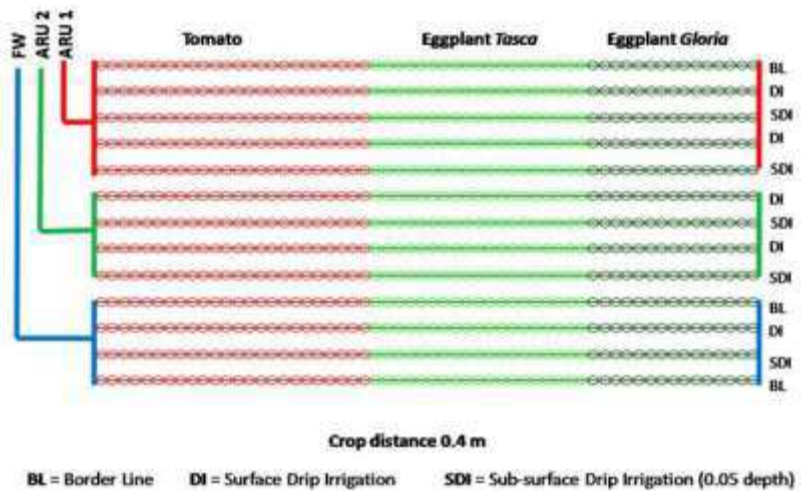


Figure 12: Experimental irrigation field for vegetable crops irrigation realized during summer 2014

In 2014, the total length of the irrigated plots was 30 m. All the plots- (supplied by ARU 1, ARU 2 and FW) were equipped with polyethylene laterals with 16 mm external diameter. All the laterals were supplied by in-line labyrinth drippers (model i-siplast Tape) with a theoretical discharge rate of 0.9 L h^{-1} at a pressure of 70 kPa.

Emitters were spaced at 0.2 m and the distance between rows was of 1.1 m.



Figure 13 Eggplant (Cv *Gloria* and *Tasca*) and Tomato (cv *Missouri*) irrigated by TWW in 2014 – summer period

Tomato (*Solanum lycopersicum* L., Cv *Missouri*) and eggplant (*Solanum melongena* L.) of two cultivars *Tasca* – Ta and *Gloria* – Gl were both transplanted in May 2014 (Figure

13), at density of 2.2 plants m^{-2} ; three replicates of 10 tomato, 5 for eggplants were considered for the all the determinations and comparisons.

For the autumn-winter cycle of the year 2014 (showed in Figure 14), on October 2014 lettuce of two variety (*Lactuca sativa* L., Cv *Canasta* and *Romana*) and cabbage (*Brassica oleracea gongylodes* var. red *Acitano* and var. white *Luna*) were planted at density of 3.7 plants m^{-2} ; three replicates of 10 lettuce and 10 for cabbage for each variety were used for the all the determinations and comparisons (Figure 15). The experimental scheme includes in-line labyrinth drippers (model i-siplast Tape) with a theoretical discharge rate of 0.8 L h^{-1} at a pressure of 55 kPa.

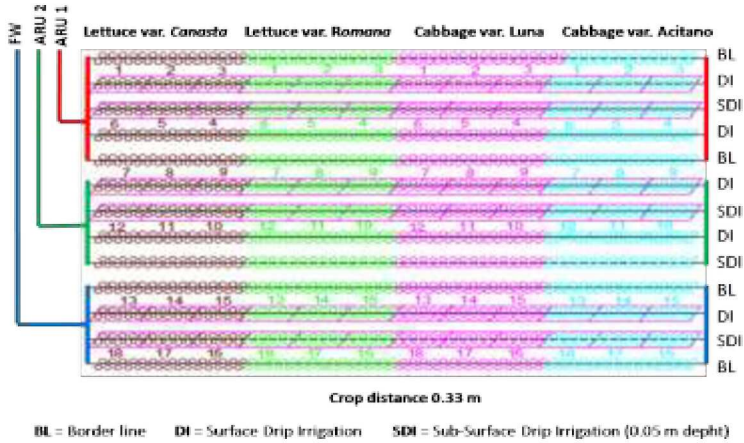


Figure 14: Experimental irrigation field for vegetable crops irrigation adopted during winter 2014



Figure15: Winter crops of Lettuce (Canasta and Romana) and Cabbage (white and red) irrigated with TWW during winter 2014

2.3.2 Irrigation scheduling for row crops

Irrigation scheduling was based on crop evapotranspiration (ET_c , mm d^{-1}) estimation. ET_c rate was calculated by multiplying the reference evapotranspiration (Et_0 , mm d^{-1}) via Penman-Monteith method (Allen et al., 1998) with the FAO-56 crop coefficient (K_c). The experimental site was equipped with an automated meteorological station, installed close to the experimental field for meteorological data monitoring. A Class A PAN evaporimeter was also installed at the field and used for data comparison. The main measured meteorological data were the following:

- Air temperature ($^{\circ}\text{C}$);
- Air humidity (%);
- Solar radiation (W m^{-2});
- Wind velocity and direction (ms^{-1});
- Rain (mm).

The value of K_c was assumed equal 1.0 during first cycle of the trial, in summer 2013, for all crops.

For second cycle, spring-summer 2014, a variable K_c was assumed for tomato and eggplant. In particular, at beginning (30 days from plantation) of the growth phase, a $K_{C\text{ ini}}$ of 0.6 was considered; then, a crop coefficient $K_{c,\text{mid}}$ of 1.1 (until 75

days from planting) was used, and a $K_{C\text{ end}} = 0.9$ was used for the final growth cycle phases.

During the winter cycle of 2014, a $K_{c,\text{ini}}=0.7$ and a $K_{c,\text{end}}=1.1$ were adopted for both lettuce and cabbage.

Because soil was mulched, a coefficient K_r to adjust this situation was applied. K_r was assumed equal to 0.65 for all the treatments in 2013 and 2014.

Irrigation water requirement (IR, mm g^{-1}) was thus estimated as:

$$\mathbf{IR} = \mathbf{ET_0} \times \mathbf{K_C} \times \mathbf{K_R} \quad (1)$$

Water volume (V , $\text{m}^3 \text{g}^{-1}$) for each irrigation was calculated as in Eq.2, where A is the experimental field area.

The time (d , hour) was calculated by Eq.3, where q is the flow rate of each emitter and n the emitters number per plant.

$$\mathbf{V} = \mathbf{IR} \times \mathbf{A} \times \mathbf{10^3} \quad (2)$$

$$\mathbf{d} = (\mathbf{V} \times \mathbf{10^3}) / (\mathbf{q} \times \mathbf{n}) \quad (3)$$

Irrigation was applied three times per week in the amount necessary to replace 100% of the established volume, since the previous irrigation. Irrigation rates were adjusted when necessary due to effect of rainfall. Because irrigation water was controlled, deep percolation and runoff were assumed as negligible. The

amount of water applied was measured with in-line flow meters; water pressure regime was also checked by manometers.

During summer 2014, the field was equipped by TDR (Time Domain Reflectometry, TDR100 CS) probes, connected to a datalogger (Campbell CR 1000), in order to analyse the soil dynamics and eventually correct the irrigation requirement rates (Figure 16).

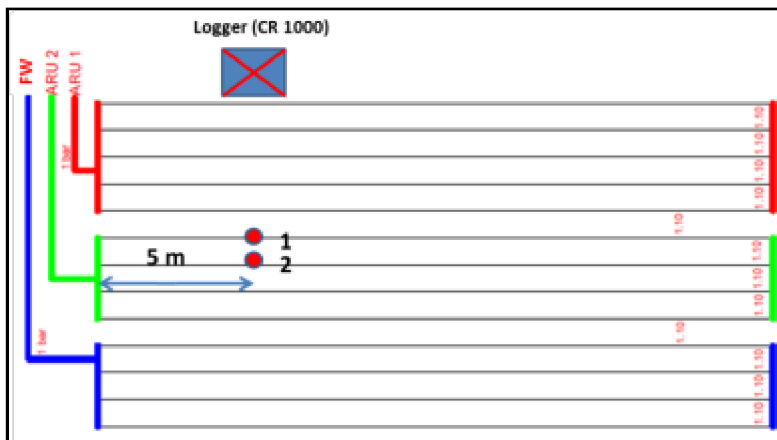


Figure 16: TDR location at the experiment plot in 2014

These probes (named from 1 to 8) were located at the experimental field, within the ARU 2 sector, at different depth in the surface row (DI) and sub-surface rows (SDI); each row was equipped by 4 probes (Figure 17).



Figure 17: TDR installation in 2014

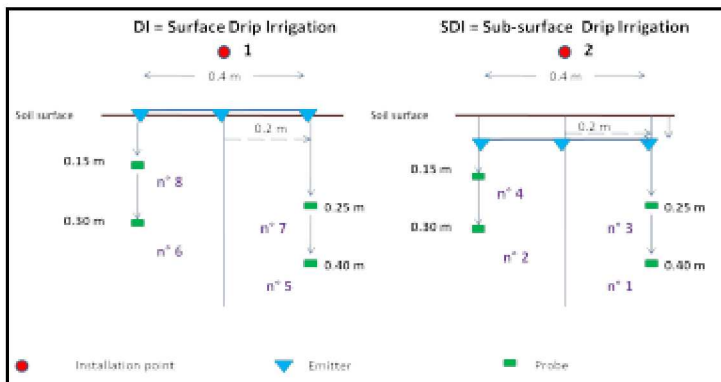


Figure 18: TDR probes position

In each investigated row (DI and SDI), soil humidity was monitored at 0.15 m, 0.25 m, 0.30 m and 0.40 m (Figure 18) from soil surface. Data were collected weekly.

2.3.3 Water sampling and irrigation system efficiency

In order to evaluate the efficiency of the different water treatment systems implemented during the years of trial (i.e. 2013-2015), TWW samples were collected in different selected points (see Figures 6, 9 and 10).

To analyse the main physical-chemical and microbiological TWW characteristics, two sterile samples of 1000 mL were collected (Figure 19).



Figure 19: TWW samples collected during 2013-2015 period

When collected TWW samples were stored at 4°C in a portable fridge and transported to laboratory for analyses. The water sampling campaign has a frequency of 2 times per months, approximately every 15 days.

Standard methods (APHA, 1998) were adopted for laboratory analyses aiming at measuring the parameter reported in Table 2.

Table 3: Physical-chemical and microbiological parameters investigated in laboratory

Water analysis	
Chemic-physic and Microbial	
pH	Sulphate (mg/l)
C.E. ($\mu\text{S}/\text{cm}$)	Potassium (mg/l)
BOD ₅ (mg/l)	CaCO ₃ (mg/l)
COD(mg/l)	Magnesio (mg/l)
TSS (mg/l)	Iron (mg/l)
TN (mg/l)	SAR
TP(mg/l)	Surfactant (mg/l)
Sodium (mg/l)	E. coli (UFC/100ml)
Chloride (mg/l)	Salmonella

2.3.4 Soil contamination and hydraulic determinations

To determine the changes induced in the soil hydraulic and transport properties, laboratory analyses were performed to determine the soil quantitative and qualitative characteristics.

Prior to planting, N (55 kg ha^{-1}), P_2O_5 (110 kg ha^{-1}) and K_2O (80 kg ha^{-1}) were applied uniformly to the experimental field for each cycle.

To ascertain the changes induced in soil hydraulic and transport properties at the end of wastewater application cycle, soil characterization analyses were performed by laboratory test. About 60 undisturbed soil samples were collected (using stainless steel cylinders of 10 cm^3 inner volume) at beginning and end of wastewater application along a diagonal transect (north-south oriented) at 0.30 m of depth (Figures 20 and 21).

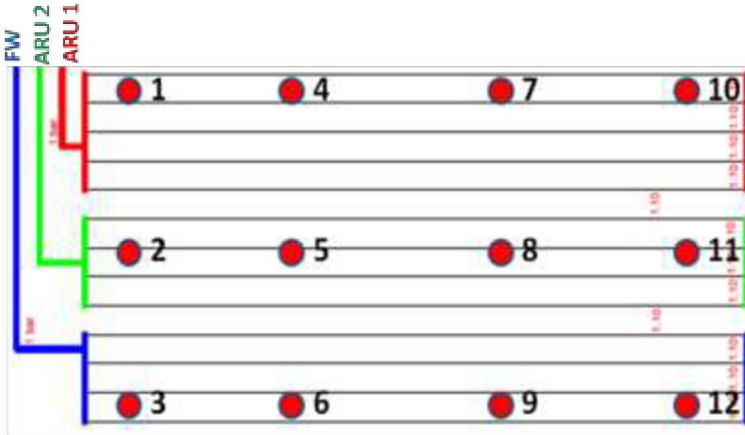


Figure 20: Soil sampling location at the experimental field

Soil contamination analysis assessed *E.coli* and *Salmonella* concentrations within soil columns (i.e. at surface, 0.15 and 0.3 m of depth) near the emitters in the drip (DI) and sub-drip irrigated (SDI) rows. Soil samples were collected in the sectors three times for each experimental cycles. Laboratory processing for soil microbial and constituent analyses were performed as outlined in APHA (2005). Soil samples microbial levels (CFU 100 mL⁻¹) were enumerated using membrane filtration techniques in 100 g of soil samples.



Figure 21: Samples of soil collected for microbial analysis during summer 2013

2.3.5 Crop response

During first experimental cycle in 2013, lettuce heads were harvested in mid-September, corresponding to their commercial stage; zucchini were harvested from mid August through the end of September with a frequency of two times

per week; eggplants were harvested, twice a week, from mid September to the first week of November 2013. During second cycle in 2014, eggplant were harvested from July 21st to September 5th, with a frequency of two times per week; the industrial tomato fruits were harvested only two times during the cycle.

During each harvest operation, for each sector and each repetition, the number of fruits and the fruit weight, were registered; for lettuce, plant weight and the number of leaves per plant were determined (Figure 22).



Figure 22: Lettuce var. *Romana* (left) and *Canasta* (right), in winter 2014

Additional laboratory analyses were carried out on fruit quality for all crops to investigate total Coliform, *Escherichia Coli*, *Salmonella* and *Listeria*. Samples of fruit were collected 3 times during each irrigation cycle for laboratory evaluations and to assess their quality characteristics. For each repetition on DI and SDI sectors, 2 fruits per plants were collected with

sterile gloves and tools. Samples were stored in a fridge at 4°C and then transported to laboratory for analyses (Figure 23).



Figure 23: Laboratory procedures on tomato fruits to determine skin colour (on the left) and antioxidant substance (on the right) – year 2014

Other investigated parameters were: plants height (cm), stem diameter (cm), fresh and dry weight (g), fruit colour, length of fruit (cm), diameters (cm), average weight fruit (g), plant production (g), number of fruit per plant, total yield (kg m²) (Figures 24-26).



Figure 24: Laboratory procedures on tomato fruits to determine fresh and dry weight – year 2014

During the years of experiment, the number of leaves and the total soluble solids (Brix) were evaluated for lettuce and cabbage. Antioxidant substances were also determined.



Figure 25: Lettuce samples in 2013



Figure 26: Eggplant and tomato samples in 2014

The effects of water quality, QW (TWW versus FW), micro-irrigation system (IS) performance, and of their interactions on crop production features (i.e. total yield, fruit numbers and fruit mean weight) were evaluated through the analysis of variance (n-ways ANOVA with randomized block design) at significant levels of $0.01 < P < 0.05$, $0.001 < P \leq 0.01$ and $P < 0.001$. An experimental split-plot design was adopted for each crop. The following experimental factors were statistically analysed: (i) water qualities (QW) (i.e. TWW and FW); (ii) irrigation systems (IS) (i.e. surface drip irrigation and sub-surface drip irrigation) and, for eggplants, lettuce and cabbage, (iii) crop cultivars (CV).

The data set obtained from the study was analysed using the “CoStat” software package (version 6.4, CoHort Software). A n-way fixed-effects general linear model (GLM) analysis of variance (ANOVA) was used to determine the effects of RW reuse. When the interaction was significant, data were presented as separate curves to show any differences in the trend. When significant differences were found, a Student-Newman-Keuls post hoc multiple comparison procedure was used to assess significant differences between the individual means.

In this study, polyphenols determinations were carried out as outlined by Soengas et al. (2012), with some modifications. HPLC (high-performance liquid chromatographic) analysis was performed using a JASCO system equipped with a diode array detector (MD-910 JASCO). The main compounds observed were caffeic, cichoric, chlorogenic, pterulic and sinapic acids. The quantification was based on calibration curves of external standards. All polyphenols were expressed in terms of sinapic acid. Peaks of polyphenol were identified by direct comparison with commercial standards and their spectral and chromatographic properties, with respect to data reported in the literature.

Fruits in 500 g-samples were used to measure microbial contamination after irrigation. *E.Coli* and TC were enumerated using membrane filtration techniques (APHA, 2005). *Salmonella* detection protocol was carried out as outlined by APHA (2005).

Contamination effects on products, evaluated in terms of *E.coli*, were determined relative to the Italian wastewater reuse standards (M.D. 185/2003) and WHO guidelines (2006).

Fruits samples of 500 g were harvested from each plot and used to measure microbial contamination. Samples were

collected at the experimental field every fortnight before irrigation. In the laboratory, 100 g of fruits, including fruit skin and flesh, were homogenised with 900 mL of sterile water by a stomacher. Then, ten-fold dilutions were conducted within the same medium. Total coliform, *E. Coli* and *Salmonella* were measured using membrane filtration techniques (APHA, 1998). The *Salmonella* detection protocol consisted of a pre-enrichment stage using a solution of buffered peptone water, which is a non-selective culture medium, to revitalise the microorganism. Then, an inoculum culture was prepared in selenite and cystimedium and incubated at 36 ± 1 °C for 48 h. After the incubation period, several cultures were inoculated and incubated in parallel on s-s agar gel to count the number of *Salmonella* colonies (Giammanco et al., 2002).

Contamination effects on products, evaluated in term of *E. Coli*, were analysed at the light of both the Italian wastewater reuse legislation (M.D. 185/2003) and WHO guidelines (2006).

2.3.6 Irrigation system performance

The performance of drip irrigation (DI) and sub-drip irrigation (SDI) laterals was analyzed by evaluating the emission uniformity (EU, %):

$$EU = 100 \cdot \frac{Q_{\min 1/4}}{Q} \quad (4)$$

the EU is the ratio between the average discharge of the emitters in the bottom 25th percentile ($Q_{\min 1/4}$) and that of all the emitters (Q), both expressed in ($L s^{-1}$) (Keller and Karmeli, 1975). The EU was determined at the beginning and at the end of the irrigation season by measuring the discharge from 30 randomly selected emitters along each pipe (DI and SDI) supplying reclaimed wastewater and FW (Figure 27).



Figure 27: Measurements of Emission Uniformity at the experimental site

For subsurface laterals, EU was determined before they were buried and at the end of the irrigation season.

2.3.7 Biofilm determination within the pipelines

During the year of trial 2014, the presence of microbial biofilm was detected within the pipelines and the filtering system. Traditional laterals (named Conv. DI and Conv. SDI) and bacteriostatic types (produced by Irritec Spa) were compared. Bacteriostatic pipelines were processed with two different bacteriostatic substances (typology “A” and “B”) at a concentration of 4%. At the field bacteriostatic pipelines (named Bact. “A” and Bact. “B”) were placed in ARU1, ARU2 and FW irrigation schemes in surface positions (Figure 28).

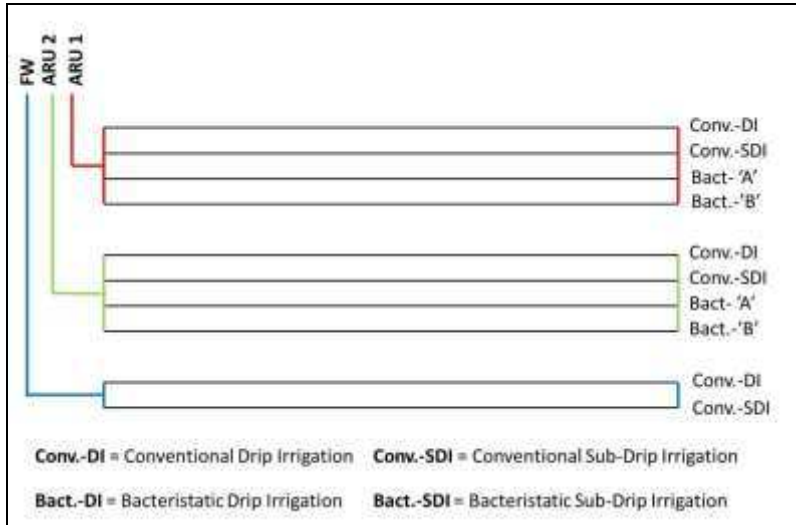


Figure 28: Irrigation scheme for biofilm growth detection

Small samples of pipelines (i.e. 0.3 m) were collected from June to September and analysed for microbial biofilm detection (Figure 29).



Figure 29: Samples of pipelines collected for microbial biofilm detection in 2014.

Laboratory procedures were adopted for detecting the total number of CFU for each centimetre of sampled pipeline (external diameter 16 mm) and the number of negative Gram bacteria.

3. RESULTS

3.1 WASTEWATER TREATMENT PLANTS RELIABILITY

Table 4 summarizes the physical-chemical and microbial analyses on TWW and FW during first crop cycle in 2013.

Table 4: Mean concentration and standard deviation of physical-chemical and microbiological parameters

Parameter	Mean value and standard deviation (in brackets)					Italian RW reuse limits (MD 185/2003)
	TWW samplin gppoint0	TWW samplingpo int 1	TWW samplingpo int 2	TWW samplingpo int 3	FW	
TSS (mg L ⁻¹)	24.5 (17.9)	9.1 (6.8)	60.0 (45.8)	39.3 (26.6)	n.d.	10
BOD ₅ (mg L ⁻¹)	30.1 (6.7)	14.4 (2.4)	26.3 (13.6)	19.4 (7.1)	n.d.	20
COD (mg L ⁻¹)	62.2 (22.8)	40.2 (25.4)	81.8 (24.3)	65.9 (32.7)	n.d.	100
pH	7.5 (0.08)	7.3 (0.03)	8.2 (0.74)	7.8 (0.3)	7.6	6-9.5
EC (dS m ⁻¹)	1.01 (0.03)	1.09 (0.05)	1.1 (0.06)	1.1 (0.06)	0.8	3.0
TN (mg L ⁻¹)	13.6	7.3 (4.4)	2.9 (3.6)	2.6 (3.3)	0.0	35

	(3.2)					
K (mg L ⁻¹)	3.3 (0.2)	2.5 (0.2)	2.5 (0.1)	2.4 (0.2)	2.5	n.d.
TP (mg L ⁻¹)	2.8 (0.3)	2.9 (0.74)	2.1 (0.2)	2.6 (0.4)	0.15	10
SO ₃ (mg L ⁻¹)	172.9 (7.3)	157.7 (19.1)	192.8 (17.2)	178.0 (37.3)	145.6	0.5
Cl (mg L ⁻¹)	82.3 (4.4)	102.4 (11.3)	117.7 (9.9)	118.9 (9.9)	43.3	250
Fe (□g L ⁻¹)	0.11 (0.09)	0.2 (0.1)	0.45 (0.34)	0.24 (0.01)	0.0	2 mg/L
SAR	0.80 (0.01)	0.47 (0.09)	0.58 (0.08)	0.6 (0.07)		10
<i>E.coli</i> (CFU/100 mL)	2x10 ⁵ (1x10 ⁵)	8x10 ³ (6x10 ³)	7x10 ³ (6x10 ³)	5x10 ³ (4x10 ³)	0	50 *
<i>Salmonella</i> (C FU/100 mL)	Absent	Absent	Absent	Absent	Absent	Absent**

*RW: Reclaimed Wastewater; FW: Fresh Water; TSS: Total suspended solids; BOD₅: Biochemical Oxygen Demand; COD: Chemical Oxygen Demand; EC: Electrical Conductivity; TN: total nitrogen; K: total potassium; TP: total phosphorous SO₃: sulphuric acid; Cl: Chlorine; F_e: Iron; SAR: structure-activity relationship; E.coli: Escherichia coli; * limit value for 80% of samples; a maximum of 200 CFU/100 mL is allowed in 20% of samples; n.d.: not detected; ** 100% of samples.*

Data annual means and standard deviations (\pm in brackets) were compared with Italian recommended limits (Ministerial Decree n. 185/2003). Water contamination by *E. coli* varied considerably during the trials at the different sampling points from 10^3 to 10^5 CFU/100 mL. None of the TWW samples analyzed in 2013 had *E. coli* concentrations below the mandatory limit of 50 CFU/100 mL. For 92% of the samples, concentration values were higher (up to 1.7×10^5 CFU/100 mL) than the permitted upper threshold (200 CFU/100 mL) (Figures 30 and 31). *Salmonella* was not detected in the RW samples. In 15% of samples, *E. Coli* contamination was above the value of 10^4 CFU/100 mL fixed by the World Health Organization; this limit was fixed for the “unrestricted irrigation” scenario, in order to reach a median design risk for rotavirus infection of 10^{-3} pppy (per patient per year), considering a 2-3 log unit reduction due to rotavirus die-off between last irrigation and consumption (Figure 5). In particular, according to WHO Guidelines (2006), TWW adopted in this experiment might be used for vegetable crop irrigation by implementing some post-treatment health protection control measures. For example, up to 5 log unit of microbial reduction could be achieved by washing the produce with clean water, disinfection and peeling. The

application of these post-treatment health-protection control measures assures the required log units reduction of *E. coli* for the tolerable rotavirus infection risk.

Results of the physical-chemical and microbiological analysis on TWW of our study case for the period 2013-2015 are reported in Figures 30-34.

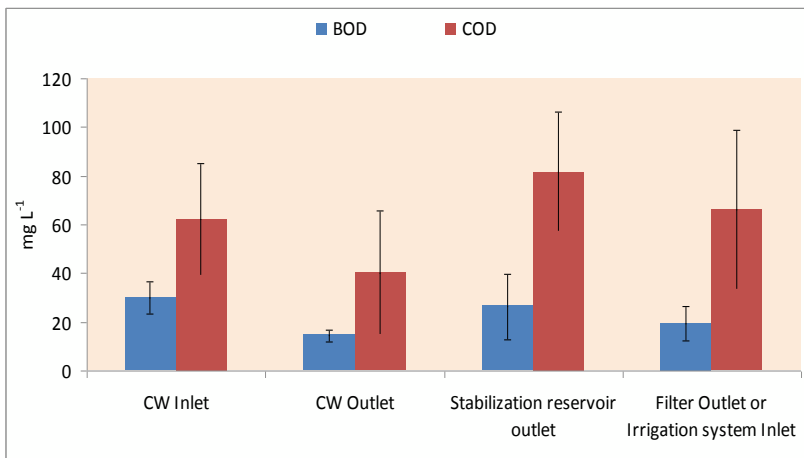


Figure 30: BOD and COD concentration in TWW during the year of trial 2013.

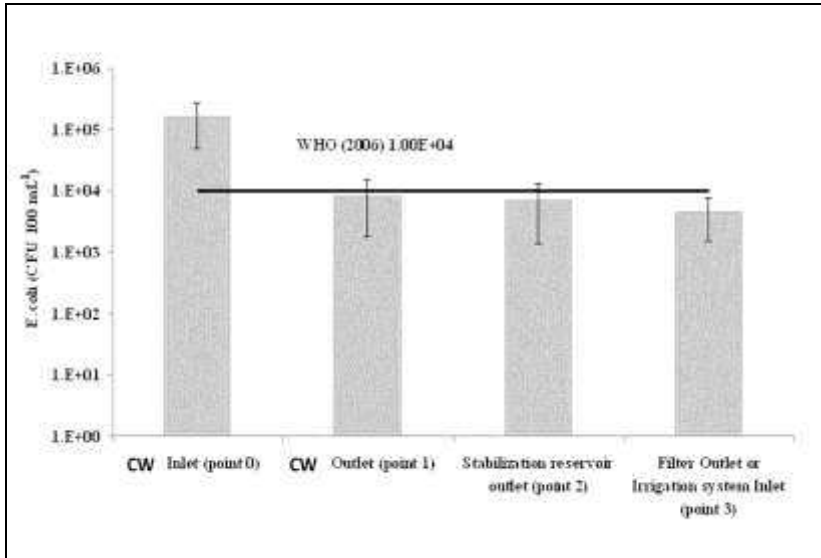


Figure 31: *E. Coli* concentrations in TWW during the year of trial 2013

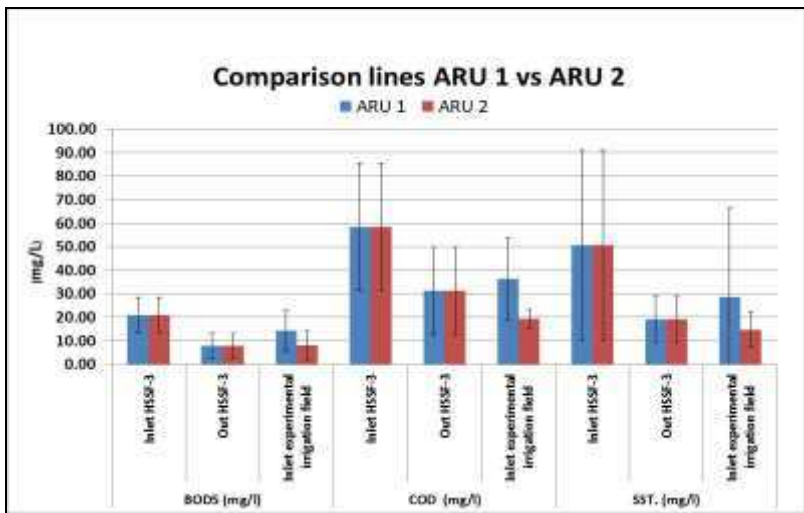


Figure 32: BOD , COD and TSS concentration in TWW during the year of trial 2014

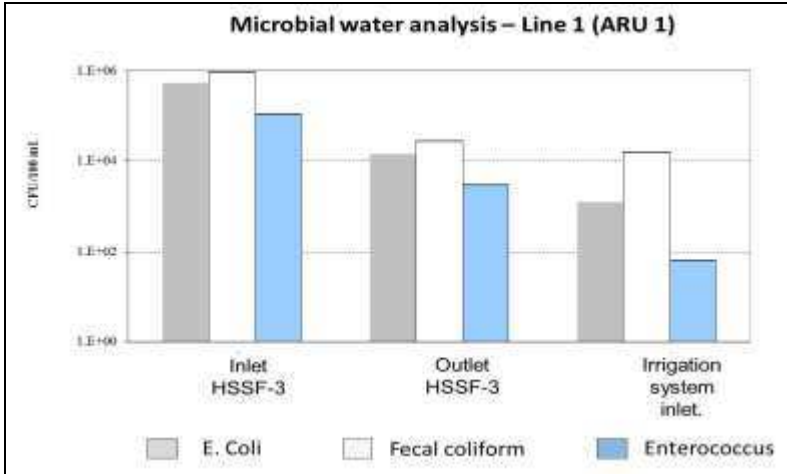


Figure 33: Microbial concentrations in TWW during the year of trial 2014 – ARU1 treatment system

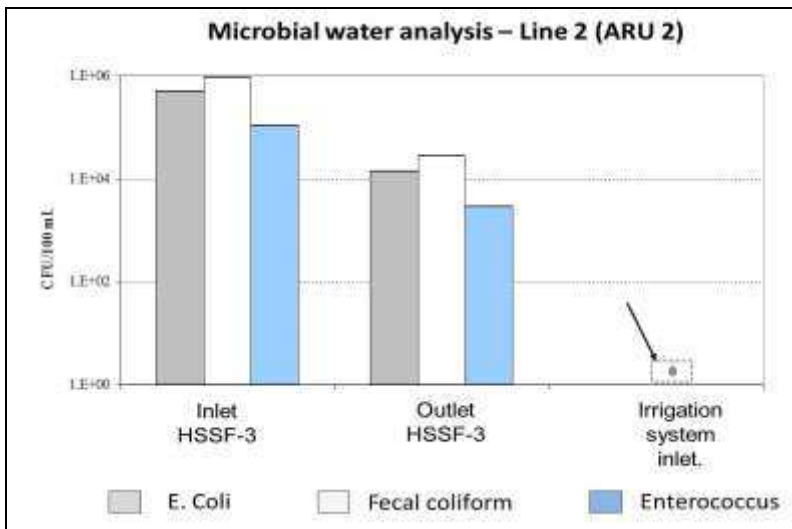


Figure 34: Microbial concentrations in TWW during the year of trial 2014 – ARU2 treatment system

The emission uniformity (first cycle – summer 2013) of DI and SDI laterals (Figure 35) indicates reductions of 12 and 18% in TWW and FW, respectively. Microbial biofilm growth within the pipelines and the emitters may have caused these reductions, as confirmed by recent literature (Hiskakis et al., 2011; Gamri et al., 2014). In particular, the presence of the microbial biofilm, (i.e. a heterogeneous and functional aggregation containing microbial community), was confirmed in our study (i.e. colonies of *Streptococcus Faecalis* and *Serratia marcescens*) by laboratory analyses. No significant variation in the EU was observed between the two micro-irrigation techniques (DI versus SDI).

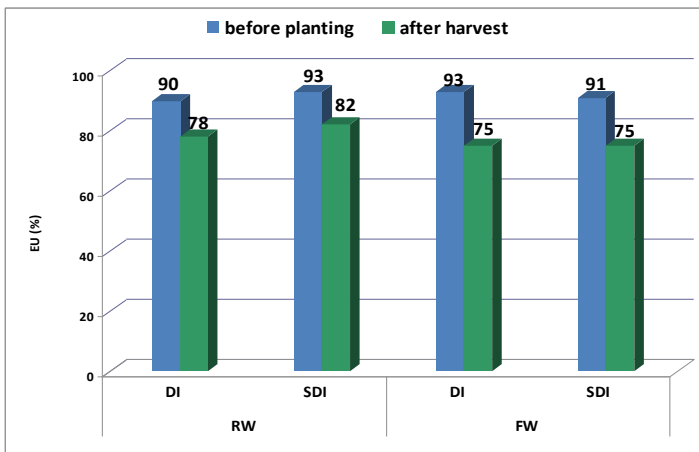


Figure 35: EU values during the year of trial 2013 for TWW (or RW) and FW irrigation systems

EU of DI and SDI laterals during the second year of trial (spring-summer 2014) indicates mean reductions of 14% all the investigated sectors (ARU 1, ARU 2 and FW) (figure 36).

During the winter trial in 2014, EU differences were not significant among the investigated sectors (figure 37).

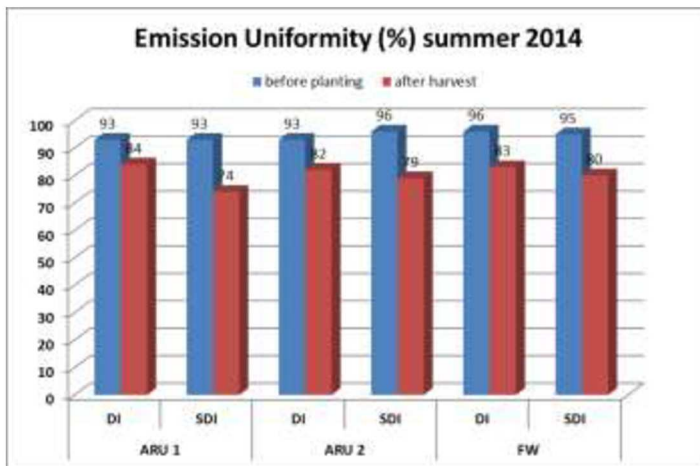


Figure 36: EU values during the year of trial 2014 (spring-summer period) for TWW (or RW) and FW irrigation systems

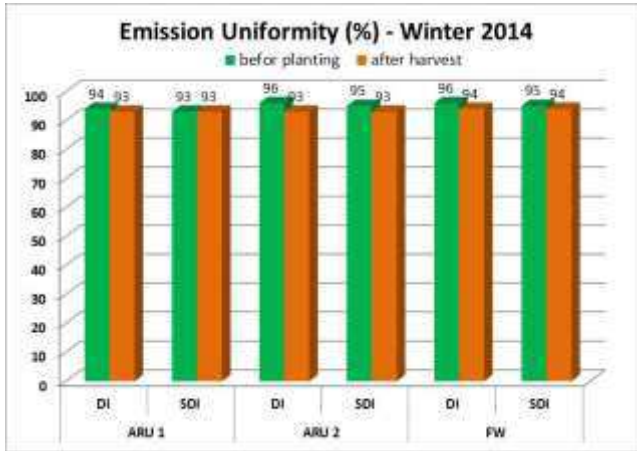


Figure 37: EU values during the year of trial 2014 (winter period) for TWW (or RW) and FW irrigation systems

3.2 SOIL MONITORING

Physical-chemical analyses carried out on soil samples were reported in Tables 5 and 6 for the year of trial 2013 and 2014, respectively.

Table 5: Physical-chemical analysis on soil samples collected in 2013

Clay (%)	50.2
Silt (%)	18.12
Sand (%)	31.67
pH	7.83
CE ($\mu\text{S}/\text{cm}$)	155
Nitrogen (%)	0.12
ORGANIC matter	2.46
Phosphorus (ppm)	87.16
Total limestone (%)	4.75
Mg (ppm)	434.37
Na (ppm)	104.5
K exchangeable (ppm)	581.5
K ₂ O (ppm)	697.8
Ca (ppm)	6615.6

Data of Table 5 refers to soil samples collected prior the TWW reuse experiment start.

Table 6: Physical-chemical analysis on soil samples collected in 2013

Clay (%)	43.15
Silt (%)	17.84
Sand (%)	39.01
pH	7.70
CE ($\mu\text{S}/\text{cm}$)	160
Nitrogen (%)	0.11
ORGANIC matter	1.37
Phosphorus (ppm)	46.23
Total limestone (%)	2.92
Mg (ppm)	456.25
Na (ppm)	122.50
K exchangeble (ppm)	643.00
K ₂ O (ppm)	771.60
Ca (ppm)	6355.21

Microbial analyses conducted during 2013 on soil sampled at different depths within the experimental field revealed the presence of *E.coli*, with a maximum concentration of $2 \cdot 10^1$ CFU g^{-1} at 0.10 m beneath soil surface (Figure 38). In this study, and as confirmed in the literature (Aiello et al., 2007; Cirelli et al., 2012), a certain microbial persistence was individuated along the investigated soil profile. No *Salmonella* contamination was detected in soil samples.

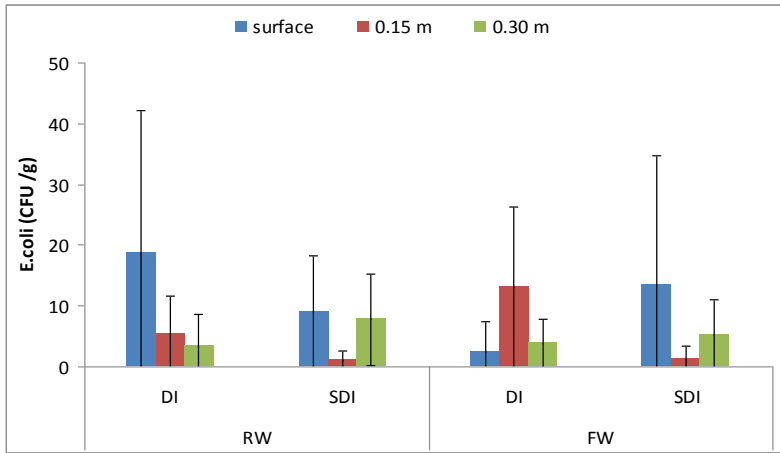


Figure 38: Microbial soil analysis at different depth during 2013

ANOVA analysis did not reveal significant differences in contamination values along the soil profile between TWW (RW in the picture) and FW. Fresh water did not cause significant microbial alterations of the irrigated soil.

In 2014, Microbial analyses on soil sampled at different depths in ARU 1 revealed the presence of *E. coli*, with a maximum concentration of $5 \cdot 10^1$ CFU g^{-1} (Figure 39).

No *Salmonella* contamination was detected.

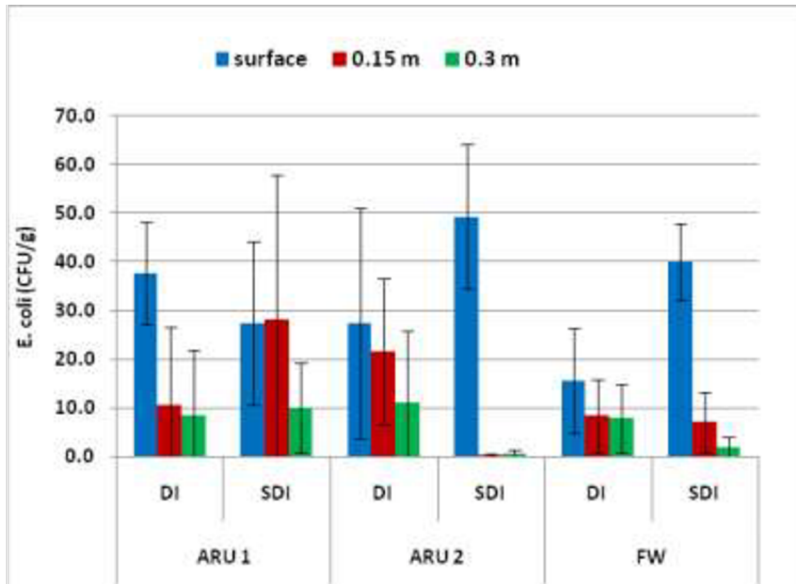


Figure 39: Microbial soil analysis at different depth during summer 2014

TWW coming from ARU2 (equipped with UV system) did not cause a significant presence of soil microbial contamination.

3.3 PLANT GROWTH, QUALITY AND BIOFILM DETECTION

Table 7 reports the main production features obtained for lettuce crops during the irrigation cycle in 2013.

Table 7: Production features of lettuce in 2013

Production features	FW		TWW	
	SDI	DI	SDI	DI
Plant vegetative plant length (day)	67.2	67.4	81.5	81.4
Stem diameter (mm)	26.1 b	24.5 b	30.6 a	29.5 a
Leaves number (n)	58.0 b	70.0 a	59.0 b	67.0 a
Plant weight (g)	1072 b	1020 c	1118 a	1106 a
Yield (kg m ⁻²)	3.0	2.8	3.1	3.1

Different letters along indicate values significantly different at probability level $P=0.05$ on the basis of Student - Newman - Keuls test.

FW: Fresh Water; TWW: Treated WasteWater; SDI: Sub-Drip Irrigation; DI: Drip Irrigation.

The basal stem diameter and the number of leaves for lettuce were influenced by the different irrigation systems (i.e. DI versus SDI). Statistically significant interactions, between the two experimental factors (water quality, QW and irrigation

system, IS), were observed for plant weight (i.e. larger leaves in TWW irrigated lettuces).

The analysis of the polyphenol acids did not reveal any significant differences induced by the different water quality (TWW versus FW); the different irrigation system (IS) has, instead, influenced the caffeic acid, which significantly increased under surface drip irrigation, DI (Table). For the other polyphenol acids, a significant interaction between QW and IS (Table 8) was observed; the highest values were observed for chlorogenic and sinapic acids in the thesis TWW-SDI, for the cichoric acids in TWW, both DI and SDI, and for pterulic acid in FW-DI.

Table 8: Analysis of polyphenol acids on lettuce

Polyphenol acids	FW		TWW	
	SDI	DI	SDI	DI
Caffeic acid	70.2	101.1	0.0	19.4
Cicoric acid	63.3 c	139.4 b	173.3 a	187.4 a
Chlorogenic acid	104.0 c	138.1 b	159.0 a	129.3 ab
Pherulic acid	33.3 c	51.3 a	43.3 b	48.3 ba
Sinapic acid	67.8 c	120.3 b	149.7 a	93.7 bc

Different letters along indicate values significantly different at probability level $P=0.05$ on the basis of Student - Newman - Keuls test.

FW: Fresh Water; TWW: Treated Waste-Water; SDI: Sub-Drip Irrigation; DI: Drip Irrigation.

The stem diameter of zucchini was significantly influenced by the interaction among the experimental factors, with the highest values observed in FW-DI thesis, where plants showed the optimal vigor (Table 9).

The weight of fruit was not influenced by the experimental factors, whereas, the number of fruits and yield were significantly different in relation to QW, with the highest values for FW (Table 9). These results may be related to the amount of total nitrogen found in TWW, which would have affect the reproductive crop phase.

Table 9: Production features of zucchini in 2013

Production features	FW		TWW	
	SDI	DI	SDI	DI
Stem diameter (mm)	22.6 ab	23.1 a	22.1 ab	19.7 c
Number of fruit per plant (n)	8.8	8.5	7.0	5.3
Fruit weight (g)	178.6 c	195.4 a	177.3 c	183.4 b
Plant production (kg)	1.6	1.7	1.3	1.0
Yield (kg m ⁻²)	2.7 a	2.8 a	2.1 b	1.6 c

Different letters along indicate values significantly different at probability level $P=0.05$ on the basis of Student - Newman - Keuls test.

FW: Fresh Water; TWW: Treated WasteWater; SDI: Sub-Drip Irrigation; DI: Drip Irrigation.

Regarding eggplants stem diameter, significant interactions among the experimental factors were observed; the highest values were detected for cv.*BI* in the theses FW-SDI and FW-DI, and in the thesis TWW-SDI for cv. *DA* (Table 10). The number of fruits per plant was not influenced by the analyzed experimental factors, whereas the fruits weight was significantly different in relation to the different cultivars (cv), with BI producing bigger fruits than DA (Table 10). The fruit production per plant and the total yield were

significantly different in relation to QW, with the highest values registered for FW. The cv BI responded less favorable than DA to irrigation with TWW, as indicated by the DA highest vigor.

Table 10: Production features of eggplants in 2013

Production features	FW				TWW			
	SDI		DI		SDI		DI	
	BI	DA	BI	DA	BI	DA	BI	DA
Stem diameter (mm)	18.2a	17.6b	18.2a	17.6b	17.3b	18.5a	17.6b	15.6c
Number of fruit /plant (n)	7.6b	8.9a	6.8b	7.3b	5.9c	5.5c	5.3c	6.6b
Fruit weight (g)	511	382	481	401	501	359	516	368
Plant production (kg)	3.9	3.4	3.5	2.8	2.9	1.9	2.7	2.4
Yield (kg m ⁻²)	6.4a	5.6b	5.8b	4.7c	4.8c	3.3c	4.5c	4.1c

Different letters along indicate values significantly different at probability level $P=0.05$ on the basis of Student - Newman - Keuls test.

FW: Fresh Water; TWW: Treated WasteWater; SDI: Sub-Drip Irrigation; DI: Drip Irrigation.

Finally, a fairly good adaptation of lettuce to TWW was observed, whereas the other crops reached their best performance under FW irrigation.

Vegetable crops with vegetative organs as edible product (i.e. lettuce) seem to be favored by TWW irrigation, in comparison with plants characterized by edible reproductive organs (i.e. eggplants and zucchini).

On vegetable products, ANOVA revealed a significant microbial contamination, both in terms of *E.coli* and Total Coliform (Figures 40 and 41). In particular, among the different vegetable crops irrigated by TWW, lettuce revealed the worst microbiological quality related to the low quality water supplied; in particular, *E. coli* reached a maximum value of about 3×10^2 CFU/g in surface drip irrigation system (DI).

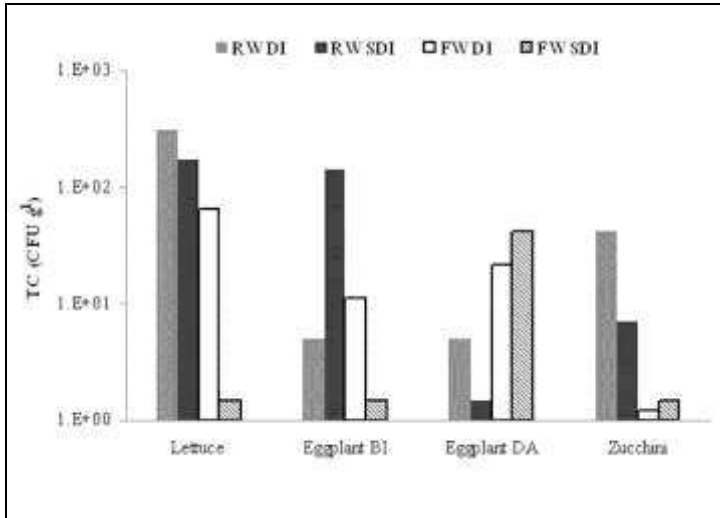


Figure 40: Total Coliform detection on vegetable crops in 2013 (RW and TWW as synonymous in the Figure)

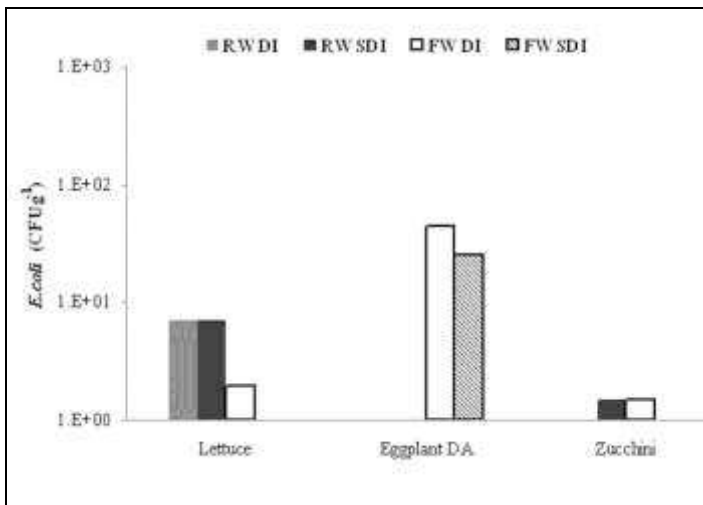


Figure 41: E. coli detection on vegetable crops in 2013 (RW and TWW as synonymous in the Figure)

Microbial contamination was also found on eggplants (var. *Dalia*), when irrigated by FW, most probably due to microbial re-growth processes acting within the reservoir (Cirelli et al., 2009; Consoli et al., 2011). *Salmonella* was not detected in all TWW irrigated products.

Table 11, summarizes the main production effects of TWW irrigation on the examined vegetable crops. Table 12 reports the analysis of polyphenols conducted on the peel of the eggplant fruits. From the analyses, not significant differences in polyphenol contents were detected among FW and TWW irrigated fruits.

Significant differences were observed in the fruit's pulp. In particular a total content of polyphenol acids of 88.4 $\mu\text{mol/ml}$ and 38.9 $\mu\text{mol/ml}$ were observed in the cv *Tasca* irrigated in AUR1 and ARU2, respectively (Table 12).

Significant differences in terms of Chlorogenic acid content in both peel and fruit pulp were observed due to the different irrigation techniques (DI versus SDI).

In particular, the values of the Chlorogenic acid content ($\mu\text{mol/ml}$) in the fruit were of 391.4, 340.8 and 160.2 $\mu\text{mol/ml}$ for ARU2, ARU1 and FW, respectively. Higher

values were registered in the pulp of the fruit sampled in the ARU 1 and ARU 2 sectors (Table 12). Quite variable values of Chlorogenic acid content ($\mu\text{mol/ml}$) were detected in cultivar *Tasca* with values between 71.2 and 6,464 $\mu\text{mol/ml}$.

During spring-summer 2014, the production features of eggplants present significant differences related to the stem diameter and the fresh weight. Plant height was higher in ARU2 for cultivar *Gloria* (0.46 m). Cultivar *Tasca* had the higher number of leaves (n. 26.3) and fruit length (0.13 m). Globally, the DI system produces the best results in terms of fruits weight, plants yield and total yield.

In 2014, tomato crop reports significant differences in plant height, number of leaves and fruit length between FW and TWW irrigated theses (Table 13).

Statistically significant interactions among water quality and irrigation system were found for plant height, fruit length (figure 42); these values registered their maximum in ARU2 sector.

Drip irrigation system shows higher value for n° of leaves, average fruit weight, yield for plant, total yield and n° of fruit for plant.

As reported in Table 14, the results of Polyphenol analysis, conducted on tomato fruit, show significant differences in terms of Total Polyphenol acid content ($\mu\text{mol/ml}$), influenced by the different quality of water adopted in the study.

The highest values were detected in ARU1 sector.

The lab-analyses were also conducted to detect the prevalent components of the Total Polyphenol acid, that are: Quercetin, Chlorogenic acid, Kaemferolo, Rutina. Concerning.

For all these forms, a significant influences of water quality and irrigation systems was recognized.

The Quercetin shows the higher value ($5.4 \mu\text{mol/ml}$) in ARU 2 sector with DI system. The FW sector irrigated with SDI presents a value of Quercetin of $5.0 \mu\text{mol/ml}$. The higher Kaemferolo was of $2.7 \mu\text{mol/ml}$ in ARU 1 with SDI system. Rutina had higher values in FW sector with SDI ($43.4 \mu\text{mol/ml}$) and in ARU 2 with DI system ($32.1 \mu\text{mol/ml}$). Chlorogenic acid does not show significant differences.

Table 11: Production features of eggplants in 2014

Eggplant Cv Gloria (G) and Tasca (T)	FW				TWW ARU1				TWW ARU2				Average						
	SDI		DI		SDI		DI		SDI		DI		QW			IS		CV	
	G	T	G	T	G	T	G	T	G	T	G	T	FW	ARU1	ARU2	SDI	DI	G	T
Height (Cm)	33.7	34.0	41.7	34.3	45.0	43.7	52.3	42.0	50.0	43.7	46.0	38.7	37.9b	45.8a	44.6a	41.6	43.8	46.1a	39.4b
Diameter(Cm)	1.1c	1.2bc	1.8a	1.2bc	1.6a	1.5ab	1.7a	1.7a	1.7a	1.2bc	1.8a	1.7a	1.3	1.6	1.6	1.4	1.6	1.6	1.4
Fresh matter (g)	123.9c	253.5bc	348.5ab	221.5bc	182.7c	355.8ab	291.8b	417.6ab	269.0b	162.6c	264.7bc	478.1a	236.9	312.0	293.6	277.2	337.0	246.8	314.9
N° leaves (n)	19.0	26.3	22.0	25.3	21.7	29.7	21.7	23.3	23.7	23.0	24.7	30.3	23.2	24.1	25.4	40.8	38.2	22.1b	26.3a
Fruit weight (g)	299.4	215.8	296.5	239.6	258.2	256.5	297.3	288.2	252.6	211.4	283.5	242.6	262.8	275.0	247.5	249b	274.6a	281.2a	242.3b
Yield for plant (g)	798.3	446.0	1146.6	718.8	1084.5	872.0	1347.5	1152.7	1060.9	479.2	1493.2	1018.8	777.4	1114.2	1013.0	790.2b	1146.3a	1155.2a	781.2b
Yield (kg m ⁻²)	1.8	1.0	2.6	1.6	2.5	2.0	3.1	2.6	2.4	1.1	3.4	2.3	1.8	2.5	2.3	1.8b	2.6a	2.6a	1.8b
N° fruit for plant (n)	2.7	2.1	3.9	3.4	4.2	3.4	4.5	4.0	4.2	2.3	5.3	4.2	2.9	4.0	4.0	3.1b	4.1a	4.1a	3.2b

FW = fresh water; ARU1 = treated wastewater line 1; AR2 = treated wastewater line 2; SDI = Sub-Surface Drip Irrigation; DI = Drip Irrigation; QW = water quality; IS = Irrigation System; CV = Cultivar; G = var. Gloria; T = var. Tasca;

Table12: Total Polyphenol acid content and Chlorogenic acid content ($\mu\text{mol/ml}$) in eggplant fruits collected in 2014

Eggplant Cv Gloria (G) and Tasca (T)	FW				ARU1				ARU2				Average							
	SDI		DI		SDI		DI		SDI		DI		WQ			IS		CV		
	G	T	G	T	G	T	G	T	G	T	G	T	FW	ARU1	ARU2	SDI	DI	G	T	
Peel																				
Total Polyphenol acid	40.4	36.2	28.2	30.6	50.4	28.0	33.5	27.1	43.9	39.2	25.9	51.1	33.9	34.7	40.0	40.7	32.7	38.3	35.2	
Chlorogenic acid	172.8	122.2	222.4	162.1	342.7	363.2	308.7	348.8	440.5	344.3	362.1	419.0	160.2c	340.8b	391.4a	272.0	307.6	306.8	306.4	
Pulp																				
Total Polyphenol acid	27.7b	23.5b	20.9b	37.9b	23.9b	88.4b	13.1b	19.5b	23.8b	36.9b	22.6b	38.9ab	27.52	36.21	30.56	40.23	25.50	47.7	22.0	
Chlorogenic acid	156.7b	166.1b	147.7b	156.5b	416.9b	71.2b	301.3b	6464.5a	222.4b	6027a	270.9b	1298.4b	456.7	1813.5	1954.7	1176.7	1439.9	259.7	2356.9	

Table 13: Production features of tomato in 2014

Tomato Cv Missouri	FW			ARU1			ARU2			Average	
	SDI	DI	Average	SDI	DI	Average	SDI	DI	Average	SDI	DI
Height (Cm)	41.7	44.7	43.2ab	36.7	41.0	38.8b	45.3	44.7	45.0a	41.2	43.4
Diameter (Cm)	1.6	1.8	1.7	1.8	1.7	1.7	1.8	1.7	1.7	1.7	1.7
Fresh matter (g)	536.1	823.3	679.7	510.0	572.8	541.4	659.4	661.3	660.3	568.5	685.8
N° leaves (n)	22.7	23.3	23.0	22.0	23.3	22.7	22.3	25.3	23.8	22.3b	24.0a
Length (Cm)	5.2	5.9	5.6b	6.1	6.3	6.2ab	6.6	7.1	6.8a	6.0	6.4
Diameter (Cm)	3.2	3.4	3.3	3.3	3.6	3.4	3.3	3.5	3.4	3.3	3.5
Polar Equatorial (Cm)	1.6	1.7	1.7	1.8	1.8	1.8	2.0	2.0	2.0	1.8	1.9
Soluble solids (Brix)	5.1	5.3	5.2	5.3	5.0	5.1	5.2	5.6	5.4	5.2	5.3
Fruit weight (g)	78.0	78.7	78.3	80.2	78.3	79.3	77.6	83.4	80.5	78.6	80.1
Yield for plant (g)	1574.2	1926.4	1750.3	1467.2	1699.6	1583.4	1747.1	1911.6	1829.4	1596.2	1845.9
Total Yield (kg m ⁻²)	3.6	4.4	4.0	3.3	3.9	3.6	4.0	4.3	4.2	3.6	4.2
N° fruit for plant (n)	20.2	25.1	22.7	18.4	21.7	20.1	22.4	22.9	22.7	20.3	23.2

Table 14: Total contents in polyphenol acid in tomato fruit ($\mu\text{mol/ml}$)

Poliphenolic acid Tomato Cv Missouri	FW			ARU1			ARU2			Average	
	SDI	DI	Average	SDI	DI	Average	SDI	DI	Average	SDI	DI
Total polyphenol acid	4.5	5.0	4.7b	20.0	20.5	20.3a	13.6	16.2	14.9a	12.7	13.9
Quercitina	5.0a	2.6c	3.8	4.5	4.0b	4.2	4.4b	5.4a	4.9	4.7	3.7
Clorogenic acid	127.4	121.9	124.6	196.8	154.1	157.4	152.3	157.9	155.1	192.7	149.8
Kaemferolo	2.2ab	2.1b	2.2	2.7a	1.4c	2.0	2.2ab	2.6ab	2.4	2.5	2.3
Rutina	43.4a	19.7c	31.5	27.1b	19.2c	23.1	30.1b	32.1b	31.1	33.2	24.1



Figure 42: Measurements of tomato fruit length

Figure 43 and 44 report the microbiological content, evaluated in terms of Total Coliform and *E. Coli* on the vegetable crops irrigated with TWW. As evidenced in 2013, a certain persistence in microbial concentrations is evident in the irrigated crops, even those coming from the ARU2 disinfection system (i.e. with UV). These concentrations were perhaps in line with those reported by WHO (2006) standards, regarding the potential risk for *Rotavirus* infection. Thus, if adequate post-treatments are adopted on the crop production, the risk of microbiological pollution would be greatly limited.

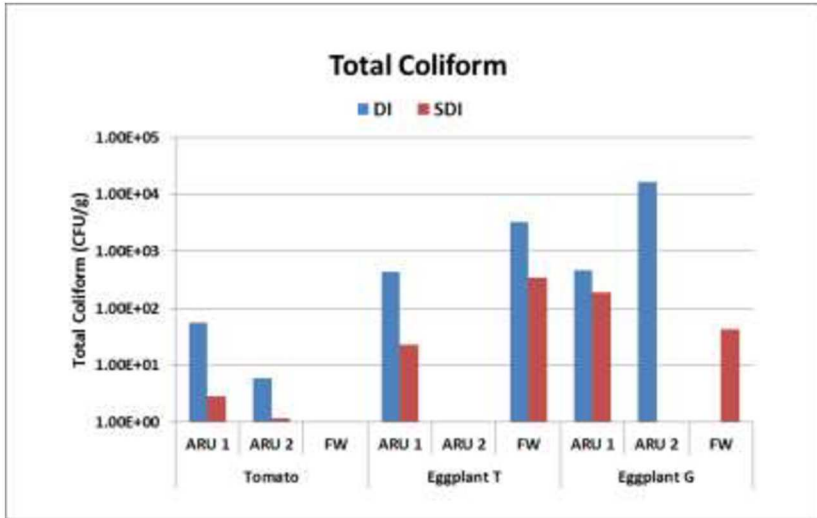


Figure 43: Total coliform on vegetable crops in 2014

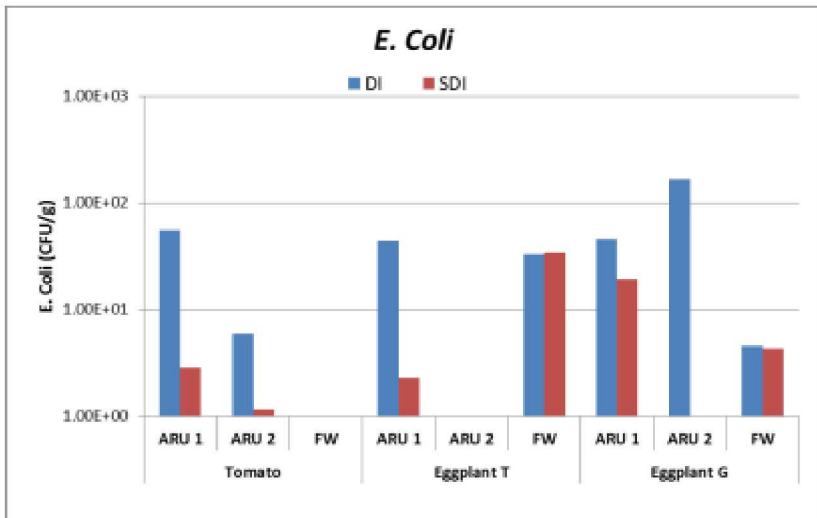


Figure 44: *E. Coli* on vegetable crops in 2014

Winter lettuces were different for leaves number and soluble solids. Water quality also influenced the plant diameter.

In the same experiment, cabbages irrigated with FW and TWW did not show significant differences on crop production features. Between the different cultivars, the higher values of plant weight, number of leaves and yield were registered in cultivar *Bianco*, instead cultivar *Rosso* had shown higher values of stem length.

Finally, the analysis of the microbial biofilm within selected segments of pipelines are illustrated in Figure 17 and 18. The biofilm attached on pipelines revealed a gradual increasing trend (data not showed) with the increasing running time of treated wastewater drip irrigation system.

Table 15: Crop production features for winter lettuce in 2014

Lettuce <i>Cv Canasta and Romana</i>	AC				ARU1				ARU2				Average							
	SDI		DI		SDI		DI		SDI		DI		WQ			IS		CV		
	C	R	C	R	C	R	C	R	C	R	C	R	AC	AR1	AR2	SDI	DI	C	R	
Heads weight (g)	317.0	474.9	356.6	471.4	330.4	449.9	326.6	457.6	348.3	380.1	313.3	339.2	405.0	391.1	345.2	383.4	377.4	332a	428.8b	
Max diameter (cm)	25.5	27.2	24.5	23.8	25.3	24.5	21.2	26.5	19.3	22.8	21.8	25.7	25.3a	24.4ab	22.4c	24.1	23.9	22.9b	25.1a	
Min Diameter (cm)	2.7	2.8	2.6	2.4	3.7	3.1	5.3	2.8	2.5	2.4	3.2	2.7	2.6	3.7	2.7	2.9	3.2	3.3a	2.7b	
length max (cm)	20.0	27.8	20.7	26.8	20.8	26.8	20.0	27.3	19.5	26.5	19.1	25.8	23.8	23.7	22.7	23.6	23.3	20.0b	26.8a	
N° of leaves (n)	12.8bc	18.3a	9.8c	20.8a	8.7c	16.3b	10.5c	15.5b	10.0c	16.7b	7.5cd	14.5b	15.5	12.8	12.2	13.8	13.1	9.9	17.0	
Stem length (cm)	3.2	4.8	2.8	4.6	2.7	4.5	3.8	4.1	2.6	4.3	2.8	3.8	3.8	3.8	3.4	3.7	3.7	3.0b	4.4a	
Dry matter (%)	6.1	6.7	5.7	5.1	5.4	5.4	5.5	6.3	5.6	5.3	5.6	6.3	5.9	5.6	5.7	5.7	5.8	5.6	5.9	
Soluble Solids (brix)	2.1d	3.9b	1.8de	4.4ab	1.8de	3.2bc	1.9c	3.3bc	1.6de	4.7a	2.1d	3.9b	3.1	2.5	3.1	2.9	2.9	1.9	3.9	
Yield (Kg m ⁻²)	1.7	2.6	1.9	2.6	1.8	2.5	1.8	2.5	1.9	2.1	1.7	1.8	2.2	2.1	1.9	2.1	2.1	1.8b	2.3a	

Tab. 16: Crop production features for winter cabbage in 2014

Cabbage <i>Cv Bianco and Rosso</i>	FW				ARU1				ARU2				Average							
	SDI		DI		SDI		DI		SDI		DI		WQ			IS		CV		
	B	R	B	R	B	R	B	R	B	R	B	R	AC	ARU1	ARU2	SDI	DI	B	R	
Plant weight (g)	595.2	424.0	581.6	539.6	531.7	514.0	503.6	406.3	528.8	471.4	538.0	491.8	535.1	488.9	507.5	510.9	510.1	546.5a	474.5b	
Max diameter (cm)	8.2	7.3	8.2	8.0	7.9	7.4	7.5	7.0	7.6	6.6	7.3	6.9	7.9	7.5	7.1	7.5	7.5	7.8	7.2	
Height (cm)	51.8	45.0	54.5	54.5	47.8	51.9	49.7	53.5	51.7	51.3	49.5	56.4	51.5	50.7	52.2	49.9b	53.0a	50.8	52.1	
N° of leaves (n)	16.0	12.2	15.7	12.7	12.7	12.5	12.3	11.2	15.3	11.2	13.3	11.0	14.1a	12.2b	12.7b	13.3	12.7	14.2a	11.8b	
Stem length (cm)	5.3	6.9	5.6	6.6	6.1	8.3	6.3	6.3	5.9	6.7	6.2	7.4	6.1	6.8	6.5	6.5	6.4	5.9b	7.0a	
Yield (Kg m ⁻²)	3.2	2.3	3.2	2.9	2.9	2.8	2.7	2.2	2.9	2.6	2.9	2.7	2.9	2.7	2.8	2.8	2.8	3.0a	2.6b	

Table 17: Results on microbial biofilm detection in 2014 (average data in CFU/cm)

		<i>Listeria spp.</i>	<i>Pseudomonas aeruginosa</i>	<i>Stafilococcus aureus</i>	<i>Enterobacteriaceae</i>	<i>Total Psychrophiles</i>
Water	Inlet ARU 1	-	1.60E+02	-	1.30E+02	3.40E+03
	Inlet ARU2	-	1.40E+02	7.00E+01	3.60E+02	4.70E+03
Pipe	Conv DI ARU 1	4.70E+03	7.00E+01	4.80E+03	2.20E+02	5.80E+04
	Conv DI ARU 2	5.10E+04	5.00E+03	1.30E+03	1.56E+04	5.70E+04
	Conv DI FW	7.40E+04	-	7.90E+03	-	4.70E+04
	Bat DI ARU 1	1.10E+05	7.40E+03	2.36E+04	7.90E+03	1.20E+06
	Bat DI ARU 2	1.50E+05	7.40E+03	4.80E+04	1.90E+03	2.20E+06

Table 18: GRAM Negative detection in 2014 (data in CFU/cm)

Pipeline	Sector	Average GRAM NEG.
Bat	ARU 1	17
Bat	ARU 2	9
Conv DI	ARU 1	19
Conv SDI	ARU 1	21
Conv DI	ARU 2	28
Conv SDI	ARU 2	25
Conv DI	FW	16
Conv SDI	FW	25

4. DISCUSSION

Reclaimed wastewater can be an alternative water resource for irrigation water in areas suffering from a water shortage or unsatisfactory water quality for agricultural irrigation. However, the safety of water reuse should be based on environmental monitoring.

Many epidemiological and microbiological studies for reclaimed wastewater irrigation were performed in upland fields (e.g., Pedrero et al. 2010; Cirelli et al. 2012), with similar characteristics that those examined in this PhD thesis.

Escherichia coli is a representative microbiological indicator of water quality as the most common type of fecal coliform, and its presence also indicates the potential for the co-existence of pathogenic organisms (An et al. 2007).

This research shows that reclaimed wastewater irrigation in vegetable crops presents no increased human health risks, as suggested by WHO standards (WHO, 2006). Based on the characteristics of the microorganisms, there were no significant effects due to the vegetable crops irrigation with urban treated wastewater compared with conventional irrigation water that satisfied agricultural water quality criteria. The results of water, soil monitoring demonstrated that the examined vegetable

crops are not generally adversely impacted by irrigation with reclaimed wastewater. This finding implies that reclaimed wastewater may be considered a practical alternative water resource for row crops irrigation and is potentially safe for the environment of cultivation, although long-term monitoring is needed to observe and understand its effects.

CONCLUSIONS

Wastewater reuse for irrigation is a valid and strategic alternative to the use of conventional water resource in agriculture. Especially, in the area of Mediterranean basin this practice may reach high level of importance in promoting and relaunching the economy of the primary sector.

Actually, to promote the reuse practice several obstacles should be still overcome, for the most part concerning the Italian regulation standards, to consolidate the reuse practice as water management strategy.

In this view, my PhD thesis aims at prove the reliability of the reuse of urban natural treated wastewater in agriculture. The study has examined the main effects of potential contamination that the reuse of TWW may cause on soil, crop production and quality and on the performance of the irrigation system.

Two different irrigation systems, with different level of disinfection, were tested during the trials.

These two systems, named ARU1 and ARU2, were based on the adoption of natural extensive treatment systems, for secondary treating WW, based on the use of constructed wetlands and lagoons. ARU1 might be a typical treatment system to be adopted in developing countries, or where the TWW quality standards are not too stringent (i.e. like the ones

suggested by WHO, 2006). ARU2, on the contrary, because proposing the UV disinfection might be adopted in those countries, like Italy, where the microbial level on TWW must be close to zero.

These two treatment systems were compared in terms of pollution effects provided on soil, plants and irrigation technologies.

The main findings obtained from comparing ARU1 and ARU2, and in general by evaluating the reuse practice, can be summarized as in the follows:

- the performance of the micro-irrigation systems adopted in the experimental trials (*surface drip irrigation* vs *subsurface drip irrigation*) was mainly influenced by water quality characteristics (ARU1 vs ARU2 and TWW vs FW), that might cause the formation of a microbial biofilm within the polyethylene laterals;
- the *E.coli* content of TWW (i.e. both in 2013 and 2014) was quite higher than the concentration limit recommended by the Italian legislation on reuse; however, accordingly to WHO (2006) standards the *E.coli* content found in TWW of San Michele di Ganzaria would not determine risk for rotavirus infection;

- the irrigated soil profiles resulted characterized by a certain persistence of microbial contamination;
- the nutraceutical crop properties did not show a significantly alterations under TWW, ARU1 and ARU2;
- the production characteristics and quality parameters of eggplants (two cultivars), zucchini, tomato were slightly modified by TWW.

Finally, this study on treated wastewater reuse in agriculture shows the importance of an integrated (technological irrigation system aspects, microbiological monitoring on water, product and soil) approach for the assessment of product quality and safety during each crop cycle.

According to the WHO Guidelines (2006), wastewater reclaimed by the Constructed Wetland system might be used for crop irrigation by implementing some post-treatment health protection control measures. Fore example, up to 5 log unit of microbial reduction could be achieved by the produce washing with clean water, disinfection and peeling. The application of these post-treatment health-protection control measures assures the required log units reduction of *E. Coli* for the tolerable rotavirus infection risk.

This study confirms the importance of a comprehensive monitoring of the soil-plant system when wastewater reuse is practiced in the agricultural context.

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